

Protecting California Crops



Commodity Protection & Quality Research Unit
(CPQRU)

Stakeholder Meeting

USDA-ARS-SJVASC Parlier, CA

October 30, 2009



Commodity Protection and Quality

Stakeholders Conference

2008

Research Summary

Commodity Protection and Quality Research Unit
Telephone: (559) 596-2720
Fax: (559) 596-2721

This report contains both published and unpublished information. The unpublished contents may not be reproduced in any form without prior written consent of the involved researchers.

Trade and company names mentioned in the report are for the benefit of the readers only, and do not constitute an endorsement by the USDA-ARS.

Table of Contents

Preface	i
Research Overview	1
Research Reports	
Charles S. Burks	3
Judy A. Johnson	7
L. P. S. Kuenen	11
David P. Obenland	15
Joel P. Siegel	19
Joseph L. Smilanick	23
Spencer S. Walse	27
Victoria Y. Yokoyama	31
Research Reprints	35
Effects of mating disruption treatments on navel orangeworm (Lepidoptera: Pyralidae) sexual communication and damage in almonds and pistachios	37
Response of postharvest tree nut lepidopteran pests to vacuum treatments	47
Bait formulations and longevity of navel orangeworm egg traps tested	55
Determinants of flavor acceptability during the maturation of navel oranges	59
New navel orangeworm sanitation standards could reduce almond damage	67
Pre- and postharvest treatments to control green mold of citrus fruit during ethylene degreening	73
Environmentally regulated abiotic release of volatile pheromones from the sugar-based oral secretions of caribflies	81
Psytalia cf. concolor (Hymenoptera: Braconidae) for Biological Control of Olive Fruit Fly (Diptera: Tephritidae) in California	95

PREFACE

California is home to one of the most productive and diverse agricultural economies in the world, worth more than \$36.6 billion in revenue, 12.8% of the US total. More than \$15 billion is from fruits, nuts and vegetable crops, producing nearly half of those grown in the US. Counties in the San Joaquin Valley of California, particularly Fresno, Tulare and Kern, lead both the state and the country in agricultural production. Tree nuts, fresh and dried fruits, and hay are also important contributors to the \$10.9 billion California export market.

Product quality is of vital importance to the marketing of agricultural products to ensure a safe and nutritious food supply and prevent loss during storage and transport, as well as maintain competitiveness in the global market. For export markets in particular, agricultural products must not only meet the demands of consumers, but must satisfy the regulatory, phytosanitary and quarantine standards of the importing countries. The Commodities Protection and Quality Research Unit of the San Joaquin Valley Agricultural Sciences Center seeks to maintain or improve the quality of US products by finding technical solutions to such issues as product decay, postharvest pathogens, and insect infestation. The unit also helps in extending foreign markets for US products by developing treatment strategies for quarantine pests. Of particular importance is the effort by researchers in the unit to develop both chemical and non-chemical treatments to replace environmentally damaging compounds such as methyl bromide.

The primary purpose of this Research Summary and the Commodities Protection and Quality Stakeholders Conference is to provide our stakeholders and collaborators a synopsis of our ongoing research. We also wish to receive comments and suggestions as to the relevance and direction of our research, so that we may better serve our constituency.

RESEARCH OVERVIEW

**Commodity Protection and Quality Research Unit
USDA-ARS San Joaquin Valley Agricultural Sciences Center
9611 South Riverbend Avenue
Parlier, CA 93648**

<http://www.ars.usda.gov/pwa/sjvasc/cpgru>

One of the primary goals of the Agricultural Research Service is to ensure high-quality, safe agricultural products while sustaining a competitive agricultural economy. Our unit seeks to achieve this by discovering new ways to control postharvest insect pests and decay in horticultural commodities. In doing so, we help provide the public with quality produce and overcome quarantine barriers to allow increased exports of U.S. crops. In addition, much of our work deals with developing non-chemical commodity treatments and integrated management systems which will reduce the use of chemical pesticides.

Appropriated Projects and Personnel

An Areawide Control Program for Navel Orangeworm

Joel Siegel
Bas Kuenen
Chuck Burks

Biological, Behavioral, and Physical Control as Alternatives for Stored Product and Quarantine Pests of Fresh/dried Fruits and Nuts

Judy Johnson
Victoria Yokoyama
Bas Kuenen
David Obenland
Chuck Burks
Joel Siegel

New Chemically Based Methods Which Reduce the Use or Emissions of Chemicals as Alternatives to Methyl Bromide for Quarantine and Postharvest Pests

David Obenland
Joel Siegel
Joe Smilanick
Victoria Yokoyama
Spencer Walse
Chuck Burks
Bas Kuenen

Emerging Technologies to Maintain Postharvest Quality and Control Decay of Fresh Commodities

Joe Smilanick
David Obenland

Charles S. Burks

Research Entomologist

My research centers on monitoring insect populations and damage as part of IPM programs. Much of my current research is focused on mating disruption as an IPM tactic against two related moth species: the navel orangeworm, which is the primary insect pest of almonds and pistachios; and the Indianmeal moth, which is a world-wide pest of stored products. California almonds and pistachios have a collective unprocessed value of >\$2.5 billion annually. Data on the value of products threatened by the Indianmeal moth are more difficult to obtain, but probably this value is greater since these tree nuts and many other commodities are threatened in their processed form.

Current Research Projects and Accomplishments**Abundance of navel orangeworm in almonds and pistachios, and movement between crops.**

An ongoing collaboration with Brad Higbee of Paramount Farming Company has examined the impact of mating disruption on navel orangeworm behavior, biology, and damage to almonds and pistachios owned by Paramount Farming Company in Kern County. Research on abundance and movement of navel orangeworm has been an important part of these studies.

Accomplishments:

- Data from pheromone traps (with virgin females as a pheromone source) and direct sampling from almond and pistachio orchards demonstrated, for the first time, a trend of greater navel orangeworm abundance in pistachios than in almonds. This finding was not previously obvious because navel orangeworm damage to almonds is frequently greater than that to pistachios.
- Mark-capture experiments and analysis of gradients of damage in almonds showed that, while females are capable of traveling a mile in several days, most egg-laying and most damage occurs within 100 yards of where the female emerges.

Mating disruption for control of navel orangeworm in almonds.

Mating disruption studies in Paramount Farming Company orchards in Kern County have examined primarily Nonpareil almonds and pollenizer varieties including Monterey, Nonpareil, and others. A more recent demonstration study, in collaboration with Brad Higbee and Kent Daane (University of California, Berkeley) examines mating disruption for control of navel orangeworm in almonds managed by various growers on the west side of Fresno County. This is part of a larger demonstration project on area-wide control of navel orangeworm, coordinated by Joel Siegel (USDA-ARS).

Accomplishments:

- We compared the impact of mating disruption on navel orangeworm behavior, biology, and damage to almonds and pistachios using 4 square-mile blocks of each crop over two years. Reduction of males in virgin female-baited traps and mating in sentinel females was more evident in pistachios, where abundance was greater, whereas reduction in navel orangeworm to the current-year crop was demonstrated in almonds but not pistachios.

Mating disruption for control of navel orangeworm in almonds (cont).

- A subsequent study, along with that mentioned previously, demonstrated that the distance over which the high-emission dispensers used for mating disruption for navel orangeworm can suppress males captured in virgin female-baited traps is far greater than the distance over which damage to almonds can be reduced by mating disruption.
- At the end of the second year of the study in western Fresno County, there is evidence of impact of mating disruption on ability of males to locate females, fertility of females, and damage to almonds. Soil conditions, grower practices, and proportional representation of almond varieties were very different in this site compared to the Kern County locations of the previous studies.

Monitoring navel orangeworm in almonds, and prediction of damage.

The ability of managers and pest control advisors (PCAs) to reliably foresee and prevent navel orangeworm damage to almonds is a potential barrier to adaptation of mating disruption. Working with Brad Higbee and Bas Kuenen (USDA-ARS), we are examining the currently-used egg traps and potential alternative trapping technologies for monitoring navel orangeworm activity and prediction of damage in almonds.

Accomplishments:

- Virgin female-baited traps were used as proxies to determine the potential of pheromone traps to monitor navel orangeworm and predict activity in almonds. These results were compared to egg traps at the same locations. Eggs per trap in the first flight and males per trap in the second flight had low but significant correlation with subsequent damage to Nonpareil almonds. Both eggs and males per trap in third flight were more highly correlated with damage to Monterey almonds, which were harvested later. These data suggest that the availability of a useful synthetic pheromone trap would represent an incremental improvement in the ability to monitor navel orangeworm in almonds.
- The number of eggs per trap was compared between traps baited with investigator-prepared ground almonds or pistachios, or an almond processor by-product in current commercial use. More eggs were found on traps contain pistachio meal or the almond by-product than the investigator prepared-almond meal; however, this was demonstrable only at high numbers of eggs per trap. When there were few eggs per trap, as is frequently the case during critical periods for monitoring, the number of traps examined was more important for detection of gravid navel orangeworm females than the type of nut meal used.
- Capturing females is useful for monitoring the impact of mating disruption treatments. We compared the trapped females and males using volatile organic compound, phenyl propionate, and almond meal. While almond meal captured only females, phenyl propionate captured both sexes. Overall more males than females were captured, but the sex ratio changed over time. Even though phenyl propionate captured more males than females, 10× more males were captured with phenyl propionate than with almond meal in both almonds and pistachios, and over a broad range of abundance.

Mating disruption for control of Indianmeal moth in processing and warehouse facilities.

I have previously examined the ability of mating disruption using a high-emission dispenser to reduce mating, reproduction, and damage of Indianmeal moth in a bean warehouse in Stanislaus County, California. Currently, in collaboration with Carlos Reyes and Joan Fisher (Suterra LLC, Bend, OR) and in cooperation with Valley Fig and Modern Commercial Pest Control, we are studying the efficacy of mating disruption using hand-applied dispensers for mating disruption under San Joaquin Valley conditions and detection of monitoring of Indianmeal moth in the presence of mating disruption.

Accomplishments:

- We compared the impact of mating disruption and fogging with pyrethrins on Indianmeal moth males captured in pheromone traps, sentinel females mated, and progeny recovered in ovipositional media. Both mating disruption and fogging substantially reduced all three measures of Indianmeal moth activity. In contrast to previous studies in other regions on corn and peanuts, Indianmeal moth remained under control in the mating disruption area in this study despite active infestation at the beginning of the study. We believe that this is because of poorer host quality and less favorable climate.

Past Research Accomplishments

- **Relative prevalence of insect pests in figs at harvest**

A multi-year study of insect damage to dried figs at harvest found that driedfruit beetle caused damage to Calimyrna figs more consistently than to navel orangeworm, the other primary pest of figs. Evidence suggested that raisin moth contributed little to damage to Calimyrnas, which are historically the most economically important fig variety in California and the most susceptible to insect damage. A subsequent study of damage in other fig varieties, which do not require fertilization the fig wasp, indicated that lepidopteran pests are more important sources of damage relative to the dried fruit beetle in these varieties and that the raisin moth causes as much damage as the navel orangeworm.

- **NIR for figs**

A collaborative study with researchers from Manhattan, Kansas developed an infrared technique for detecting insect-infested and defective figs during processing. Analysis using near-infrared spectra for partial least squares analysis with cross-validation, showing a sensitivity of 97% and a specificity of 21-25% for the two varieties. The sensitivity and specificity of the infrared technique was as good as human sorters.

Selected Publications

- Burks, C.S., Higbee, B.S., Kuenen, L.P.S., and Brandl, D.G. 2009.** Monitoring *Amyelois transitella* males and females with phenyl propionate traps in almonds and pistachios. Entomol. Exp. Appl. (Accepted for publication).
- Higbee, B. S., and C. S. Burks. 2008.** Effects of mating disruption treatments on navel orangeworm (Lepidoptera: Pyralidae) sexual communication and damage in almonds and pistachios. J. Econ. Entomol. 101: 1633-1642.
- Burks, C. S., B. S. Higbee, D. G. Brandl, and B. E. Mackey. 2008.** Sampling and pheromone trapping for comparison of abundance of *Amyelois transitella* in almonds and pistachios. Entomol. Exp. Appl. 129: 66-76.
- Burks, C. S., and D. G. Brandl. 2005.** Quantitative assessment of insect pest damage to figs. Online. Crop Management doi:10/1094/CM-2005-0510-01-RS.
- Leal, W. S., Parra-Pedrazzoli, A.-L., Kaissling, K.-E., Morgan, T. I., Zalom, F. G., Pesak, D. J., Dundulis, E. A., Burks, C. S., and Higbee, B. S. 2005.** Unusual pheromone chemistry in the navel orangeworm: novel sex attractants and a behavioral antagonist. Naturwissenschaften 92: 139-146.
- Burks, C. S., and D. G. Brandl. 2004.** Seasonal abundance of navel orangeworm (Lepidoptera: Pyralidae) in figs and effect of peripheral aerosol dispensers on sexual communication. 8 pp. J. Insect Science 4: 40.
- Burks, C. S., F. E. Dowell, and F. Xie. 2000.** Measuring fig quality using near-infrared spectroscopy. J. Stored Products Res. 36: 289-296.
- Burks, C. S., J. A. Johnson, D. E. Maier, and J. W. Heaps. 2000.** Temperature, pp. 73-104. In B. Subramanyam and D. W. Hagstrum [eds.], Alternatives to Pesticides in Stored-Product IPM. Kluwer, Boston.
- Burks, C. S., and D. W. Hagstrum. 1999.** Rapid cold hardening capacity in five species of coleopteran pests of stored grain. J. Stored Products Res. 35: 65-75.
- Burks, C. S., D. W. Hagstrum, and J. E. Baker. 1999.** Selection of cold injury treatments to facilitate release of the parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) reared on the rice weevil (Coleoptera: Curculionidae). J. Econ. Entomol. 92: 473-479.

Judy A. Johnson

Research Entomologist

Much of the U.S. production of dried fruits and tree nuts occurs in the central valley of California. An important problem for processors of these and other durable commodities are insect infestation. Processors must control postharvest insect pests both to provide domestic consumers with quality product, as well as to access export markets. Currently, much of the industry depends on the use of chemical fumigants, many of which have environmental, regulatory, and safety concerns. The growing organic industry also requires postharvest treatments that do not rely on chemical control. My current research involves the development of non-chemical treatments as alternatives to fumigants. I am concerned primarily with physical treatments using temperature extremes or vacuum, but I am also interested in insect parasitoids as control agents. The target pests for most of this work include both field pests of postharvest significance (navel orangeworm, codling moth, peach twig borer, raisin moth) and stored product pests (Indianmeal moth, red flour beetle, cowpea weevil).

Current Research Projects and Accomplishments

Non-chemical postharvest insect control in lentils using radio frequency energy.

Working with collaborators Shaojin Wang and Juming Tang of Washington State University, Pullman, we are developing radio frequency energy as heat disinfestation treatments to control postharvest pests of lentils. Phosphine or methyl bromide are normally used to meet phytosanitary requirements for exported product, but insect resistance and regulatory actions are making these fumigants more difficult and expensive to use.

Accomplishments:

- We have determined the effect of temperature and moisture content on the dielectric properties of several legumes, and used this information to develop the necessary treatment conditions to optimize heating uniformity. Comparing the dielectric properties of the legumes with those of the target insects indicates that the insects may heat faster during radio frequency treatments, and should result in greater efficacy with less risk to product quality.
- Heat block studies have shown that the pupal stage of the cowpea weevil is the most heat tolerant stage of this insect. Our preliminary results indicate that it requires 4 minutes of exposure to 58°C for complete mortality of pupae treated in mung beans, making it far more tolerant than the previously studied Indianmeal moth, which only 3 minutes of exposure to 50°C for complete mortality. Some of this tolerance in the pupal stage may be due to insulation by the mung bean, but even adult beetles exposed directly to the heat survived 15 minute exposure to 50°C with only 20% mortality. These results indicate that treatments designed for cowpea weevil should be affective against Indianmeal moth.

Low pressure treatments for control of postharvest insects in tree nuts.

The development of low-cost containers using flexible PVC capable of withstanding pressures of 50 mm Hg has made low pressure (vacuum) treatments more practical. Low pressure treatments may be useful to organic or small processors, providing relatively rapid treatment without the use of chemical fumigants.

Low pressure treatments for control of postharvest insects in tree nuts (cont).

Accomplishments:

- We determined the necessary exposures to 50 mm Hg at 25 and 30°C for Indianmeal moth eggs and diapausing larvae, codling moth eggs, non-diapausing and diapausing larvae, and navel orangeworm eggs and non-diapausing larvae. Eggs and diapausing larvae were found to be the most tolerant stages. Preliminary data at 20°C indicates that at lower temperatures, diapausing larvae are more tolerant than eggs.
- The effect of product moisture and relative humidity on efficacy of low pressure treatments was determined. High product moisture or relative humidity reduced the efficacy of 50 mm Hg treatments against non-diapausing and diapausing Indianmeal moth larvae at both 25 and 30°C by reducing moisture loss in treated larvae. Diapausing larvae were more tolerant of low pressure treatments, probably due to their resistance to desiccation.
- We conducted field trials with shelled almonds, inshell almonds and inshell walnuts, using 5 MT flexible PVC containers. The ambient temperature had a strong effect on the length of time needed to obtain control; treatments during winter months when average temperatures were about 6°C required more than 13 days for complete control, with diapausing codling moth being the most tolerant stage at low temperatures. During summer months when average temperatures were above 25°C, complete control was possible within 72 hours, comparable to phosphine fumigation.

Use of the parasitoid *Habrobracon hebetor* to control overwintering (diapausing) populations of Indianmeal moth.

Studies in a Fresno culler fig warehouse showed that adult *Habrobracon (Bracon) hebetor*, a common parasitoid of postharvest pyralid larvae, were active throughout the winter. These adults were capable of stinging and paralyzing raisin moth and Indianmeal moth larvae on warm winter days. This observation suggests that *H. hebetor* could be used to control overwintering populations of Indianmeal moth in bulk stored dried fruits and nuts, thereby reducing the number of moths emerging in the spring and also reducing the need for disinfestation treatments. Critical to such an effort, however, is developing data to obtain an exemption from FDA regulations, allowing the addition of *H. hebetor* to dried fruits and nuts.

Accomplishments:

- We have completed several trials releasing *H. hebetor* into 50 gallon barrels of inshell almonds infested with diapausing Indianmeal moth and held under ambient winter conditions. The emergence of adult Indianmeal moths in the spring was significantly reduced from barrels to which *H. hebetor* were added. Relatively small numbers of released *H. hebetor* were capable of controlling overwintering Indianmeal moth, and high numbers of released *H. hebetor* actually reduced the number of *H. hebetor* produced, without affecting control efficacy. The latter is due to competition; adult *H. hebetor* are known to actively destroy the offspring of competing females.

Past Research Accomplishments

- **Radio frequency treatments for inshell walnuts**

Working with collaborators Shaojin Wang (WSU), Juming Tang (WSU) and Elizabeth Mitcham (UCD), a practical and effective radio frequency heat treatment for inshell walnuts was developed and demonstrated at a walnut processing plant. The treatment caused 100% mortality in all treated insects (5th instar navel orangeworm larvae, determined to be the most tolerant stage in laboratory studies) without compromising product quality.

- **Systems approach for codling moth in cherries**

We conducted field trapping studies in cherry, walnut, pear and apple orchards to provide further evidence that cherries are a poor host for codling moth. The research was used to develop a systems approach as an alternative to fumigation with methyl bromide as a quarantine protocol for California and Pacific Northwest cherries exported to Japan. The systems approach has been approved by Japan MAFF, and the first shipments from the Pacific Northwest arrived in Japan in July, 2009. Using the systems approach, because no fumigation is required, cherry quality is much improved at the consumer level.

- **Low temperature treatments for dried fruits and nuts**

We determined the exposures needed to kill 95% (LT₉₅) of eggs, non-diapausing larvae and pupae of navel orangeworm and Indianmeal moth at 0, 5, and 10°C, and diapausing Indianmeal moth larvae at -20, -15 and -10°C. Refrigeration temperatures of 0-5°C was found to be useful in disinfesting product contaminated with non-diapausing insects, with storage times of 3 weeks needed for adequate control. We showed that relatively brief storage in commercial freezers, provided that the temperature throughout the product was below -15°C for at least 48 hours, also shows potential as a disinfestation treatment, and it is necessary when diapausing Indianmeal moth larvae are present.

- **Use of commercial freezers to control cowpea weevils in organic garbanzo beans**

Commercial organic bean producers use freezing as a method to disinfest product of cowpea weevil infestations, but were unsure of the treatment times necessary. We did laboratory studies that identified eggs as the most cold-tolerant stage, and then determined the length of time, under commercial freezer conditions, that was necessary to get complete kill. We found that the processor could shorten his treatment time by 1-2 weeks, and still get adequate control.

- **Combining non-chemical treatments for postharvest insect control in dried fruit and nuts**

We developed a treatment strategy combining an initial disinfestation treatment (0.4% O₂) with one of three protective treatments (10°C storage, 5% O₂, and Indianmeal moth granulosis virus) as an alternative for chemical fumigation of dried fruits and nuts for control of postharvest insect populations. The initial disinfestation treatment was effective against navel orangeworm and raisin moth while all three protective treatments prevented development of damaging Indianmeal moth populations. Quality analysis showed that overall product quality for all protective treatments was maintained at levels acceptable by industry standards.

Selected Publications

- Johnson, J. A., and Zettler, J. L. 2009.** Response of postharvest tree nut lepidopteran pests to vacuum treatments. *J. Econ. Entomol.* 102: 2003-2010.
- Johnson, J. A., and Hansen, J. D. 2008.** Evidence for the non-pest status of codling moth on commercial fresh sweet cherries intended for export. *Crop Protection.* 27: 1415-1420.
- Johnson, J. A. 2007.** Survival of Indianmeal moth and navel orangeworm (Lepidoptera: Pyralidae) at low temperatures. *J. Econ. Entomol.* 100: 1482-1488.
- Wang, S., Monzon, M., Johnson, J. A., Mitcham, E. J., Tang, J. 2007.** Industrial-scale radio frequency treatments for insect control in walnuts I: Heating uniformity and energy efficiency. *Postharvest Bio. and Tech.* 45: 240-246.
- Wang, S., Monzon, M., Johnson, J. A., Mitcham, E. J., Tang, J. 2007.** Industrial-scale radio frequency treatments for insect control in walnuts II: Insect mortality and product quality. *Postharvest Bio. and Tech.* 45: 247-253.
- Wang, S., Johnson, J. A., Tang, J., Yin, X. 2005.** Heating condition effects on thermal resistance of fifth-instar *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae). *J. Stored Products Res.* 41: 469-478.
- Johnson, J. A., Valero, K. A., Wang, S., Tang, J. 2004.** Thermal death kinetics of red flour beetle (Coleoptera: Tenebrionidae). *J. Econ. Entomol.* 97: 1868-1873.
- Mitcham, E. J., Veltman, R. H., Feng, X., de Castro, E., Johnson, J. A., Simpson, T. L., Biasi, W. V., Wang, S., Tang, J. 2004.** Application of radio frequency treatments to control insects in in-shell walnuts. *Postharvest Bio. and Tech.* 33: 93-100.
- Johnson, J. A., and Valero, K. A. 2003.** Use of commercial freezers to control cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae), in organic garbanzo beans. *J. Econ. Entomol.* 96: 1952-1957.
- Johnson, J. A., Wang, S., and Tang, J. 2003.** Thermal death kinetics of fifth-instar *Plodia interpunctella* (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 96: 519-524.
- Wang, S., Tang, J., Johnson, J. A., Mitcham, E., Hansen, J. D., Cavalieri, R. P., Bower, J., and Biasi, B. 2002.** Process protocols based on radio frequency energy to control field and storage pests in in-shell walnuts. *Postharvest Bio. and Tech.* 26: 265-273.
- Wang, S., Tang, J., Johnson, J. A., and Hansen, J. D. 2002.** Thermal-death kinetics of fifth-instar *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae). *J. Stored Products Res.* 38: 427-440.
- Johnson, J. A., Vail, P. V., Brandl, D. G., Tebbets, J. S., and Valero, K. A. 2002.** Integration of nonchemical treatments for control of postharvest pyralid moths (Lepidoptera: Pyralidae) in almonds and raisins. *J. Econ. Entomol.* 95: 190-199.
- Johnson, J. A., Valero, K. A., Hannel, M. M., and Gill R. F. 2002.** Seasonal occurrence of postharvest dried fruit insects and their parasitoids in a culled fig warehouse. *J. Econ. Entomol.* 93: 1380-1390.

L.P.S. Kuenen

Research Entomologist

An important problem for growers and processors of durable commodities are insect infestation. Reduction of pests entering the marketing chain from the field is one aspect of insect control for post harvest. In addition, further elucidation of insect biology and behavior may lead to development of methods that may be adjuncts to fumigant alternatives. Therefore my research focuses primarily on the biology of pest insects and the semiochemicals (signaling chemicals) they use for mate and host location, which may be used to monitor these insects and/or alter their behavior to reduce or eliminate their reproduction. I am also interested in fundamental aspects of insect behavior which may offer new avenues for insect control. The target pests for most of this work include a field pest of postharvest significance (navel orangeworm) and stored product pests (Indiameal moth and cowpea weevil).

Current Research Projects and Accomplishments**Elucidation of the Navel Orangeworm Sex Pheromone**

Working with collaborators Jocelyn Millar and Steven McElfresh of the University of California at Riverside, we are elucidating critical sex pheromone blends of the female sex pheromone of the navel orangeworm. The primary sex pheromone component was described in 1979; even though some males can be trapped with this component alone it is not very effective. We showed in 2001 that traps with three unmated females as trap baits captured approx. 20 fold more males than traps baited with the synthetic primary component. Several research teams had attempted to elucidate other active compounds prior to our effort, but none were successful.

Accomplishments:

- We found that sex pheromone gland-extract elicited upwind flight of 99+% of all males tested in our laboratory wind tunnel. Thus we were able to use gland extracts as a standard to compare with new synthetic blends. Subsequent fractionation of pheromone gland extract by liquid chromatography followed by wind tunnel bioassays indicated that the pheromone likely consisted of components with aldehyde and alcohol moieties and “plain” hydrocarbon molecules. The recombined pheromone fractions were as active as the original extract, indicating that no active components had been lost during fractionation. We used electroantennographic detectors on the effluent of a gas chromatographic separation of pheromone extract to identify the most electro physiologically active components; these corresponded with chemical groups isolated by the earlier wind tunnel bioassays.
- After syntheses of these isolated compounds, extensive bioassays were conducted in laboratory wind tunnel (blends always contained equal amounts of the primary pheromone component [aldehyde – 4 female equivalents, as determined by gas chromatographic analyses]) and in field trapping assays we always used unmated females as a positive control to contrast the efficacy of our synthetic blends. Two years of these assays led us to a four component blend that was as attractive to males as pheromone gland-extract in wind tunnel assays. In field trapping assays this four component blend was as good as or better than female baited traps, but 1) the baits were short lived and 2) different synthetic batches of primary component.

Development of a trap bait/lure for Navel Orangeworm

We are continuing our work with the sex pheromone of the navel orangeworm with our collaborators, Jocelyn Millar (UC Riverside) and Spencer Walse (CPQ), to develop a field lure that consistently traps navel orangeworm males. Success will likely depend on both determining the pheromone release rates and component ratios from female pheromone glands and from assessing the cause of failure/short field life of current standard formulations.

Accomplishments:

- We have determined that sex pheromone titer in the female gland is approx. 3 ng of the primary component. Thus we do not expect a high release rate of pheromone from a live female nor from an effective lure, thus technique enhancements are critical to obtain an adequate signal-to-noise ratio in the volatile collections. We have optimized our GC/mass spectrometry protocols for measuring low levels of the known pheromone components and recent volatile collections appear to meet our signal-to-noise ratio criteria.

Cowpea weevil: release of the natural sex pheromone and responses to female volatiles.

We are collaborating with Spencer Walse (CPQ) to purify synthetic cowpea weevil pheromone provided by Richard Petroski (ARS, Peoria, IL). Our collaboration also extends to the collection and measurement of female sex pheromone from female beetles and determination male responses to them and synthetic components.

Accomplishments

- There are two “forms” of the cowpea weevil, the so-called “flight form” and the “flightless” form. By adapting methods from the literature, we have developed protocols for rearing both forms in our lab. These forms are morphologically distinct externally and we have confirmed that the flight form’s internal reproductive organs are not fully developed whereas the flightless form has fully developed internal reproductive organs. We continue to collect the pheromone volatiles from unmated females of both forms to determine the release rate and ratios of the identified sex pheromone components to determine differences and/or similarities in this process and to determine if the differences in reproductive organ development is related to behavioral responses of the males toward the female sex pheromone. With the purified synthetics for standards, we will determine the time course of the release rates and ratios of the putative sex pheromone of these beetles in both forms.
- In the first ever analysis of a beetle’s flight maneuvers while flying toward a female sex pheromone (we have collected the sex pheromone volatiles from live cowpea weevil females) we found that males’ flight responses showed only a few dissimilarities to the way that male moths fly upwind toward the sex pheromone from conspecific females and like moths they adjusted their course and airspeed in different wind speeds to maintain a constant upwind velocity. We are currently testing cowpea weevil males’ flight responses to a range of pheromone dosages; preliminary tests indicate that again, males adjust their course and airspeed in a manner similar to male moths. Most importantly, males land on the pheromone source indicating a clear ability to locate a point source of sex pheromone rather than just landing near a source as many bark beetles do.

Attractants for female navel orangeworm: phenyl propionate, almond meal, crude almond oil and pistachio volatiles.

Twelve years before the elucidation of the primary sex pheromone component of the navel orangeworm, attractants for female navel orangeworm were developed; the first reported attractant was phenyl propionate (and related chemicals) for use in water pail traps. Subsequently navel orangeworm rearing diet or almond press cake plus crude almond oil (CAO) were placed in traps to elicit female egg laying. The latter method remains in field use today as a measure of female navel orangeworm activity. We are collaborating with Spencer Walse (CPQ) to elucidate host attractants from pistachios that elicit host location and oviposition on these nuts.

Accomplishments

- We re-examined attractancy of phenyl propionate and found that both males and females were attracted in contrast to nearly exclusively females in published reports. We are working to develop traps that capture both males and females and others that are attractive to females only and this may relate to trap color and texture.
- Almond meal is a by-product of crude almond oil (CAO) extraction and replaced almond press-cake from the almond oil processor approx. 1990. We determined that almond meal plus 3% CAO was as an optimal mixture for “capturing” female navel orangeworm eggs and these egg traps last for at least 10 weeks in the field.
- We have developed laboratory assays to assess female upwind flight toward prospective pistachio volatiles. We have obtained up to 50% upwind female flight to CAO which was a standard in the late 1980’s and we have obtained nearly 100% upwind flight to a recently isolated volatile complex that will be analyzed for its chemical constituents.

Past Research Accomplishments

- **Fundamental investigations of insect flight behavior**

The process of insect orientation and locomotion toward an attractive odor source has been studied for decades, yet insects’ integration of all cues required for odor source location is only partially understood. Research of fundamental mechanisms in science has always been the precursor to successful applied science, therefore we are supplementing past research with new work to continue investigations of parameters that affect insects’ arrival at an odor source. We have focused our attention primarily on the influence of visual cues on the flight parameters of moths flying upwind toward sex pheromone sources. Moths fly faster when they fly higher above the ground and when ventral visual cues are smaller. Although smaller objects lead to faster flight we recently showed that moths steer their flights differently over transverse stripes vs. circles. We also found that increased flight speed with higher flight height has an upper limit, i.e., when moths fly higher they do not increase their flight speed any further even though they are capable of much greater airspeeds. Furthermore, the limit of increased flight speed is not the same among different moth species.

- **Varroa mites**

Prior to working in Parlier, CA, I worked at the ARS facilities at Beltsville, MD (BARC) and Cornell Univ. on mite pests of honey bees. We found that Varroa mites select their preferred larval host (drones) on the basis of physical cues, specifically the raised edges

of drone cells amid worker cells. Drones in the center of an even-aged patch of drones were not infested higher than worker larvae/cells in the surrounding comb. We found that worker larvae/cells that were experimentally raised (mimicking drone cells) were infested at the same rate as drone larvae/cells. Although no chemical cues appear necessary for larval host location we did demonstrate that selection of the preferred adult host (nurse bees) was dependent on chemical cues emanating from the bees. We also demonstrated that Varroa mites could follow a bee odor plume upwind to its source; this was the first demonstration that any mite could follow and odor plume upwind to its source.

Selected Publications

- Kuenen, L.P.S. and Calderone, N.W. 1997.** Transfers of *Varroa* mites from newly emerged bees: Preferences for age- and function-specific adult bees (Hymenoptera: Apidae). *J. Insect Behavior*. 10(2):213-228.
- Kuenen, L.P.S. and Calderone, N.W. 1998.** Positive anemotaxis by *Varroa* mite: responses to bee odour plumes and single clean-air puffs. *Physiol. Entomol.* 23:255-264..
- Kuenen, L.P.S. and Calderone, N.W. 2000.** *Varroa* mite infestations in elevated honey bee brood cells: effects of context and caste. *J. Insect Behavior*. 13(2): 201-215.
- Kuenen, L.P.S., Rowe, H.C. and Vail, P.V. 2002.** Leaf feeding by early instar codling moth (Lepidoptera: Tortricidae) Proceedings of the 86th Annual Meeting of the ESA Pacific Branch, Tahoe, CA.
- Kuenen, L.P.S., Rowe, H., Garcia, J., Bentley, W., Ribeiro, B. 2003.** Pistachio volatiles to attract female navel orangeworm, *Amyelois transitella*. California Pistachio Commission Production Research Reports. 181-187.
- Kuenen, L.P.S., Brandl, D.G., and R.E. Rice. 2005.** Modification of assembly of Pherocon 1C traps speeds trap liner changes and reduces in-field preparation time. *Can. Entomol.* 137:117-119.
- Kuenen, L.P.S., and Rowe, H.C. 2006.** Cowpea weevil flights to a point source of female sex pheromone: analyses of flight tracks at three wind speeds. *Physiol. Entomol.* 31:103-109.
- Siegel, J. P., Kuenen, L. P. S, Higbee, B. S., Noble, P., Gill, R., Yokota, G. Y., Krugner, R., and Daane, K. M. 2008.** Postharvest survival of navel orangeworm assessed in pistachios. *Calif. Agric.* 62(1):30-35.
- Kuenen, L.P.S., Bentley, W., Rowe, H.C. and Ribeiro, B. 2008.** Bait formulations and longevity of navel orangeworm egg traps tested. *Calif. Agric.* 62(1):36-39.
- Charles S. Burks, Higbee, B. S., Kuenen, L. P. S., and Brandl, D. G.** “Monitoring *Amyelois transitella* males and females with phenyl propionate traps in almonds and pistachios” *in press*.
- Kuenen, L.P.S., McElfresh J, S. and Millar, J.G.** Identification of critical secondary components of the sex pheromone of the Navel Orangeworm, *Amyelois transitella*. *Journal of Economic Entomology.* *in press*.

David P. Obenland

Research Plant Physiologist

The quality of fresh fruit is very important in determining its acceptance by consumers. My primary research goal is to determine ways to help maintain or enhance fresh fruit quality by increasing the understanding of what determines quality both before and after harvest. The primary commodities I work with are citrus and stone fruit, although I have also worked with grapes and avocados. Part of my research involves seeking both non-chemical and chemical alternatives to the fumigant methyl bromide, with the emphasis on the treatment effect on fruit quality. The other main portion of my research deals with increasing the understanding of the impacts of both pre- and postharvest factors on fresh fruit flavor and determining the physiological and chemical basis of the changes that occur.

Current Research Projects and Accomplishments

Development of non-chemical and chemical methyl bromide fumigation alternatives

Most of my efforts in this area have been in evaluating the use of heat as a non-chemical alternative to methyl bromide fumigation. Heating is an attractive method in that it can effectively kill insect pests and does so without the use of controversial chemicals and without the concern for chemical residues. Of all of the heat treatment methods that have been evaluated, application of heat to agricultural commodities in the form of forced hot air, with the humidity maintained at a level that prevents water from condensing on the fruit, is thought to be the least injurious to quality. I have been working for a number of years with Lisa Neven, an ARS entomologist in Wapato, WA, to develop a forced hot air method for peaches and nectarines. The California Tree Fruit Agreement has funded a great deal of this research. The treatment, also known as CATTS, combines forced hot air with low oxygen (1%) and high carbon dioxide. The altered atmosphere acts to greatly reduce the needed treatment time below what would be needed for forced hot air alone and reduces the chance of fruit injury.

In order for CATTS treatment to be viable commercially it is desirable that it be implemented on fruit that is packed into boxes which have been stacked onto pallets. This is an added challenge in that the hot air must flow through all of the boxes and still heat the fruit in an even manner. Testing of this treatment application led to the development and construction of a large test chamber that is located at the Parlier ARS facility capable of simultaneously treating two commercial pallets of stone fruit.

I also participate in research to find alternative chemicals to replace methyl bromide fumigation. This primarily involves evaluating fruit following fumigation and subsequent storage for overall fruit quality and identifying potential fumigation-related quality loss. This work is mainly performed by interaction with colleagues at the Parlier facility that are involved in fumigation research.

Accomplishments:

- Initial work with CATTS on a laboratory scale indicated that peach and nectarine quality was not adversely affected by a treatment that was effective at oriental fruit moth and codling moth disinfestation (Obenland et al., 2005). This included the flavor of the fruit which was found by a series of formal taste panels conducted to not be significantly altered by treatment. Research using the large CATTS chamber has shown that it is

possible to heat fruit that are boxed and stacked onto pallets to core temperatures that achieve quarantine kill in a timeframe that would be commercially feasible.

- Heat was also shown to have some beneficial effects on fruit quality in that it can slow the development of internal breakdown in susceptible cultivars (Obenland and Neipp, 2005b) as well as lessen the rate of ripening. A slower rate of ripening could be advantageous in maintaining fruit quality for a longer period of time. It was also found that the treatment was very effective in the elimination of brown rot decay.

Fruit flavor quality

This work is done in collaboration with Mary Lu Arpaia (University of California) and involves using both semi-trained and consumer sensory panels as well as analysis of flavor components to better understand what impacts and determines flavor quality in citrus. The goal is to provide information that can be used to alter both pre- and postharvest practices to improve overall flavor quality. A large part of the research has been funded by the Citrus Research Board.

Accomplishments:

- We examined the changes in flavor and flavor components that occurred during the maturation of navel oranges and found that the current maturity standard in California, that is based solely on the amount of sugar and acid in the fruit, was inadequate to fully describe the flavor changes that were occurring (Obenland et al., 2009). This has led to the proposal of a new maturity standard known as BrimA that better defines what fruit should be harvested and allowed into the marketplace. Also, the work identified volatile flavor compounds that are important to the development of flavor quality during navel orange maturation.
- Research performed to examine the effect of packing and handling on navel orange fruit flavor quality showed that both the waxing of the fruit that occurs and mechanical impacts imposed by a commercial packing line likely interact to cause a loss in flavor quality (Obenland et al., 2008a). These changes were associated not with changes in the sugar and acid present in the fruit but instead with an alteration in flavor volatiles content.
- We determined that mandarin oranges are very susceptible to the development of poor flavor during storage and that the temperature that the fruit are stored at makes a large difference in determining the degree of poor flavor that is observed. The flavor changes appear to be mainly due to alterations in the amount of flavor volatiles present within the fruit.

Past Research Accomplishments

- **Use of chlorophyll fluorescence to identify areas of peel injury in citrus**

In this project it was shown that areas of peel injury in lemons, as caused by hot water treatment, could be visualized by the use of chlorophyll fluorescence prior to the appearance of visible peel injury (Obenland and Neipp, 2005a). The practical use of this finding was that it enabled the study of the early mechanisms involved in causing this type of injury by identifying areas of the peel that would develop injury.

- **Use of volatile chemicals emitted from fruit to indicate quality loss**

A series of experiments were conducted to find chemical markers that would be predictive of fruit quality following exposure of the fruit to extremes in temperatures. We determined that the release of peel oil in lemons was linked to the development of peel injury following either treatment with high or with low temperatures and that the peel oil is likely involved in the development of chilling injury. In addition, it was found that navel oranges emit volatiles following freezing that are predictive of whether or not the internal fruit quality has been injured (Obenland et al., 2003).

- **Investigations into the biochemical basis of mealiness development in peach**

Mealiness is a poorly understood storage disorder in peaches that causes the flesh to become dry and inedible. We analyzed changes in proteins that occurred during the development of mealiness and identified a number of proteins that were altered in amount in fruit that became mealy (Obenland et al., 2008b). A large loss in the amount of a protein involved in ripening may be important to the development of the disorder.

Selected Publications

Obenland, D., S. Collin, B. Mackey, J. Sievert, K. Fjeld, and Arpaia, M.L. 2009.

Determinants of flavor acceptability during the maturation of navel oranges. *Postharvest Biol. Technol.* 52:156-163.

Obenland, D., S. Collin, J. Sievert, K. Fjeld, J. Doctor, and Arpaia, M.L. 2008a.

Commercial packing and storage of navel oranges alters aroma volatiles and reduces flavor quality. *Postharvest Biol. Technol.* 47:159-167.

Obenland, D. and Neipp, P. 2005a.

Chlorophyll fluorescence imaging allows early detection and localization of lemon rind injury following hot water treatment. *HortScience* 40:1821-1823.

Obenland, D. and Neipp, P. 2005b.

Forced hot air treatment of stone fruit to inhibit the development of mealiness. *Acta Hort.* 682:1171-1178.

Obenland, D., P. Neipp, B. Mackey, and Neven, L. 2005.

Peach and nectarine quality following treatment with high-temperature forced air combined with controlled atmosphere. *HortScience* 40:1425-1430.

Obenland, D.M., W.H. Vensel, and Hurkman, W.J. 2008b.

Alterations in protein expression associated with the development of mealiness in peaches. *J. Horticult. Sci. Biotechnol.* 83:85-93.

Obenland, D.M., L.H. Aung, D.L. Bridges, and Mackey, B.E. 2003.

Volatile emissions of navel oranges as predictors of freeze damage. *J. Agric. Food Chem.* 51:3367-3371.

Joel P. Siegel

Research Entomologist

The navel orangeworm *Amyelois transitella* is a primary pest of almonds and pistachios and a secondary pest of walnuts. These three crops comprise more than 1.2 million acres and there has been unprecedented expansion of almond and pistachio acreage in the last five years. Their 2005 total farm gate value was \$3.46 billion (almond \$2.34 billion, walnut \$540 million, and pistachio \$580 million) and these crops contribute substantially to the export balance of trade. Damage by navel orangeworm can exceed 30% in almonds and pistachios, and infested nuts face an increased likelihood of mycotoxin (aflatoxins B1, B2, G1, G2) contamination, which is a serious food safety concern. The stringent European Union standards present a significant trade barrier impacting grower returns. Currently, the primary way to ensure meeting this standard is to improve control of navel orangeworm. Different control strategies are required for the Central Valley because the northern and central regions are characterized by different climatic patterns than the southern growing areas. Given the economic value of these crops and increased concern about food safety, the groundwork has been laid for a reevaluation of current management strategies for these nut crops.

Current Research Projects and Accomplishments

Areawide program to control navel orangeworm in almonds, pistachios, and walnuts

I am currently the coordinator of this program, which will reduce navel orangeworm damage through adoption of control strategies consisting of cultural control, reduced risk insecticides, and nonchemical methods, primarily mating disruption, optimized for the different growing regions of the Central Valley. These strategies will be validated in representative counties in the north, middle and south valley. This focus on an IPM approach will include linking producer data on navel orangeworm damage and aflatoxin contamination within and between commodities, thereby identifying high incidence areas for further study. Coordinating control measures in these three commodities as well as linking grower data will reduce navel orangeworm damage and reduce the use of in season sprays. A total of 3 ARS researchers from the Commodity Protection and Quality Unit, 2 researchers from UC Berkeley, 2 researchers from UC Davis, 1 researcher from Paramount Farming Company, 2 UCCE researchers based at UC Davis, 2 UCCE farm advisors from Kings and Kern counties, several PCAs, and numerous growers are involved in the project.

Accomplishments in determining the population dynamics of navel orangeworm

- The currently published development studies of navel orangeworm were conducted using the Nonpareil almond variety and did not include pistachios. I repeated these studies using Nonpareil almonds as well as the commercially important almond varieties Butte, Carmel and Padre, Kerman and Kalegouchi variety pistachios, and wheat bran diet as a control. My research demonstrated that the navel orangeworm developed faster on pistachios than on almonds as well as at different rates among varieties, and that larval survivorship differed among varieties as well. In almonds, navel orangeworm had the highest survivorship in the varieties Butte and Nonpareil, and the lowest survivorship when reared on Padre almonds, while in pistachios survivorship was higher on the Kalegouchi variety than on the Kerman variety. The differences in development rate described above have implications for the peak emergence of navel orangeworm in the

field, which in turn affects the choice of control strategy. I have confirmed my laboratory studies in almonds by quantifying the pattern of adult emergence from Butte and Padre mummy pistachios collected in the late spring, and in pistachios by quantifying the emergence pattern from Kerman mummies. In collaboration with Bas Kuenen, a research entomologist in this unit, we evaluated the pattern of male capture in the field in order to validate the mummy emergence studies.

- For the past five years I have investigated the overwintering mortality of navel orangeworm in pistachios and almonds in Madera and Tulare counties. This research was conducted in collaboration with Bas Kuenen, a research entomologist in this unit. We identified the emergence peaks of navel orangeworm in the spring (first flight) by a combination of monitoring adult emergence from mummies collected during the winter and using several types of female-baited traps to capture males. These data, when combined with the developmental studies previously mentioned, enable us to predict the population peaks and then establish the optimal time to apply insecticides. Recently, as part of the areawide project, I am collaborating with Frank Zalom, an entomologist at UC Davis, to contrast the survival of navel orangeworm in the Sacramento Valley with the San Joaquin Valley. For the past two years my laboratory has infested more than 20,000+ almonds, which were then placed in Tehama and Butte counties and monitored by Frank Zalom. Survival over the winter was determined and contrasted between these northern sites, while I evaluated survival in Madera County using naturally infested Butte and Padre almonds. These studies will help us to develop navel orangeworm control strategies for the different counties within the Central Valley.
- There are several newly registered insecticides for use in almonds and pistachios to control navel orangeworm. I am evaluating their efficacy with Gary Weinberger, Weinberger & Associates, and with James Bettiga, S&J Ranch, in Madera County and am coordinating my research with Bradley Higbee, Paramount Farming Company, who is conducting large scale trials evaluating the efficacy of several of these insecticides in Kern County. My interest is determining the duration of protection afforded by these insecticides using laboratory bioassays of field-collected material. In collaboration with Spencer Walse, a research chemist in our unit, these studies will be extended to determine the environmental stability of these insecticides as well as the actual concentration deposited on tree nuts and the rate of insecticide breakdown. These studies will help identify the insecticides with the greatest duration of protection and help determine the optimum application times to enhance control of navel orangeworm.

Accomplishments in Almonds:

- Bradley Higbee, research entomologist at Paramount Farming Company, conducted a four year study to evaluate the role of multiple factors, including sanitation efficiency and previous year history, on navel orangeworm damage to Nonpareil almonds. I helped analyze his dataset and we confirmed that both mummies on the tree and on the ground, harvest date, proximity to pistachio plantings and previous year damage contributed to navel orangeworm damage in Nonpareil almonds. We extended the analysis to pollenizer varieties of almonds and noted that the single greatest predictor of their damage was the damage sustained by the Nonpareil almonds that year. I have developed a simple spreadsheet based on my statistical analysis that can be used as an educational tool so that

growers can vary several parameters and see their impact on damage. I am conducting additional studies in collaboration with Frank Zalom, an entomologist at UC Davis, to validate these findings in the northern regions of the Central Valley.

Accomplishments in Pistachios:

- Pistachios are far more difficult to sanitize than almonds because they cannot be destroyed by flail mowing. In collaboration with Bradley Higbee, Paramount Farming Company, Gary Weinberger, Weinberger and Associates, James Bettiga, S&J Ranch, Ali Orandi, Orandi Farm Management, and Rob Frits, Valent Chemical Company, I have evaluated the efficacy of entomopathogenic nematodes for use against navel orangeworm surviving the winter inside pistachio mummies on the ground. We developed a method to apply entomopathogenic nematodes through the irrigation system, and demonstrated that their use reduced the population of overwintering navel orangeworm. Chemigation is a cost effective and flexible method to apply these nematodes if growers wish to target the overwintering population.
- I have developed a post harvest treatment, using insecticides targeting navel orangeworm eggs and newly emerging larvae before they infest nuts as an alternative to physically destroying mummy pistachios. For the past four years I have been evaluating the efficacy of this strategy, which can reduce the overwintering population as much as 80%, and can also be used in conjunction with other strategies such as application of entomopathogenic nematodes or insecticides in mid to late spring. I am identifying the insecticides that will provide the greatest duration of control, using a combination of bioassay and chemical analysis in collaboration with Spencer Walse, the chemist in our research unit. When used as part of an integrated strategy to control navel orangeworm this technique should reduce the standing population. It may also be applicable to almonds in special cases.

Selected Publications

- Niu, G., Siegel, J. P., Schuler, M. A. and Berenbaum, M. R.** Comparative toxicity of mycotoxins to navel orangeworm (*Amyelois transitella*) and corn earworm (*Helicoverpa zea*). *J. Chem Ecol* (in press).
- Higbee, B. S. and Siegel, J. P.** New navel orangeworm sanitation standards could reduce almond damage. *Calif. Agricul.* 63 (1):24-28. 2009.
- Siegel, J. P., Kuenen, L. P. S, Higbee, B. S., Noble, P., Gill, R., Yokota, G. Y., Krugner, R., and Daane, K. M.** Postharvest survival of navel orangeworm assessed in pistachios. *Calif. Agricul.* 62 (1):30-35. 2008.
- Bentley, W., Siegel, J. P., Holtz, B.A., and Daane, K. M.** Navel orangeworm and obliquebanded leafroller as pests of pistachio. In Ferguson, L. F., Beede, R. H., Haviland, D. H., Holtz, B. A., Kallsen, C. E., and Sanden, B. L. (eds.) *Pistachio Production Manual, 5th Edition* 2008, 179-191. 2008.
- Garczynski, S. and Siegel, J. P.** Bacteria, pp. 175-198. In Lacey, L. and Kaya, H. (eds.) *Manual of techniques in invertebrate pathology, Second Edition. Application and evaluation of pathogens for control of insects and other invertebrate pests.* Springer, Dordrecht, The Netherlands. 868 pp. 2007.
- Shapiro-Ilan, D., Lacey, L. A., and Siegel, J. P.** Microbial control of insect pests of stone fruit and nut crops, pp. 547-566. In Lacey, L. and Kaya, H. (eds.) *Manual of techniques in invertebrate pathology, Second Edition. Application and evaluation of pathogens for control of insects and other invertebrate pests.* Springer, Dordrecht, The Netherlands. 868 pp. 2007.
- Federici, B. and Siegel, J. P.** Safety assessment of BT and BT crops used for insect control, pp. 45-102. In Hammond, B. G. (ed.) *Food safety of proteins in agricultural biotechnology.* Taylor and Francis Group, Boca Raton, Florida. 299 pp. 2007.
- Siegel, J. P., Lacey, L. A., Higbee, B. S., Noble, P., and Fritts, R. Jr.** Effect of application rates and abiotic factors on *Steinernema carpocapsae* for control of overwintering navel orangeworm (Lepidoptera: Pyralidae, *Amyelois transitella*) in pistachios. *Biol. Control.* 36 (3):324-330. 2006
- Siegel, J. P., Lacey, L. A., Fritts, R. Jr., Higbee, B. S., and Noble, P.** Use of steinernematid nematodes for post harvest control of navel orangeworm (Lepidoptera: Pyralidae, *Amyelois transitella*) in fallen pistachios. *Biol. Control.* 30 (2):410-417. 2004.

Joseph L. Smilanick
Research Plant Pathologist

More than 95% of all the table grapes and more than 75% of all the fresh citrus fruit grown in the United States originate in California. Both fruits are susceptible to decay losses after harvest, most caused by rot fungi, and measures to manage this problem by packinghouse and cold storage managers. Producers must control postharvest decay losses both to provide domestic consumers with quality product, as well as to access export markets. Currently, the table grape industry depends on the use of sulfur dioxide fumigation to stop decay losses, while the citrus industry uses a variety of postharvest fungicides applied on packing lines. The growing organic industry also requires postharvest treatments that do not rely on chemical control. My current research involves the development of 'reduced-risk' fungicides, thermal treatments, generally recognized as safe substances, and preharvest practices to refine and improve decay management while reducing or eliminating conventional fungicides. With table grapes, current projects include the use of ozone gas in various regimes to replace or augment postharvest sulfur dioxide fumigation and preharvest practices, such as potassium salt solution or reduced-risk fungicide regimes, to reduce postharvest decay losses. With citrus fruit, current projects include ammonia fumigation to control postharvest decay and improve fungicide performance, thermal treatments combined with generally recognized as safe substances such as potassium phosphite or potassium sorbate, packinghouse sanitation by chemical and thermal means to replace formaldehyde fumigation. The goals of this work are to provide practical tools for both conventional and organic growers to better protect their products after harvest.

Current Research Projects and Accomplishments

Postharvest fumigation of table grapes.

The primary decay pathogen of table grapes is *Botrytis cinerea*. In prior work, we developed means to better manage and control sulfur dioxide fumigation that is used to control this pathogen. Currently, we are evaluating ozone fumigation of table grapes to control postharvest decay, using equipment provided by commercial collaborators and valuable assistance from University of California coworkers. Sulfur dioxide has been the technology of choice for many years, but its negative impact on berry appearance and flavor, regulatory issues associated with its toxicity to workers and residues, and prohibition of its use on 'certified' organic grapes, have made the development of alternatives valuable.

Accomplishments:

- In work done in the early 1990s, we determined the minimal doses of sulfur dioxide needed to control gray mold, the primary storage decay pathogen, expressed this dose in a concentration and time product, introduced dosimeters to measure this dose, and developed a total utilization fumigation schedule to eliminate atmospheric venting after fumigation to stop environmental releases of the gas.
- Evaluation of ozone fumigation has been an on-going project for several years. Brief, very high (up to 10,000 ppm) fumigation with ozone under a partial vacuum was found to have efficacy similar to initial sulfur dioxide fumigation. It is promising but relatively costly, and a few prototype installations were made at cold storages.

- Long term, constant low dose (100 to 300 ppb) fumigation is currently under laboratory and commercial evaluation. Several commercial storage employ this technology, and we have found it promising. Our objectives are to evaluate low dose ozone toxicity to decay fungi and the influence of package materials, vent area, and air velocity on ozone penetration into packages.

Preharvest actions to manage postharvest decay of table grapes.

The approaches of this project are two: 1) applications of ‘reduced-risk’ fungicides in practical regimes and assess their impact on subsequent postharvest decay; 2) evaluate cluster-directed sprays of nutrients to accelerate maturity and improve berry quality, and control postharvest decay; and 3) combinations of these, where fungicide applications and nutrient cluster spray applications are combined.

Accomplishments:

- We found, that among the vineyard fungicides registered and available today, that fenhexamid (Elevate) was consistently the best performing preharvest fungicide to control postharvest decay, followed by pyrimethanil (Scala). In a survey of the fungicide resistance present in gray mold populations in the San Joaquin Valley, fenhexamid resistance was very rare, while resistance to all of the other fungicides was common.
- Among cluster-applied nutrient solutions, we found many cultivars responded to those containing potassium. Potassium solutions applied after veraison increased soluble solids contents as much as 5%, deepened color, and increased firmness of the cultivars evaluated at harvest. Because harvest was earlier by as much as three weeks, less postharvest decay developed among the treated fruit.
- We conducted laboratory and field trials combining reduced-risk fungicides and potassium solutions. The most effective treatment has been potassium sorbate, which has been fortunate since it has antifungal activity itself, it is inexpensive, exempt from residue tolerances, and is not hazardous to workers. Our goal is to find a regime where postharvest decay is minimized, quality is enhanced, and early harvest is accomplished.

New technologies to control postharvest decay of citrus fruit.

The primary decay pathogen of concern in California is *Penicillium digitatum*. This pathogen rapidly develops very high spore populations within packinghouses and with groves whenever warm rainy periods occur. It rapidly develops fungicide resistance. A large part of this work is conducted using the research packline and groves of the University of California Lindcove Research and Extension Center in Exeter and assistance provided by them made much of this work possible.

Accomplishments:

- Work completed in the 1990s showed rates of the fungicides imazalil and thiabendazole could be reduced by as much as 90% compared to the older practice of mixing fungicides in fruit waxes by using heated, aqueous solutions to apply them instead. Aspects of this work continue, including experiments to refine this practice and assess the mode of application evaluations of newer reduced-risk fungicides, mixture of the fungicides with safe substances such as sodium bicarbonate, potassium phosphite, and potassium sorbate, and tests to develop thermal regimes of several seconds in duration to four minutes.

- Disinfection of packinghouses is a critical element in the management of this pathogen, and I am actively examining alternatives to the traditional option of formaldehyde fumigation. Relatively mild thermal treatments inactivate spores of this pathogen. Alternative chemical sanitizers, mostly oxidizing compounds such as hydrogen peroxide, chlorine dioxide, and ozone are under evaluation in commercial facilities. Currently chlorine dioxide is the subject of repeated tests this season.
- Fumigation with ammonia gas, an old technique, has been revived as a subject of investigation and has recently shown promise to solve modern problems. A single fumigation of two hours duration markedly reduced subsequent postharvest decay without harm to the fruit. The high pH imparted by ammonia in wounds in the fruit, sites of infection by the decay fungi, greatly enhanced the performance of the popular fungicide imazalil. Little is known of its insecticidal properties, and assessment of this aspect is planned by coworkers.

Past Research Accomplishments

- **Postharvest biological control**

Working with commercial, university, and USDA collaborators in the mid 1990s to develop formulations, efficacy studies, host range determinations, and other information required by regulators, two postharvest biological control products (BioSave 10, BioSave 11) were registered and entered commercial use on citrus fruit in the USA and elsewhere.

- **Ethanol as a fungicide**

In a collaboration with ARO coworkers in Israel, the use of ethanol as a fungicide on table grapes and stone fruit was developed and demonstrated to perform to commercially useful levels to control decay and extend the shelf and shipping life of these products.

- **Fungicide molecular modes of resistance**

In 2006, we determined the molecular modes of resistance to two common conventional fungicides, imazalil and thiabendazole, in the citrus pathogen *Penicillium digitatum*. Among 68 isolates examined, thiabendazole resistance was the consequence of transition at codon 200 in the beta-tubulin gene from thymine to adenine, which caused an amino acid change of phenylalanine to tyrosine in the tubulin protein that inhibited thiabendazole binding to it. Among 109 isolates examined, imazalil resistance was the consequence of over-expression of cytochrome p450-dependent 14 alpha de-methylase, the target of this fungicide, which occurred as a consequence of one of two insertions in the regulatory of the gene encoding this enzyme.

Selected Publications

- Mlikota Gabler, F., Julien Mercier, J.I. Jiménez and J. L. Smilanick. 2009.** Compatibility of *Muscodor albus* with ozone or sulfur dioxide fumigation for decay control in table grapes. *Postharvest Biol. Tech.* *in press*
- Mlikota Gabler, F., J. L. Smilanick, M. F. Mansour, Hakan Karaca. 2009.** Influence of fumigation with high concentrations of ozone gas on postharvest gray mold, quality, and fungicide residues on table grapes. *Postharvest Bio. Tech.* *in press*
- Gianfranco Romanazzi, Franka Mlikota Gabler, Dennis Margosan, Bruce E. Mackey, and Joseph L. Smilanick. 2009.** Effect of chitosan dissolved in different acids on its ability to control postharvest gray mold of table grapes. *Phytopathology* 99(9):1028-1036.
- Montesinos Herrero, C., Smilanick, J. L., Hurley, J. M., and Palou, L. 2009.** Potassium sorbate residue levels and persistence in citrus fruit as detected by a simple colorimetric method. *J. Agric. Food Chem.* 57(9):3458–3463.
- Palou, L., Smilanick, J. L., Crisosto, C. H. 2009.** Evaluation of food additives as alternative or complementary chemicals to conventional fungicides for the control of major postharvest diseases of stone fruit. *J. Food Protection* 72(3):1037-1046.
- Leesch, J. L., Smilanick, J. L. and Tebbets, J. C. 2008.** Methyl bromide fumigation of packed table grapes: Effect of shipping box on concentrations and phytotoxicity. *Postharvest Bio. Tech.* 49: 283-286.
- Lluís Palou, Joseph L Smilanick, and Samir Droby. 2008.** Alternatives to conventional fungicides for the control of citrus postharvest green and blue moulds. *Stewart Postharvest Reviews* 2
- Smilanick, J. L., Mansour, M. F., Mlikota Gabler, F., and Sorenson, D. 2008.** Control of citrus postharvest green mold and sour rot by potassium sorbate combined with heat and fungicides. *Postharvest Bio. Tech.* 47: 226–238.
- Usall, J., Smilanick, J. L., Palou, L., Denis-Arrue, N., Teixido, N., Torres, R., and Vinas, I. 2008.** Preventive and curative activity of combined treatments of sodium carbonates and *Pantoea agglomerans* CPA-2 to control postharvest green mold of citrus fruit. *Postharvest Bio. Tech.* 50: 1-7.
- Ghosoph, J. M., Schmidt, L. S., Margosan, D.A., and Smilanick, J. L. 2007.** Imazalil resistance linked to a unique insertion sequence in the *PdCYP51* promoter region of *Penicillium digitatum*. *Postharvest Bio. Tech.* 44: 9-18.
- Pervin Kinay, M. F. Mansour, F. Mlikota Gabler, D.A. Margosan, and J. L. Smilanick. 2007.** Characterization of fungicide-resistant isolates of *Penicillium digitatum* collected in California. *Crop Protection* 26: 647-656.
- Romanazzi, G., O. A. Karabulut and J. L. Smilanick. 2007.** Combination of chitosan and ethanol to control postharvest gray mold of table grapes. *Postharvest Bio. Tech.* 45: 134-140.
- Smilanick, J. L., and Mansour, M. F. 2007.** Influence of temperature and humidity on survival of *Penicillium digitatum* and *Geotrichum citri-aurantii*. *Plant Disease* 91: 990-996.

Spencer S. Walse

Research Chemist

Research efforts focus on solving chemically-based problems in agriculture. Research activities involve the development and integration of predictive chemical kinetics, modeling strategies, and field/*in situ* results as they relate to quantitatively understanding the interaction of molecules with their surroundings. We look at molecules that are produced naturally, as well as, those that are produced by man, anthropogenically. Specific services provided to customers include: commodity fumigation and residues, agrochemical fate and transport, and natural products discovery and utilization.

Specialty crop fumigation and residues

The movement of specialty crops to foreign markets is of vital economic importance. At any time, importing countries can confront industry with quality, quarantine, and residue requirements with the potential to terminate trade. A substantial portion of my research program is dedicated to the protection of CA-grown commodities in these postharvest trade and marketing channels. We routinely develop chemical, physical, biological, and toxicological strategies for controlling populations of stored product and quarantine pests in perishables, such as fresh fruits, and durables, such as nuts and dried fruit. By in large, the existing infrastructure of the specialty crop industry dictates that chamber fumigations be used for this protection. Therefore, we design and apply, in concert with industry needs, chamber-based techniques for the specific purpose of overcoming consequential trade barriers.

Natural products

Our natural products work focuses on molecules that are released/utilized into the environment as chemical signals for inter-organism communication. Generically termed semiochemicals, these signaling molecules include: pheromones, plant/insect defense compounds, algal neurotoxins, quorum promoters. Curious from an engineering perspective, very little has been published with respect to how tracing the environmental signatures of these natural products can be used to inspire the development of sustainable chemical technologies for agriculture and beyond.

Agrochemical fate and transport

Man-made agrochemicals are investigated in a wide-range of environments, such as orchards, irrigation canals, post harvest commodity treatment facilities, and organisms. Examples of these chemicals we routinely analyze include: fumigants, pesticides, fungicides, disinfection byproducts, engineered nanomaterials, and endocrine disruptors. Considerable effort is spent delineating the spatio-temporal effects of concentration-dependent phenomenon, at both the molecular and systemic levels, on the formation, degradation, transport, and toxicity (targeted and nontargeted) of these chemicals within a particular environment.

Current Research Projects and Accomplishments

Schedule development for chamber fumigations: quarantine scenarios.

Industry, APHIS, and ARS must work together to establish effective postharvest quarantine treatments for CA insect pests. The dose-mortality data that our group collects, with and without commodity loadings, frequently represents the foundation of quarantine schedule development. Quarantine/pre-shipment (QPS) uses of methyl bromide fumigant are permitted; however, regulatory trends suggest that this postharvest allowance will not continue indefinitely. Therefore, my research team also explores the efficacy of methyl bromide alternatives in quarantine scenarios. Current examples of quarantined insects we are targeting include: light brown apple moth (LBAM), peach twig borer (PTB), Asian citrus psyllid (ACP), and cherry vinegar fly (CVF).

Sulfuryl fluoride as a fumigant for insect pests of dried fruit and nuts.

Sulfuryl fluoride, originally produced and marketed as the structural fumigant Vikane®, has transitioned toward use in durable commodities as ProFume®. Substantial laboratory- and commercial-scale data exists on its ability to curb insect infestation in milling scenarios; however, relatively little (empirical data) is known about its insecticidal efficacy, and degradation, when dried fruit and nut pests of CA are considered, particularly for eggs which are the most tolerant life stage. This research program has detailed treatment schedules at atmospheric pressure (NAP) and reduced pressure (-100 mmHg) for eggs of: navel orangeworm, diapausing codling moth, red flour beetle, Indianmeal moth, and dried fruit beetle.

Mathematical modeling of fumigant effectiveness.

Multivariable experimental designs, which facilitate the analyses and interpretation of data, can be used to simultaneously delineate the contribution of various factors that influence the overall effectiveness of a fumigant. Using this statistics-based approach, existing or novel fumigants can be rapidly and thoroughly screened for optimal dose-duration responses, applicability toward a particular commodity, and physicochemical behavior within a commodity, the target organism(s), and the environment. With this design strategy, we have been able to provide the CA walnut industry with a toll that allows them to delineate the influence of sulfuryl fluoride dose, pressure, temperature, and exposure duration on both insect mortality, as well as, levels of SF₂O₂, FSO₃⁻, and F⁻ residues.

Application of the Horn phosphine method to CA pests and infrastructure.

The main goal of this research is to develop chamber fumigations using the Horn method of high-concentration phosphine fumigant, registered in the US as Vaporphos® (Cytec), at temperatures that will not break the cold-chain of the fruit in storage (~5 °C). This method is being used successfully by our Chilean reciprocal-trade counterparts, albeit on different insect pests. If this method is successful in controlling CA pests and is scalable for CA industries, benefits of this research will include: economic gains associated with breaking trade barriers, the ability to fumigate effectively during cold-storage, and human and environmental health improvement as phosphine leaves less residue than MB in commodity and has a markedly lower atmospheric ozone depleting potential.

Development of low-emission chamber fumigations.

In light of current and emerging limitation (and regulation) of agriculturally-related fumigant emission into the atmosphere, the aim of this project is to proactively assess the practical and economic feasibility of conducting postharvest chamber fumigations with reduced or negligible atmospheric impact. Important features of this research include: the comparative evaluation of contemporary containment/reuse and destruction methods for methyl bromide (MB) versus registered alternative fumigants (i.e. phosphine, ozone, sulfuryl fluoride, propylene oxide), the development of novel technologies to reduce and eliminate atmospheric emissions of fumigants, the utilization of an experimental scale-up approach that begins in laboratory chambers and culminates in commercial chambers with commodity-specific industry input, and an economic cost analysis of promising technologies, particularly those applicable to quarantine & pre-shipment postharvest (QPS) scenarios.

Degradation of economically important tree nuts.

We explore abiotic factors such as hydrolysis and sunlight photolysis, and biotic factors, such as tree species and fungal colonization. Our goal is to have a comprehensive understanding of the entire degradation process in order to maximize tree nut production. An overall aim is to apply pesticides more efficiently in orchards and /or develop attractants, based on degradation-related chemicals, which can be used instead of pesticides to control insects such as the navel orangeworm and peach twig borer.

Design of pheromone release matrices.

In general, the “Achilles heel” of pheromone-based insect pest management is the inability of current-use formulation technologies to mimic the natural pheromone ratios and release rates used by a particular species. What is curious about this dilemma is that we have not yet characterized and exploited the most logical resource, the vast arsenal of abiotic strategies for pheromone release that are already used by insects. The evolution of abiotic strategies for pheromone release, which serve to compliment the relatively well-documented biotic strategies, is of paramount utility to the many species of insect pests that initiate aggregation and mating via tracking volatile pheromones through the environment. When such pheromones are released from an insect (i.e., biologically), that insect’s ability to perceive other sources is often completely suppressed due to a “muffling” of the remote signal. Aggregation to remote locations can occur, however, if abiotic strategies are in place that release pheromone when the biological synthesis and release are not occurring. Our research program seeks to characterize and exploit insect-derived abiotic strategies of volatile pheromone release for the purpose of promoting pheromone-based insect pest management and other forms of sustainable agriculture.

U.S. Patents

Walse, S.S.; Fang, L.; Alborn, H.T.; Teal, P.E.A. “Glycoside linkage of pheromone component as a slow release formulations for insect attractions” provisional serial No. 60/195,218

Silhacek, D.; Murphy, C.; Walse, S.S; Teal, P.E.A. “Wheat germ attractants for larvae of the Indian Meal Moth, *Plodia interpunctella* (Hubner) provisional serial No. 60/254,342

Dossey, A. T.; Walse, S.S.; Conle, O.; Rocca, J.R.; Edison, A.S. “Parectadial compounds, methods of synthesis, and methods of use” serial No. 60/909,827

Selected Publications

- Walse, S. S.; Mitch, W. A. 2008.** N-nitrosamine carcinogens also swim in pools. *Environ. Sci. Technol.* 42: 1032-1037.
- Dossey, A. T.; Walse, S. S.; Edison, A. S. 2008.** Developmental and geographical variation in insect chemical ecology. *J. Chem. Ecol.* 34: 584-590.
- Walse, S. S.; Alborn, H. T.; Teal, P. E. A. 2008.** Suspensoside, a pheromone glucoconjugate from the oral secretions of *Anastrepha suspense*. *J. Nat. Prod.* 71: 1726-1731.
- Walse, S. S.; Alborn, H. T.; Teal, P. E. A. 2008.** Naturally occurring abiotic formation and release of pheromones by tephritid fruit flies. *Green Chem. Let. Rev.* 1: 205-217.
- Walse, S. S.; Pennington, P. L.; Scott, G. I.; Ferry, J. L. 2004.** The fate of fipronil in modular estuarine mesocosms. *J. Environ. Monit.* 6: 58-64.
- Walse, S. S.; Morgan, S. L.; Kong, L.; Ferry, J. L. 2004.** The role of dissolved organic matter, nitrate, and bicarbonate in the photolysis of aqueous fipronil. *Environ. Sci. Technol.* 38: 3908-3915.
- Volz, D. C.; Wirth, E. W.; Fulton, M. H.; Scott, G. I.; Strozier, E.; Block, D. S.; Ferry, J. L.; Walse, S. S.; Chandler, G. T. 2003.** Effects of chlorpyrifos and fipronil on endocrine-related endpoints in female grass shrimp (*Palaemonetes pugio*). *Bull. Environ. Contam. Toxicol.* 71: 497-503.
- Walse, S. S.; Scott, G. I.; Ferry, J. L. 2003.** Stereoselective degradation of aqueous endosulfan in modular estuarine mesocosms: identification of endosulfan γ -hydroxycarboxylate. *J. Environ. Monit.* 5: 373-379.
- Walse, S. S.; Shimizu, K. D.; Ferry, J. L. 2002.** Surface-catalyzed transformations of aqueous endosulfan. *Environ. Sci. Technol.* 36: 4846-4853.

Victoria Y. Yokoyama

Research Entomologist

California is the only producer of canned olives in the U.S. and the olive fruit fly has become a major pest in commercial olive orchards since it was discovered in Los Angeles in 1998. The biology of the pest and non-chemical control techniques have been studied as a means to mitigate insect populations. A biological control program was implemented with an imported parasitoid to reduce pest numbers in highly infested areas. Research is in progress to develop better biological control agents and economical methods such as mass trapping to control the pest in the central valley.

Hay exported from the western states is valued at \$550 million annually. Timothy hay from Washington, and alfalfa, oat, Bermuda and Sudan grass hays, primarily from California are shipped to ports in the Pacific Rim. Japan is the primary buyer and existing and emerging markets are available in Taiwan, South Korea, Hong Kong, China, and Vietnam. Hessian fly is a pest of regulatory concern in exported hay and procedures must be developed to ensure that the insect is not accidentally introduced into foreign countries in bales shipped from the western states. Several quarantine treatments have been previously developed for different styles of baled hay. Work is in progress to modify these treatments using novel fumigants, new hay production procedures, and modern bale compressors.

Current Research Projects and Accomplishments

Biological and cultural control of olive fruit fly in olives.

Research in cooperation with the California Olive Committee provides potentially sustainable and economical control methods for olive fruit fly in olives.

Accomplishments:

- Olive fruit fly biology was studied in the laboratory, greenhouse, and field to determine optimum conditions for survival at different temperatures and humidities and in the presence and absence of food. Reproduction in relation to insect age and size of olive fruit were compared and the developmental times for the immature stages were described. The newly emerged adults and last larval instars of the pest were found to have great dispersal capacity in laboratory tests. The data was related to potential control strategies and the abundance of the pest in the different olive growing regions of California.
- A parasitic wasp was imported from the USDA, APHIS, PPQ, Moscard laboratory in Guatemala and shown to parasitize olive fruit fly in laboratory and field cage tests. The parasitoid did not interact with non-target fruit fly species in laboratory tests. A permit was obtained for free releases in California and the parasitoid was imported and released in olive fruit fly infested orchards in five regions of the state. A method to mass produce the parasitoid was developed using sterile Mediterranean fruit fly larvae in Guatemala. The effect of environmental conditions on parasitoid survival was studied in laboratory and greenhouse tests and related to the biology and distribution of olive fruit fly.

Biological and cultural control of olive fruit fly in olives (cont.).

- Two new parasitoids are under review for permits to import into the UCB quarantine facility to determine potential for biological control of olive fruit fly.
- Two traps used for monitoring olive fruit fly adults were compared and an attract- and-kill trap used in Europe evaluated in the field. New trap designs are under investigation to determine potential use in mass trapping programs to further reduce low numbers of olive fruit fly in the central valley including a novel trap developed for subtropical fruit fly species at the ARS, Hilo, HI laboratory. Field sanitation was found to be the most economical method to control overwintering populations of olive fruit fly in commercial olive orchards and urban olive trees.
- A colony of olive fruit fly developed by UCR is maintained on formulated diet in the laboratory for potential use in a sterile-insect-technique program to eradicate olive fruit fly. The ARS laboratory in Albany, CA is collaborating to study the potential of x-ray sterilization to sterilize olive fruit fly.

Quarantine strategies to control Hessian fly in exported hay.

Research was planned with the National Hay Association to develop new procedures to control of Hessian fly in hay exports to existing and emerging markets in Asia.

Accomplishments:

- A Hessian fly colony of the Great Plains biotype is maintained on wheat seedlings in the greenhouse in collaboration with the USDA-ARS, West Lafayette, IN to provide the insect puparia stage for testing.
- Basic tests were conducted in laboratory incubators to determine the effect of simulated timothy hay harvesting conditions in Ellensburg, WA on the survival of Hessian fly puparia. Studies will continue to evaluate hay harvesting conditions in Oregon and California.
- Basic tests were conducted in collaboration with the Dried Fruit Association of California, Fresno to determine the efficacy of phosphine and carbon dioxide gas mixtures to control Hessian fly puparia, the least susceptible stage to fumigation.
- Research plans were developed to reduce the 7-day aluminum phosphide fumigation to 4-days to control Hessian fly in the certified quarantine treatment for hay exports to Japan.
- Newly available Hessian fly pheromone traps are under investigation for use as possible detection devices in freight containers of exported hay.

Past Research Accomplishments

• Olives

Low temperature storage at 2-3°C in bins and brine solutions were developed for postharvest control of olive fruit fly.

Biology, life history, seasonal populations, and effect of olive production practices on olive fruit fly populations were evaluated. The pest was shown to be abundant in cool and humid coastal conditions and less prevalent in the warm and arid interior valleys.

Olive fruit fly specimens were collected from different regions of California for genetic analysis by colleagues who showed that the California invasion was of eastern Mediterranean origin. The parasitoid imported from Guatemala for biological control of olive fruit fly was submitted for genetic analysis and found to be distinct from other parasitoids in the same complex and resulted in a scientific name change.

● **Exported Hay**

Certified quarantine treatments were developed to control Hessian fly in hay exported to Japan and approved by regulatory agencies as follows: 1996 Standard Compressed Bales; 1997 Film-Wrapped Standard Compressed Bales; 2005 Large-Size, Polypropylene Fabric-Wrapped Bales.

Quarantine treatments to control cereal leaf beetle in hay exported to Canada including inter-province shipments, and for interstate shipments to California are as follows: Single treatments of standard bale compression or hydrogen phosphide fumigation; and multiple quarantine treatments of standard bale compression plus one day storage, and bale compression combined with fumigation.

● **Stone Fruit and Grape Exports**

First in-carton fumigation approved by Japan developed to control of codling moth in exported nectarines. Nectarines were demonstrated to be a commodity group eliminating the need for varietal testing.

Methyl bromide fumigation treatment developed to control oriental fruit moth in stone fruit exported to British Columbia and Mexico. Low temperature storage treatment developed to control oriental fruit moth and used to export apples from Washington to Mexico.

A pest-free period and poor host status of stone fruit for walnut husk fly developed and implemented for peaches, nectarines, plums, and fresh prunes exported to New Zealand, Brazil, Colombia, Ecuador, Argentina, and Chile.

Low temperature treatment and in combination with slow release sulfur dioxide pads developed to control omnivorous leafroller, western flower thrips, and two-spotted spider mite in exported table grapes.

Selected Publications

Yokoyama, V. Y., P. A. Rendón, and J. Sivinski. 2008. *Psytalia* cf. *concolor* (Hymenoptera: Braconidae) for biological control of olive fruit fly (Diptera: Tephritidae) in California. *Environ. Entomol.* 37: 764-773.

Yokoyama, V. Y. and G. T. Miller. 2007. Olive fruit fly biology and cultural control practices in California. *IOBC/WPRS Bull.* 30: 277-285.

Yokoyama, V. Y., G. T. Miller, J. Stewart-Leslie, R. E. Rice, and P. A. Phillips. 2006. Olive fruit fly (Diptera: Tephritidae) seasonal populations in relation to region, trap type, season, and availability of fruit. *J. Econ. Entomol.* 99: 2072-2079.

Yokoyama, V. Y. and G. T. Miller. 2004. Quarantine Strategies for olive fruit fly (Diptera: Tephritidae): Low-temperature storage, brine, and host relations. *J. Econ. Entomol.* 97: 1249-1253.

- Yokoyama, V. Y. and G. T. Miller. 2003.** Quarantine control of Hessian fly (Diptera: Cecidomyiidae) in exported hay: A new treatment for large-size polypropylene fabric-wrapped bales and a 3-d fumigation for compressed standard bales. *J. Econ. Entomol.* 96: 1340-1344.
- Yokoyama, V. Y. and G. T. Miller. 2002.** Bale compression and hydrogen phosphide fumigation to control cereal leaf beetle, *Oulema melanopus* (L.), in exported rye straw. *J. Econ. Entomol.* 95: 513-519.
- Yokoyama, V. Y., G. T. Miller, and C. H. Crisosto. 2001.** Pest response in packed grapes to low temperature storage combined with slow release sulfur dioxide pads in basic and large-scale tests. *J. Econ. Entomol.* 94: 984-988.
- Yokoyama, V. Y. and G. T. Miller. 2000.** Response of omnivorous leafroller (Lepidoptera: Tortricidae) and onion thrips (Thysanoptera: Thripidae) to low temperature storage. *J. Econ. Entomol.* 93: 1031-1034.
- Yokoyama, V. Y., G. T. Miller, P. L. Hartsell, and J. G. Leesch. 2000.** Large-scale, on-site confirmatory, and varietal testing of a methyl bromide quarantine treatment to control codling moth (Lepidoptera: Tortricidae) in nectarines exported to Japan. *J. Econ. Entomol.* 93: 1025-1030.
- Yokoyama, V. Y., G. T. Miller, P. L. Hartsell, and T. Ely. 1999.** On-site confirmatory test, film wrapped bales, and shipping conditions of a multiple quarantine treatment to control Hessian fly (Diptera: Cecidomyiidae) in compressed hay. *J. Econ. Entomol.* 92: 1206-1211.

HORTICULTURAL ENTOMOLOGY

Effects of Mating Disruption Treatments on Navel Orangeworm (Lepidoptera: Pyralidae) Sexual Communication and Damage in Almonds and Pistachios

BRADLEY S. HIGBEE AND CHARLES S. BURKS¹

Paramount Farming Company, 33141 E Lerdo Highway, Bakersfield, CA 93308

J. Econ. Entomol. 101(5): 1633–1642 (2008)

ABSTRACT Two experiments in 2003 examined the effects of different ways of dispensing the principal sex pheromone component on sexual communication among and crop damage by the navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae) in Nonpareil almonds and pistachios. A third experiment in 2004 compared the effect on navel orangeworm damage to several almond varieties using one of these dispensing systems by itself or with phosmet, phosmet alone, and an untreated control. Additional data are presented estimating release rates from timed aerosol release devices (PuffersNOW, Suterra LLC, Bend, OR) and hand-applied membrane dispensers. In 2003, puffers placed peripherally around 16-ha blocks, evenly spaced Puffers, and hand-applied dispensers reduced males captured in virgin-baited traps by $\geq 95\%$ and mating in sentinel females by $\geq 69\%$, with evenly placed Puffers showing greater reduction of males captured and females mated compared with the other dispensing systems. Mating disruption with gridded Puffers or hand-applied devices in almonds resulted in an $\approx 37\%$ reduction of navel orangeworm damage (not significant), whereas peripheral Puffers resulted in a 16% reduction of navel orangeworm damage to almonds. In pistachios neither peripheral nor gridded Puffers reduced navel orangeworm damage, whereas insecticide reduced damage by 56%. In 2004, Puffers alone, insecticide alone, and both in combination significantly reduced navel orangeworm damage in Nonpareil almonds. In other, later harvested varieties, the insecticide treatments reduced damage, whereas the mating disruption treatment alone did not. We discuss application of these findings to management of navel orangeworm in these two crops.

KEY WORDS mating disruption, almonds, pistachios, *Amyelois transitella*

The navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), is a key pest of both almonds, *Prunus amygdalus* Batsch, and pistachios, *Pistacia vera* L., in California (Wade 1961, Bentley et al. 2003, Zalom et al. 2005). Current practices for control of navel orangeworm damage in almonds and pistachios are intimately linked to cultural practices in these crops. Almonds are usually grown with different varieties in alternating rows. Often the variety Nonpareil comprises 50% of the orchard, and the other 50% is one or more of several varieties collectively referred to as pollenizers. Nonpareil almonds typically mature first and are harvested sometime in August, several weeks ahead of the pollenizer varieties. Approximately 40–60 d before harvest, the hulls begin to split, exposing the nut inside the shell. The risk of navel orangeworm infestation in almonds is very low before hullsplit, and increases markedly thereafter. The shell

of Nonpareil almonds is relatively light and porous, and the nut meat is considered more susceptible to infestation compared with “hard shell” varieties (Soderstrom 1977). Pistachios have a single commercially important variety, Kerman, and splitting of the hull occurs much closer to harvest, if at all. Harvest of both crops results in a significant number of nuts remaining to serve as host material for development and overwintering of navel orangeworm.

The development of mating disruption for suppression of navel orangeworm damage has progressed slowly. Until recently, only the principal component of the female sex pheromone was known (Coffelt et al. 1979, Leal et al. 2005). This component, (Z,Z)-11,13-hexadecadienal (Z11,Z13-16:Ald), is not sufficient to efficiently bring males to a point source (Leal et al. 2005) and is particularly vulnerable to degradation in the field (Curtis et al. 1985).

Mating disruption formulations in current commercial use have been broadly categorized as microencapsulated dispensers, hand-applied dispensers, and high-emission dispensers (Sarfranz et al. 2006). Previously trials with Z11,Z13-16:Ald released from hand-applied dispenser in 6–8-ha plots demonstrated

This article reports the results of research only. Mention of a proprietary product or pesticide does not constitute an endorsement or a recommendation for its use by USDA.

¹ Corresponding author: San Joaquin Valley Agricultural Sciences Center, USDA-ARS, 9611 S. Riverbend Ave., Parlier, CA 93648 (e-mail: charles.burks@ars.usda.gov).

greatly reduced capture of males in virgin-baited flight traps and reduction of mating in sentinel females (Curtis et al. 1985). In two of four trials, significant reduction of crop damage resulted. The dispensers were replaced 3–5 times over ≈ 85 d, and a total of ≈ 12.5 g of Z11,Z13-16:Ald per ha was placed in the field (theoretically 146 mg/ha/d), but most of this material was never released (Curtis et al. 1985). A high-emission dispenser, in which the pheromone is stored in a liquid organic solvent before being released at timed intervals, reduced this problem (Shorey and Gerber 1996). Studies with this and other lepidopteran pests have suggested that these high-emission dispensers (Puffers) placed around the perimeter of plots of up to 16 ha can reduce males captured in pheromone traps as effectively as such devices placed evenly throughout the plot, thereby saving labor costs (Shorey and Gerber 1996, Shorey et al. 1996).

Mating disruption treatment blocks of ≥ 16 ha were previously recommended (Shorey and Gerber 1996) because the navel orangeworm has a high capacity for dispersal. A mark–release–recapture study examining eggs colored by fat soluble dye in the maternal diet found that, over two to four nights after eclosion, females oviposited equally at all distances up to 375 m from the release point (i.e., an area of 44 ha) (Andrews et al. 1980). However, sanitation trials indicated that 20-ha almond blocks were large enough to obtain benefit from sanitation despite being surrounded by unsanitized almonds (Curtis 1976), suggesting that blocks around this size were large enough to avoid obscuring the effect of treatments within the block by immigration from outside the block.

Here, we present results of experiments in 2003 and 2004 comparing the effects of release systems for Z11,Z13-16:Ald on sexual communication and nut damage in almonds and pistachios located across the southern San Joaquin Valley. The objective of this study was to 1) to compare changes in release rate over time, under field conditions, between a hand-applied membrane dispenser (CheckmateNOW, Sutterra LLC, Bend, OR) and Puffers (PufferNOW, Sutterra LLC, Bend, OR); 2) to compare effect on sexual communication and damage of peripherally-placed Puffers with Puffers or hand-applied devices placed evenly throughout the plot; and 3) to further examine evenly spaced Puffers alone or in combination with insecticide on navel orangeworm damage in several almond varieties.

Materials and Methods

General Procedures. Traps using virgin females as a pheromone source were used to monitor relative abundance of males and their ability to locate females. Groups of three females were sealed in a mesh bag, which was then suspended from inside of a wing trap (Pherocon IC, Trécé Inc., Adair, OK). Females placed in the field in this manner were shown previously to live and call for 4–7 d in summer conditions in this region (Burks and Brandl 2004). Moths for this experiment were obtained as eggs from a laboratory

colony originally obtained in 1966 from the University of California, Berkeley, and maintained on a wheat bran diet (Tebbetts et al. 1978). Three newly eclosed females were enclosed in plastic mesh bags and placed in traps in the field within 24 h. Traps were checked and females replaced weekly, and liners were removed and replaced if they contained moths or were dirty. Old liners were taken to the laboratory to confirm field counts and identification.

Effects of the treatments on mating were examined with sentinel females using methods and apparatus similar to those described by Curtis et al. 1984). A 473-ml round polypropylene cup was suspended from the top of a wing trap by clips, and used to contain a second such cup with the top half coated with Fluon (ICI, London, United Kingdom). A portion of the wings on one side were clipped on freshly eclosed females, which were individually placed in plastic vials for transport the same day to mating assay locations in the field. The next week, females were again placed in plastic vials for transport to the laboratory where they were evaluated for mating based on the presence or absence of spermatophore(s) in the bursa.

The ranches used were owned and managed by Paramount Farming (Bakersfield, CA), and their location codes are used here to distinguish these sites. Four of these ranches (3410, 3710, 3740, and 3940) contained almonds of the varieties Nonpareil, Carmel, Fritz, and Monterey, and four (4010, 4260, 4540, and 4840) contained Kerman pistachios. These ranches were within a 65 by 31 km (east–west and north–south, respectively) area of Kern County, CA; an area roughly encompassed by Highways 99 and 33 on the east and west, respectively, and Seventh Standard Road and County Line Road on the south and north, respectively. The distance between each of these ranches and the next nearest location in this study ranged from 3 to 14 km, with a median of 5 km.

Percentage of reduction of males captured in virgin female-baited traps was calculated as $[1 - (\text{no. males captured in pheromone plot} / \text{no. males captured in untreated}) \times 100]$. SAS software (SAS Institute 2004) was used for all analysis, and proportions of nuts with harvest damage were transformed as arcsine(\sqrt{x}) before analysis.

Release Rate of Hand-Applied Dispensers and Puffers under Field Conditions. Concurrently with the two mating disruption experiments in 2003 examining effects of mating disruption treatments on sexual communication (see below), an experiment was performed examining rate of loss of Z11,Z13-16:Ald from hand-applied dispensers under field conditions. Dispensers were placed on the north and south side of almond trees far from mating disruption experiments, and removed from 10 trees at various intervals for analysis. One set of dispensers was placed in the field on 26 March and sampled 20, 41, 54, 68, 96, 110, 155, 202, and 225 d later, and a second set was placed in the field on 17 July and sampled on days 21, 42, 56, 89, and 112. Gas chromatography was used to determine the amount of Z11,Z13-16:Ald that could be extracted from the dispensers. The difference between phero-

October 2008

HIGBEE AND BURKS: NAVAL ORANGEWORM MATING DISRUPTION

1635

mone content of hand-applied dispensers removed from the north and south side of trees on the same day was compared using Student's *t* test (TTEST procedure), with a Bonferroni correction for multiple comparisons (Zar 1999). The average rate of emission over each of the intervals was estimated as the difference in average Z11,Z13-16:Ald content divided by the interval in days, emission rate versus time (d) fit to first-order decay $\{\text{mg Z11,Z13-16:Ald} = a \times \exp(-b*d)\}$ and linear equations $\{\text{mg Z11,Z13-16:Ald} = a - b*d\}$ by using the NLIN and REG procedures. Changes in Z11,Z13-16:Ald content also were compared over the first 115 d between the first and second application by using linear and first-order decay equations, and the predicted mean and 95% confidence limits (CL) for the decay equation were used to estimate the hourly rate of pheromone release over the season.

In 2003, the emission rate from timed release aerosol dispensers (Puffers) was examined by determining the crude weight of a single puff 77 d after activation in two almond blocks ($n = 79$ and 59 Puffers), and 99 d after activation in an almond and a pistachio block ($n = 155$ and 161 , respectively). The crude weight was multiplied by the proportion and purity of Z11,Z13-16:Ald in the canister. Fixed effects analysis of variance (ANOVA) (GLM) with a Tukey-Kramer adjustment for multiple comparisons was used to examine the differences between the four block-time combinations.

In 2004, procedures similar to those for hand applied dispensers in 2003 were used to examine changes in emission rate of Puffers over the season. The Puffer consisted of a cabinet containing a programmable electronic timer and an aerosol canister. Aerosol canisters used in 328 Puffers as part of an experiment (see below) were weighed when first placed in service, weighed again after 30–50 d, and then weighed after four to five additional intervals of 27–30 d. The difference in weight, divided by the interval and multiplied by the proportion Z11,Z13-16:Ald, was used as an estimate of the emission rate over this interval. Fixed effects ANOVA with Dunnett's test for difference of means from a control was used to examine differences in emission rate over time, with the average daily pheromone emission as the dependent variable and time (in days) between Puffer activation and the interval midpoint as the independent variable.

To facilitate comparison between the emission rate of Puffers and membrane dispensers, estimates of the mean and standard error of the hourly release rate per dispenser in both 2003 and 2004 were multiplied by the density of dispensers per hectare and reported as milligrams per hour per hectare.

Comparison of Mating Disruption Treatments in Almonds and Pistachios in 2003. Two similar randomized complete block mating disruption experiments were performed in almonds and pistachios. Four treatments were applied to 16-ha treatment plots centered in each corner of four blocks, making up square 256-ha plantings of almonds or pistachios (i.e., a part or all of a ranch) (Fig. 1). In the first mating disruption experiment, in almonds, treatments included 1) a con-

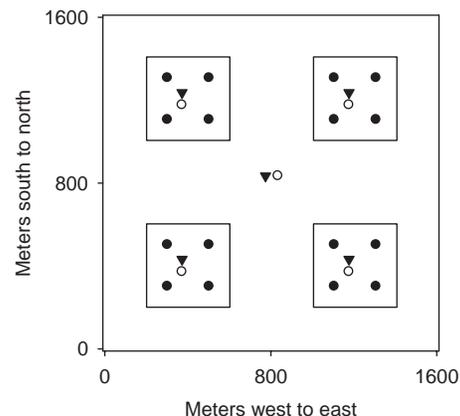


Fig. 1. Plot arrangement for randomized complete block design in almonds and pistachios in 2003, representing one of four 256-ha blocks (replicates) each in almonds and pistachios. The smaller squares represent 16-ha treatment plots. Effects of treatments were compared using wing traps baited with virgin females (dark circles), unbaited wing traps (dark triangles), and sentinel females (open circles). A blank wing trap and sentinel female were placed in the center of the block between the treatment plots.

2) mating disruption treatment targeted against navel orangeworm, 3) mating disruption with Puffers placed around the perimeter of the treatment plot, 4) mating disruption with Puffers placed in an even grid throughout the block, and 5) mating disruption with hand-applied membrane dispensers placed on each tree in the block. A 96-ha limit on experimental treatments precluded us from using hand-applied dispensers in both almonds and pistachios, so in the second mating disruption experiment, in pistachios, one of the four treatment blocks was instead treated 30 d before harvest with a residual insecticide, azinphosmethyl (Guthion 50 WP, Research Triangle Park, NC) at a rate of 2.26 kg of active ingredient (AI) in 1,893 liters water per ha. Response variables for each of the two experiments were males captured in traps baited with virgin females, mating status in sentinel females, and navel orangeworm damage to harvest samples of Nonpareil almonds.

Mating disruption treatments were applied from 3 April through mid-October in almonds or mid-September in pistachios. Puffers were placed peripherally or evenly throughout the experimental block at a density of five dispensers per ha, and emitted a target of 40 mg of solvent and propellant containing 1.09% Z11,Z13-16:Ald of 87.4% purity, for a target of 0.38 mg of Z11,Z13-16:Ald every 15 min from 6 PM to 6 AM (PDT) (i.e., 91 mg/ha/d Z11,Z13-16:Ald over 160–200 d). Hand-applied dispensers were placed in the trees at a rate of 375 per ha (two per tree) on 26 March, a second and equal application was placed on 17 July.

Disruption of male trap capture was compared between treatments by using four virgin-baited wing traps in each 16-ha treatment plot, placed 1.5 m above the ground, 200 m from the nearest other traps in the

plot, and 100 m from the edge of the block (Fig. 1). The distance of these traps from the nearest Puffer in any direction was ≥ 24 m in the gridded Puffers and ≥ 95 m for the peripherally placed Puffers. Blank flight traps, without virgin females, also were placed at the center of each treatment plot and at the center of the 256 ha ranch (block). These data were collected for 24 consecutive weeks in pistachios and 23 wk in almonds, from 31 March to 8 September 2003. We used sentinel females, placed at the center of each treatment plot and in the center of the ranch, to compare the relative probability of female mating.

Impact of the treatments on crop damage was compared by taking nut samples at 16 points within each of the plots, which were pooled to form samples of 4,265–7,967 almonds and 10,738–19,310 pistachios per plot. Each nut was opened and examined under magnification by Paramount Farming research personnel. Harvest dates for Nonpareil almonds were 17 and 21 August for ranches 3940 and 3740, and 28 August for ranches 3440 and 3710. Harvest dates for pistachios were 16 and 17 September for ranches 4010 and 4510 and 24 and 25 September for ranches 4260 and 4840.

The effect of treatments on males captured in virgin female baited traps in almonds and pistachios over the 23–24-wk observation period was analyzed using generalized linear mixed models (GLIMMIX) with a negative binomial distribution (Agresti 2007). The dependent variable was the sum of males captured in plots in almonds and pistachios over the 23–24-wk observation period, ranch was a random effect, the mating disruption or other treatment was a fixed effect, and a Tukey adjustment was used for multiple comparisons. The number of males captured in blank traps was negligible in both almonds and pistachios, and blank traps were therefore not included in the analysis. Differences in female mating were examined using contingency table analysis for multiple proportions (FREQ) with a Tukey style test for multiple comparisons (Zar 1999). Navel orangeworm damage was compared using a 2-way mixed model ANOVA (MIXED) in almonds and pistachios, with treatment as a fixed effect and ranch as a random effect.

Comparison of Mating Disruption in Almonds with and without Insecticide Treatments in 2004. A third mating disruption experiment in 2004 examined the effect of mating disruption with gridded Puffers in several almond varieties, alone or with a hullsplit insecticide treatment. Treatments were 1) untreated controls, 2) mating disruption with gridded Puffers, 3) a hullsplit treatment with residual insecticides, and 4) a combination of both the mating disruption and residual insecticide treatments. Response variables for this experiment were males captured in traps baited with virgin females, mating status in sentinel females, and navel orangeworm damage to harvest samples. Treatment effects on navel orangeworm damage were examined for Nonpareil and two pollenizer varieties, Carmel and Monterey.

A Latin square experimental design (Zar 1999) was used in Ranch 3710 to obtain greater replication within an area of homogeneously high navel orange-

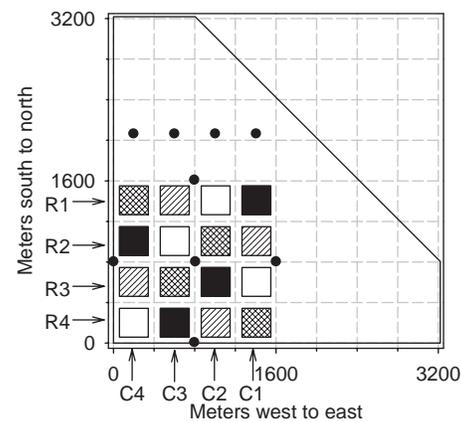


Fig. 2. Plot arrangement for Latin square experiment in almonds in 2004. Squares represent 8-ha treatment plots, with untreated control plots represented by white squares, plots treated with Puffers represented by diagonal lines, plots treated with insecticide represented by cross hatches, and plots treated with both mating disruption and insecticide represented by black squares. Row and columns grouping of plots (Table 4) are indicated by the letters and arrows. Wing traps baited with virgin females outside of the Latin square test area and within the area outside the plots are represented by dark circles. There were four virgin-baited flight traps in each treatment plot, arranged as in Fig. 1.

worm activity. A square 256-ha block was divided into 16 square eight ha (284 by 284 m) plots separated from each other by 118 m, and from the sides of the block by 59 m (Fig. 2). Each treatment was represented once in each east–west row and once in each north–south column. The 256-ha block of almonds examined was bordered by additional sections of almonds to the north and east, and by highways to the south and west. Puffers were placed at two-thirds canopy height evenly throughout the treated plots at a density of five per ha, and mating disruption with 105 mg/ha/d Z11,Z13-16:Ald was applied from the beginning of March until the end of the Monterey harvest. A hullsplit insecticide treatment consisting of 4.18 kg of phosmet (AI) (Imidan 70 WP, Gowan, Yuma, AZ) and 113 g of permethrin (AI) (Pounce 3.2 EC, FMC Agricultural Products, Philadelphia, PA) in 1,893 liters water per ha, was applied on 10 July. Areas between the treatment plots simultaneously received a hullsplit insecticide treatment with 4.18 kg of phosmet (AI) in 1,893 liters water per ha but without permethrin.

Traps baited with virgin females were used to compare disruption of male trap captures inside treatment plots, and in the buffer regions between treatment plots, with activity in an adjacent block of almonds completely outside the 4 by 4 grid of treatment plots (Fig. 2). Within each treatment, plot four wing traps were placed 142 m from each other and 71 m from the side of the treatment block. In mating disruption treatment blocks, these traps were ≥ 15 m from the nearest Puffer and ≥ 18 m from the nearest upwind Puffer. Outside the 256-ha site containing the 16 treatment plots, four traps were placed 500 m north of the north

October 2008

HIGBEE AND BURKS: NAVAL ORANGEWORM MATING DISRUPTION

1637

edge and parallel with the center of each of the four columns of treatment plots (Fig. 2). Within this site, a trap was placed in the center of the section and one each in the middle of the west, north, east and south sides of the section 46 m from the edge. Females were placed in the flight traps on 1 March, 2004 and replaced weekly until 24 August.

Samples of Nonpareil almonds were taken from the ground after trees were shaken for harvest and dislodged nuts had been piled in the center of alleys for harvest. Dates of harvest were 20–23 August for Nonpareil, 17 September for Carmel, and 27 September to 4 August for Monterey. Samples of 474 ± 6.4 (mean \pm SD) Nonpareil, 89 ± 1.2 Carmel, and 86 ± 1.2 Monterey almonds each were taken from a point in a windrow within 16 equal sectors in each treatment plot. These 16 subsamples for each variety were pooled for determination of infestation as described previously.

The GLIMMIX procedure was used to perform generalized linear models analysis, with a negative binomial distribution and all fixed effects, for comparison of treatment effects on males captured in virgin female-baited traps. The dependent variable was total males captured in the four traps in each plot over the monitoring period, and the independent variables were row, column, and treatment. Fixed effects ANOVA was used to examine row, column and treatment effects on navel orangeworm damage to almonds in the treatment plots at harvest, and Fisher protected least significant difference (LSD) was used for separation of means. Separate analyses were performed for damage in Nonpareil, Carmel, and Monterey almonds.

Results

Release Rate of Hand-Applied Dispensers under Field Conditions. The hand-applied dispensers released a calculated $32.3 \text{ g/ha Z11,Z13-16:Ald}$ over the season. Nonlinear regression of estimates of emission rate on time in field by using a first-order decay model was significant ($F = 40.19$; $df = 2, 24$; $P < 0.0001$) and accounted for much of the observed variation ($r^2 = 0.77$). Estimates of the initial rate and the decay were $0.603 \pm 0.12 \text{ mg/d}$ (mean \pm SE) and $-0.0096 \pm 0.0035 \text{ mg/d/d}$, respectively. Dispensers on the south side of the tree lost significantly more Z11,Z13-16:Ald ($P < 0.05$ after Bonferroni adjustment) than those on the north side on days 54, 68, and 96 of the first application (19 May to 30 June), but not at any time in the second application (Fig. 3A). The first-order decay equation fit the data for changes in membrane weight over the first 115 d better than linear regression ($r^2 = 0.99$ versus 0.89 – 0.91), and parameter estimates were not significantly different between the two applications with either linear or nonlinear regression. The daily mean release rate, as estimated using first order decay, is plotted in Fig. 3A for the first and second applications of hand-applied devices.

In 2003, the single-puff emission rate for Puffers in the almond block was 8.5 ± 0.17 and $8.8 \pm 0.23 \text{ mg/ha/h Z11,Z13-16:Ald}$ in the two almond blocks on day

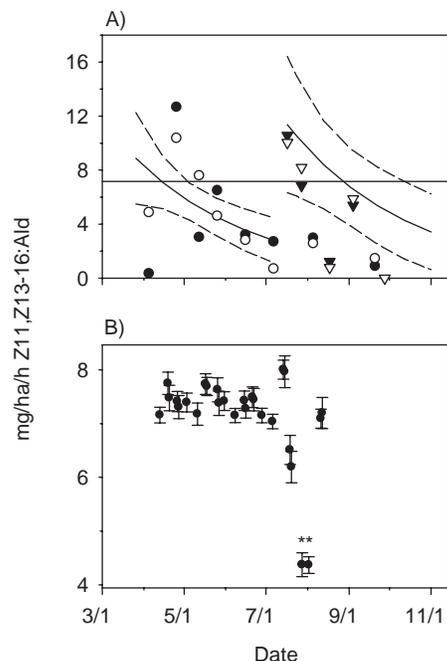


Fig. 3. Effect of time in field on the emission rate of two types of dispensers. (A) Hand-applied dispensers in 2003: Estimated emission rate of groups of 10 membranes placed in the field on 26 March (circles) or 17 July (triangles) on the north (white symbols) or south (black symbols) side of the tree. Solid and dashed lines indicate the mean and 95% CI predicted by nonlinear regression for dispensers from the first application before 17 July, and from combined emission from dispensers from both applications after 17 July. The vertical reference is the mean for the first rate determined for Puffers in 2004. (B) Emission of Puffers between 7 AM and 7 PM (PDT) in 2004 (mean and SE). There were no significant changes in the rate of emission for the first 120 d in the field. Observations with asterisks differ significantly ($P < 0.05$) from the first rate determined (day 35). Note that the vertical axis differs between A and B.

77 and 8.7 ± 0.14 and $7.3 \pm 0.23 \text{ mg/ha/d Z11,Z13-16:Ald}$ in almonds and pistachios, respectively, on day 99 (i.e., 88–106 mg/ha/d). There were significant differences among these means ($F = 20.34$, $df = 3, 450$; $P < 0.0001$). The pistachios on day 99 were significantly different than the other three measurements, which were not significantly different from each other.

In 2004, 93% of the Puffer units functioned throughout the entire season with no maintenance. The most common maintenance was replacing Puffer canisters, which occurred most frequently on days 141 and 146. There were significant differences in emission rates between days ($F = 24.79$, $df = 26, 1,380$; $P < 0.0001$). Emission rates were significantly less than the reference value (i.e., the first measurement) on days 141 and 146 ($P < 0.0001$) (Fig. 3B).

Comparison of Mating Disruption Treatments in Almonds and Pistachios in 2003. There were differences between mating disruption treatments in al-

Table 1. Treatment and replicate effects (mean \pm SE) on navel orangeworm males captured per plot in 16-ha plots in almonds between 31 March and 15 September 2003

Factor	Level	Males per plot	% reduction
Treatment	Gridded Puffers	2.5 \pm 0.9a	99.7
	Peripheral Puffers	13.5 \pm 11.5ab	98.2
	Hand-applied dispensers	31.3 \pm 12.6b	95.7
	Untreated	729.8 \pm 383.5c	
Replicate	Ranch 3740	34 \pm 18	
	Ranch 3440	118 \pm 114	
	Ranch 3940	139 \pm 133	
	Ranch 3710	487 \pm 451	

Means followed by different letters are significantly different ($P < 0.05$).

monds in the number of males captured in virgin-baited traps ($F = 26.10$, $df = 3, 9$; $P < 0.0001$) (Table 1). Significantly fewer males were captured in all mating disruption treatment plots compared with untreated controls, significantly fewer males were captured in plots treated with gridded Puffers compared with peripheral Puffers, and the number of males captured in plots treated with hand-applied dispensers was not significantly different from those captured in plots treated with either gridded or peripheral Puffers (Table 1). A plot of trap sums by week shows that, in almonds, most males in virgin-baited traps were captured after 1 August and, before 24 August, more males were captured in plots treated with peripheral Puffers compared with hand-applied dispensers (Fig. 4). Over the observation period, three males were captured in blank traps in almonds, compared with the 10 females captured in female-baited traps in gridded Puffer plots.

In pistachios, there were significant differences between treatments in sums of males captured in virgin-baited flight traps ($F = 76.21$, $df = 3, 9$; $P < 0.0001$). There were significantly fewer males captured in plots treated with gridded Puffers than in those treated with peripheral Puffers, and far fewer males were captured in plots treated with either of the mating disruption treatments compared with the untreated control or the pesticide treatment (Table 2). Over the observation period, six males were captured in blank traps in pistachios compared with the 44 males in female-baited traps in gridded Puffer plots.

There were significant differences between treatments in the proportion of sentinel females mated in both almonds ($\chi^2 = 33.17$, $df = 4$, $P < 0.0001$) and pistachios ($\chi^2 = 90.26$, $df = 4$, $P < 0.0001$). In almonds, significantly fewer females were mated in the plots treated with peripheral or gridded Puffers compared with females in the untreated control plot or in the center between plots, whereas the proportion of females mated in plots treated with hand-applied dispensers were not significantly different from that in either the Puffer-treated plots or the untreated control plots (Table 3). In pistachios significantly more females were mated in the untreated control plot compared with the center position and plots treated

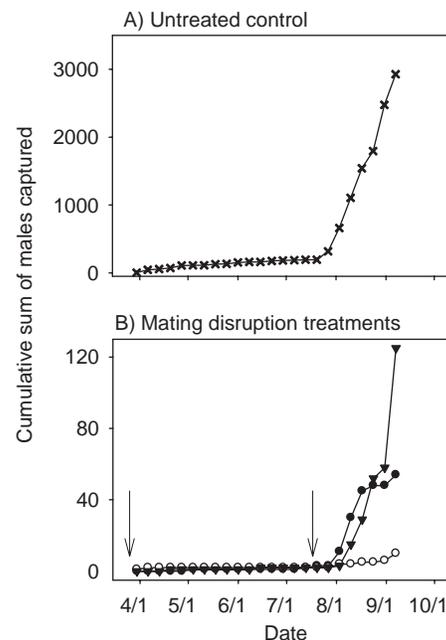


Fig. 4. Cumulative sum of navel orangeworm males captured in virgin female-baited traps in almonds in 2003 in untreated control plots (A) and mating disruption plots. (B). Symbols: males captured in untreated control plots (Xs); males captured in plots treated with peripherally-placed Puffers (black circles); males captured in plots treated with hand-applied dispensers (black triangles); and males captured in plots treated with gridded Puffers (white circles). Arrows indicate dates of first and second applications of the hand-applied dispensers.

with either gridded or peripheral Puffers had significantly fewer females were mated compared with any nonmating disruption treatment (Table 3).

The percentage of almonds damaged by navel orangeworm was 3.6 ± 2.8 (mean \pm SE), 3.0 ± 2.4 , 2.3 ± 1.6 , and $2.3 \pm 1.7\%$ in the untreated control, peripheral Puffer, hand-applied dispenser, and gridded Puffer plots, respectively. A mixed models ANOVA found no significant differences among these treatments ($F = 1.80$, $df = 3, 9$; $P = 0.2178$). The mean navel orange-

Table 2. Treatment and replicate effects (mean \pm SE) on navel orangeworm males captured per plot in 16-ha plots in pistachios between 31 March and 8 September 2003

Factor	Level	Males per plot	% reduction from untreated
Treatment	Gridded Puffers	11 \pm 3a	99.3
	Peripheral Puffers	72 \pm 35b	95.7
	Azinphosmethyl	2,853 \pm 449c	
	Untreated	1,662 \pm 456c	
Replicate	Ranch 4260	965 \pm 766	
	Ranch 4010	1,013 \pm 592	
	Ranch 4510	1,019 \pm 655	
	Ranch 4840	1,601 \pm 909	

Means followed by different letters are significantly different ($P < 0.05$).

October 2008

HIGBEE AND BURKS: NAVAL ORANGEWORM MATING DISRUPTION

1639

Table 3. Effect of treatments on mating status of sentinel females recovered from mating assays in 2003 in the center of each 16-ha treatment plot and in the center of the 256-ha block, equidistant between the treatment plots

Mating disruption?	Treatment	Almond		Pistachio	
		n	% mated	n	% mated
No	Center of 256-ha block	81	14.8ab	76	35.5b
	Untreated control	83	19.3a	81	61.0a
Yes	Azinphosmethyl			77	53.1ab
	Hand-applied dispensers	84	3.6bc		
	Peripheral Puffers	84	2.4c	81	11.4c
	Gridded Puffers	86	0.0c	79	3.8c

Percentages within the same column followed by different letters are significantly different ($P < 0.05$).

worm damage to almonds (across treatments) was $9.1 \pm 1.2\%$ in ranch 371 and ranged from 0.2 to 1.1% in the remaining three ranches. In pistachios there were significant differences in navel orangeworm damage between treatments ($F = 5.70$, $df = 3, 9$; $P = 0.0182$). The percentage of pistachios with navel orangeworm damage was 1.2 ± 0.33 , 1.3 ± 0.39 , 1.2 ± 0.31 , and $0.5 \pm 0.13\%$ in the untreated control, peripheral Puffer, gridded Puffer, and insecticide-treated plots, respectively. There was significantly less damage in insecticide-treated pistachios ($P < 0.0001$), but no significant differences in damage among the remaining treatments. The mean percentage of navel orangeworm damage by ranch ranged from 0.4 to 1.6%.

Comparison of Mating Disruption in Almonds with and without Insecticide Treatments in 2004. Over the 26-wk monitoring period, $1,287 \pm 110$ males (mean \pm SE) per trap were captured in four traps in an adjacent block of almonds, compared with 44 ± 21 males captured in five traps between treatment plots in the Latin square plot arrangement. Within the 16 plots, there were significant differences in the sum of males captured over the season between treatments ($F = 54.58$, $df = 3, 6$; $P < 0.0001$), but not between rows ($F = 2.38$, $df = 3, 6$; $P = 0.1688$) or columns ($F = 2.85$, $df = 3, 6$; $P = 0.1274$). There were significant difference in males

captured between mating disruption and nonmating disruption plots, but not between the untreated control and hullsplit insecticide treatment or between plots treated with mating disruption with or without a hullsplit insecticide treatment (Table 4).

For navel orangeworm damage to Nonpareil almonds at harvest, the ANOVA model was significant ($F = 10.21$, $df = 9, 6$; $P = 0.0052$). There were significant differences in the proportion of Nonpareil almonds with navel orangeworm damage between treatments ($F = 18.99$, $df = 3, 6$; $P = 0.0018$) and columns ($F = 8.12$, $df = 3, 6$; $P = 0.0156$), but not between rows ($F = 3.53$, $df = 3, 6$; $P = 0.0884$). The untreated control had significantly greater damage than all other treatments, there was no difference in Nonpareil damage between the hullsplit treatment with or without gridded Puffers, and the gridded Puffer treatment by itself had intermediate damage (Table 4).

For navel orangeworm damage to Carmel almonds at harvest the ANOVA model was significant ($F = 5.21$, $df = 9, 6$; $P = 0.0300$). There were significant differences in the proportion of Carmel almonds with navel orangeworm damage between treatments ($F = 9.75$, $df = 3, 6$; $P = 0.0101$), but not between columns ($F = 3.19$, $df = 3, 6$; $P = 0.1052$) or rows ($F = 2.40$, $df = 3, 6$; $P = 0.1666$). The plots treated with a hullsplit insecticide, with or without the gridded Puffer treatment, had less navel orangeworm damage than the untreated control, whereas there was no significant difference in damage to Carmel almonds between the gridded Puffer treatment by itself and the untreated control. The same pattern as for Carmel was seen in damage to Monterey almonds (Table 4). In that case, the overall model was significant ($F = 9.35$, $df = 9, 6$; $P = 0.0300$), and there were significant differences between treatments ($F = 19.60$, $df = 3, 6$; $P = 0.0017$), but not between columns ($F = 3.96$, $df = 3, 6$; $P = 0.0714$) or rows ($F = 4.50$, $df = 3, 6$; $P = 0.0559$).

Discussion

These data demonstrate that mating disruption can significantly reduce navel orangeworm damage in al-

Table 4. Latin square experiment in almonds in 2004: treatment, row, and column effects on males (mean \pm SE) captured in virgin female baited traps and navel orangeworm damage in Nonpareil (NP), Carmel (Ca), and Monterey (Mo) almonds at harvest

Factor	Level	Males per trap	% dmg NP	% dmg Ca	% dmg Mo
Treatment	Untreated	97.56 \pm 22.71a	6.46 \pm 1.19a	7.69 \pm 2.52a	10.58 \pm 1.86a
	Phosmet + permethrin	187.13 \pm 51.75a	3.00 \pm 0.17c	1.87 \pm 0.20b	4.81 \pm 0.69b
	Puffers	0.44 \pm 0.22b	4.52 \pm 1.04b	5.16 \pm 0.45a	9.52 \pm 1.69a
	Both	0.31 \pm 0.12b	2.46 \pm 0.27c	2.22 \pm 0.51b	3.79 \pm 0.63b
Row	Row 1 (north)	145 \pm 53	4.54 \pm 0.39	5.77 \pm 2.28	8.91 \pm 2.37
	Row 2	18 \pm 6	4.59 \pm 0.99	3.70 \pm 1.08	7.06 \pm 2.00
	Row 3	38 \pm 19	4.36 \pm 1.38	4.99 \pm 2.34	7.83 \pm 2.31
	Row 4 (south)	85 \pm 32	2.94 \pm 0.41	2.49 \pm 0.62	4.90 \pm 0.85
Column	Column 1 (west)	121 \pm 52	3.12 \pm 0.39b	2.44 \pm 0.71	5.26 \pm 0.79
	Column 2	13 \pm 5	5.31 \pm 1.52a	3.50 \pm 1.13	7.85 \pm 2.14
	Column 3	44 \pm 22	3.02 \pm 0.63b	5.04 \pm 2.41	6.57 \pm 2.25
	Column 4 (east)	107 \pm 33	4.98 \pm 1.22a	5.96 \pm 2.07	9.03 \pm 2.38

Means followed by different letters in the same column and level-within-factor grouping are significantly different ($P < 0.05$). Rows and columns are as shown in Fig. 2. There were no significant differences among row means of damage to Nonpareil, and among row and column means of damage to Carmel and Monterey.

monds. The first mating disruption experiment, in almonds in 2003, found mean reductions of navel orangeworm damage to Nonpareil almonds of 16% by peripheral Puffers and 37% by either hand-applied membranes or gridded Puffers. Because of high variation due to very low damage at three of the four sites, none of these differences were statistically significant. The third mating disruption experiment, in almonds in 2004, found a 31% reduction in navel orangeworm damage to Nonpareil almonds using gridded Puffers, and this difference was statistically significant.

The second mating disruption experiment, in pistachios in 2003, did not demonstrate reduction of navel orangeworm damage in pistachios treated with the mating disruption technique that was most effective in almonds (i.e., gridded Puffers). The trap data (males per replicate and males per plot in the untreated plots) suggest that males were significantly more abundant in the pistachio sites than in the almond sites that were examined in 2003. Other data indicate that this greater abundance in pistachios compared with almonds is a general trend in the southern San Joaquin Valley (our unpublished data). In contrast to the abundance data, navel orangeworm damage to pistachios, although economically important, is generally more limited in worse-case situations compared with almonds (B.S.H., unpublished data). Previous studies have commented on the relative susceptibility of Nonpareil almonds to navel orangeworm infestation (Soderstrom 1977), and lesser susceptibility of pistachios (Crane 1978). These observations suggest that navel orangeworm infestation in almonds, particularly Nonpareil, is due to a relatively susceptible crop exposed to moderate abundance, whereas in pistachios it is due to a less susceptible crop exposed to higher abundance. Mating disruption often works better with low initial abundance (Cardé and Minks 1995); therefore, this difference in the relationship between abundance and damage in the two crops suggests that using mating disruption for reduction of navel orangeworm damage in pistachios will be a greater challenge compared with almonds. However, the nonbearing period for pistachios (Klonisky et al. 1998) is greater than that for almonds (Kester and Asay 1975), and it is possible that use of mating disruption in pistachios beginning in the early years in the life of the orchard would prevent the development of a potentially damaging navel orangeworm population.

Peripheral placement of Puffers around control blocks has been suggested as a method of reducing treatment cost (Shorey and Gerber 1996). This study, like others (Shorey and Gerber 1996, Shorey et al. 1996, Burks and Brandl 2004), found that Puffers placed peripherally around 16-ha blocks significantly reduced males captured in traps baited with virgin females, thereby demonstrating interference with sexual communication. But the male trapping data from pistachios, where these treatments were challenged with higher abundance, shows 96% reduction of trap capture with peripheral Puffers versus 99% reduction with gridded Puffers, a significant difference. In 2003, sentinel females in the center position between the

plots were mated less frequently than those in the untreated control plot; the control plot was also farther from plots receiving mating disruption treatments. In 2004, the average of the mating disruption treatments with or without hullsplit insecticide (targeted against larvae) represented a 99.7% reduction in males captured versus non-MD treatments. By comparison, the traps between the treatment plots showed a 70% reduction, and the traps far outside the test area captured 9x more males. We recognize that these comparisons are complicated by unequal numbers and spacing of the between-plot and outside traps compared with those in the treatment plots. These data nonetheless suggest that male trap capture was depressed throughout the area of the 2004 Latin square experiment. The data from the Latin square experiment also suggested a pattern of fewer males captured in the inner treatment plots compared with the outer plots (i.e., rows and columns two and three versus one and 4), whereas no such pattern was evident in the damage data from these plots. These observations indicate that Puffers are able to reduce males captured in flight traps baited with virgin females and the proportion of sentinel females mated over greater distances than they are able to influence navel orangeworm damage to almonds. We conclude that the peripheral Puffer arrangement, as proposed previously (Shorey et al. 1996), should not be considered further.

Puffers and the hand-applied membrane dispensers used in this study represent very different ways of implementing mating disruption, with different advantages and disadvantages in terms of mechanism and equipment and labor costs (Sarfratz et al. 2006). The hand-applied dispensers are simple and relatively economic devices that release pheromone steadily at a continuously declining rate, as demonstrated in Fig. 3A. Logically, hand-applied devices might be more sensitive to temperature, which would explain the low emission rate estimates in April and the greater difference in emission rates between the sunny and shady sides of the tree before versus after 1 June. The method used in this study to estimate the emission rate examines the amount of active ingredient lost from the device, which may be greater than the amount emitted due to chemical instability or sequestration within the walls of the dispenser. Although there are analytical methods that account for this possibility by measuring the amount of active ingredient recovered from absorbent placed in an enclosed chamber with the dispenser (Mayer and Mitchell 1998), the present estimates serve to illustrate that the rate of emission of the hand-applied devices was more variable over the season compared with the Puffers.

Compared with the hand-held devices, Puffers are more complicated, expensive, and vulnerable to possible mechanical problems. They are also potentially more economical to place in the field because far fewer devices per ha are used. Because the Puffers were active only 12 h each day whereas hand-applied dispensers continuously emitted pheromone, and the number of dispensers per ha and amount of phero-

mone released per dispenser was vastly different between Puffers and hand-applied membrane, it was necessary and appropriate to compare release rates of membranes and Puffers by multiplying per-device hourly release rates by the number of devices per ha. Navel orangeworm mating activity occurs mostly within 2–3 h of the end of scotophase under warmer summer conditions, but begins earlier as temperature decreases (Landolt and Curtis 1982); thus, the hourly rate of pheromone dispensed during scotophase is of primary interest. During the period between dusk and dawn the mean emission rate per hectare per hour was greater in the plots treated with hand-applied devices compared with Puffers for the first 15 d after the first application and the first 27 d after the second, but in other times the amount of pheromone emitted dusk to dawn was greater in Puffers compared with hand-applied devices. A significant reduction in the amount of pheromone dispensed was noted in Puffers in late July and early August of 2004, but this was primarily due to problems with canisters and emission rates returned to normal when the canisters were replaced.

The inflection on the plot of cumulative males captured in untreated control plots indicates that the third flight began in the final week of July. For the next 2 wk, the cumulative number of males captured in plots treated with hand-applied devices was intermediate between that of gridded Puffers and that of peripheral Puffers. For the final three weeks of monitoring with traps baited with virgin females, more males were captured in the plots treated with hand-applied dispensers than in those treated with gridded Puffers. This final 3 wk coincides with the period after the Nonpareil harvest. The decreased suppression of males captured in female-baited traps in this period may indicate that the hand-applied devices were more susceptible to shaking and dust of harvest. (Puffers in Nonpareil trees were moved to an adjacent pollenizer row when their original tree was shaken for harvest.) However, the greater number of males captured in plots treated with hand-applied dispensers between the start of the third flight and the Nonpareil harvest suggests that the hand-applied dispensers did not interrupt sexual communication as effectively as the gridded Puffers, despite the sources being more densely distributed. The ability of gridded Puffers to disrupt chemical communication might have been due to mechanistic differences between the two methods of dispensing pheromone, or it might have been because the hourly release rate at night was lower at important points during the growing season.

The mechanisms involved in successful disruption of mating vary depending on delivery systems and species (Sarfaz et al. 2006) and may include point source competition, camouflage, neurophysiological effects, and shifting rhythms of diurnal response (Cardé and Minks 1995, Sarfaz et al. 2006). The former two mechanisms require an attractive blend, whereas the latter two do not (Cardé and Minks 1995, Sarfaz et al. 2006). For both neurophysiological effects and shifts in diurnal rhythm, it is conceivable that emission of pheromone only during the 12 h including scoto-

phase and not the entire diurnal cycle contributed to greater efficacy in Puffers versus the hand-held device, but further research is needed to clarify this point.

In mating disruption targeted against other species, sometimes less technically efficient formulations are used because they provide acceptable results in a manner that is more cost-efficient or more compatible with grower practices, e.g., use of microencapsulated formulations instead of hand-applied devices (Kovanci et al. 2005). Also, some practitioners use targeted rather than season-long control, by using mating disruption against one flight or generation, and residual insecticides against others (Gut et al. 2004). Treatment with hand-applied dispensers, whereas not suppressing males in female-baited traps as well as gridded Puffers in third flight, resulted in similar damage in almonds compared with gridded Puffers and numerically less damage than peripheral Puffers.

If hand-applied membranes and gridded Puffers result in similar damage, then it is likely that mating disruption treatments with membranes would be more economical and therefore more cost-effective. However, only gridded Puffers were examined further in 2004 because control of navel orangeworm in pollenizer varieties was considered as important as control in Nonpareil. These varieties are exposed to more of the third flight than Nonpareil, and the pheromone trap and mating assay data from the experiment in almonds in 2003 suggested that gridded Puffers disrupted chemical communication in the third flight more effectively than membrane dispensers. In the third mating disruption experiment, in almonds in 2004, gridded Puffers significantly reduced navel orangeworm damage in Nonpareil almonds but not in the pollenizer varieties Carmel or Monterey. Subsequent research suggests that reduction of damage in pollenizer varieties can be obtained using larger treatment blocks or a more complete pheromone blend (our unpublished data). Nonetheless, further research should examine the feasibility of targeted control with mating disruption for reduction of damage in almonds, by using hand-applied devices and/or Puffers starting shortly before the second flight.

In summary, we conclude that 1) mating disruption can significantly reduce navel orangeworm damage in Nonpareil almonds; 2) mating disruption, under the conditions in this study, does not seem promising for reducing navel orangeworm damage to pistachios in mature orchards with abundant navel orangeworm populations, but might help prevent abundant navel orangeworm populations if used from when pistachios first come into bearing; 3) the strategy of concentrating Puffers peripherally around large treatment blocks with no Puffers within the block results in greater damage than hand-applied dispensers or Puffers applied evenly throughout the block; and 4) the hand-applied membranes examined do not disrupt navel orangeworm sexual communication as well as Puffers after the start of the third flight, but may protect Nonpareil almonds as effectively as Puffers.

Acknowledgments

We thank David Brandl and Tom Larsen for assistance with key aspects of this work; Amanda Bulls, Michael Bryant, Jennifer Estrada, Reuben Larrios, Jose Madrigal, Maria Madrigal, and Lori Smith for technical support; and Ring Cardé, Larry Gut, Orkun Kovanci, L.P.S. Kuenen, Brendon Reardon, David Williams, and Frank Zalom for reviewing previous versions of this manuscript. The Almond Board of California and the California Pistachio Commission provided partial funding. Suterra LLC provided mating disruption dispensers, and Paramount Farming provided orchards for experiments.

References Cited

- Andrews, K. L., M. M. Barnes, and S. A. Josserand. 1980. Dispersal and oviposition by the navel orangeworm. *Environ. Entomol.* 9: 525–529.
- Agresti, A. 2007. An introduction to categorical data analysis, 2nd ed. Wiley, Hoboken, NJ.
- Bentley, W. J., W. J. Beede, K. M. Daane, T. J. Michailides, B. L. Teviotdale, and B. B. Westerdahl. 2003. UC IPM pest management guidelines: pistachio. Publication 3471. University of California Agriculture and Natural Resources, Oakland, CA.
- Burks, C. S., and D. G. Brandl. 2004. Seasonal abundance of navel orangeworm (Lepidoptera: Pyralidae) in figs and effect of peripheral aerosol dispensers on sexual communication. *J. Insect Sci.* 4: 40.
- Cardé, R. T., and A. K. Minks. 1995. Control of moth pests by mating disruption: successes and constraints. *Annu. Rev. Entomol.* 40: 559–585.
- Coffelt, J. A., K. W. Vick, P. E. Sonnet, and R. E. Doolittle. 1979. Isolation, identification and synthesis of a female sex pheromone of the navel orangeworm, *Ameylois transitella*. *J. Chem. Ecol.* 5: 955–966.
- Crane, J. C. 1978. Quality of pistachio nuts as affected by time of harvest. *J. Am. Soc. Hort. Sci.* 103: 332–333.
- Curtis, C. E. 1976. Economics of NOW control and implementing orchard cleanup. *Almond Facts* 41: 5–8.
- Curtis, C. E., R. K. Curtis, and K. L. Andrews. 1984. Progression of navel orangeworm (Lepidoptera: Pyralidae) infestation and damage of almonds on the ground and on the tree during harvest. *Environ. Entomol.* 13: 146–149.
- Curtis, C. E., P. J. Landolt, and J. D. Clark. 1985. Disruption of navel orangeworm (Lepidoptera: Pyralidae) mating in large-scale plots with synthetic pheromone. *J. Econ. Entomol.* 78: 1425–1430.
- Gut, L. J., L. L. Stelinski, D. R. Thomson, and J. R. Miller. 2004. Behaviour-modifying chemicals: prospects and constraints in IPM, pp. 73–121. In O. Koul, G. S. Dhaliwal and G. W. Cuperus [eds.], *Integrated pest management: potential, constraints and challenges*. CAB International, Wallingford, United Kingdom.
- Kester, D. E., and R. Asay. 1975. Almonds, pp. 387–419. In J. Janick and J. N. Moore [eds.], *Advances in fruit breeding*. Purdue University Press, West Lafayette, IN.
- Klonsky, K., P. Livingston, R. H. Beede, M. W. Freeman, L. Hendricks, B. Holtz, C. Kallsen, S. Sibbett, and L. Ferguson. 1998. Sample costs for establishing a pistachio orchard and producing pistachios. *Acta Hort.* 470: 481–492.
- Kovanci, O. B., C. Schal, J. F. Walgenbach, and G. G. Kennedy. 2005. Comparison of mating disruption with pesticides for management of oriental fruit moth (Lepidoptera: Tortricidae) in North Carolina apple orchards. *J. Econ. Entomol.* 98: 1248–1258.
- Landolt, P. J., and C. E. Curtis. 1982. Effects of temperature on the circadian rhythm of navel orangeworm sexual activity. *Environ. Entomol.* 11: 107–110.
- Leal, W. S., A.-L. Parra-Pedraza, K.-E. Kaissling, T. I. Morgan, F. G. Zalom, D. J. Pesak, E. A. Dundulis, C. S. Burks, and B. S. Higbee. 2005. Unusual pheromone chemistry in the navel orangeworm: novel sex attractants and a behavioral antagonist. *Naturwissenschaften* 92: 139–146.
- Mayer, M. S., and E. R. Mitchell. 1998. Rapid measure of sex pheromone emission from plastic rope dispensers: example of utility in sexual communication disruption of the diamondback moth, *Plutella xylostella*. *Phytoparasitica* 26: 117–125.
- Sarfraz, R. M., M. L. Evenden, B. A. Keddie, and L. M. Dossdall. 2006. Pheromone-mediated mating disruption: a powerful tool in insect management. *Outlooks Pest Manag.* 17: 36–45.
- SAS Institute. 2004. SAS/STAT 9.1 user's guide. SAS Institute, Cary, NC.
- Shorey, H. H., and R. G. Gerber. 1996. Use of puffers for disruption of sex pheromone communication among navel orangeworm moth (Lepidoptera: Pyralidae) in almonds, pistachios, and walnuts. *Environ. Entomol.* 25: 1154–1157.
- Shorey, H. H., C. B. Sisk, and R. G. Gerber. 1996. Widely separated pheromone release sites for disruption of sex pheromone communication in two species of Lepidoptera. *Environ. Entomol.* 25: 446–451.
- Soderstrom, E. L. 1977. Seal of almond shells and resistance to navel orangeworm. *J. Econ. Entomol.* 70: 467–468.
- Tebbetts, J. S., C. E. Curtis, and R. D. Fries. 1978. Mortality of immature stages of the navel orangeworm stored at 3.5°C. *J. Econ. Entomol.* 71: 875–876.
- Wade, W. H. 1961. Biology of the navel orangeworm, *Parameylois transitella* (Walker), on almonds and walnuts in northern California. *Hilgardia* 31: 129–171.
- Zalom, F. G., C. Pickel, W. J. Bentley, R. L. Coviello, R. A. Van Steenwyck, M. W. Freeman, W. D. Bublter, J. E. Adaskaveg, R. Duncan, J. J. Stapleton, et al. 2005. UC IPM Pest Management Guidelines: Almond. Publication 3431. University of California Agriculture and Natural Resources, Oakland, CA.
- Zar, J. H. 1999. *Biostatistical analysis*, 4th ed. Prentice Hall, Upper Saddle River, NJ.

Received 31 October 2007; accepted 9 May 2008.

STORED-PRODUCT

Response of Postharvest Tree Nut Lepidopteran Pests to Vacuum Treatments

J. A. JOHNSON¹ AND J. L. ZETTLER²

San Joaquin Valley Agricultural Sciences Center, USDA-ARS, 9611 South Riverbend Avenue, Parlier, CA 93648

J. Econ. Entomol. 102(5): 2003–2010 (2009)

ABSTRACT Industry concerns over insect resistance, regulatory action, and the needs of organic processors have renewed interest in nonchemical alternative postharvest treatments to fumigants used for California tree nuts. The development of inexpensive polyvinyl chloride containers capable of holding low pressures has increased the practicality of vacuum treatments for durable commodities such as tree nuts. To develop vacuum treatment protocols, we determined the relative tolerance to vacuum (50 mmHg) at 25 and 30°C of different life stages of three postharvest pests of tree nuts: codling moth, *Cydia pomonella* (L.), navel orangeworm, *Amyelois transitella* (Walker), and Indianmeal moth, *Plodia interpunctella* (Hübner). At both temperatures, nondiapausing codling moth larvae were the least tolerant stage tested. LT₉₅ values for diapausing Indianmeal moth larvae were similar to Indianmeal moth eggs at both temperatures. Indianmeal moth diapausing larvae and eggs were the most tolerant at 25°C, whereas navel orangeworm eggs were most tolerant at 30°C. Field tests using GrainPro Cocoons (GrainPro, Inc., Concord, MA) to treat shelled almonds, *Prunus dulcis* (Mill.) D.A. Webb, in bins at vacuum levels of 18–43 mmHg at average winter temperatures (6–10°C) showed that diapausing codling moth larvae were the most tolerant under these conditions and that exposures of 7–13 d provided incomplete control. Summer field tests treating in-shell almonds in bags at average temperatures of 25–30°C provided complete control with 48 h exposure to average vacuum levels of 50 mmHg, and navel orangeworm eggs were the most tolerant stage.

KEY WORDS vacuum treatment, tree nuts, codling moth, navel orangeworm, Indianmeal moth

The central valley of California produces nearly all of the almonds, *Prunus dulcis* (Mill.) D.A. Webb; pistachios, *Pistacia vera*; and walnuts (*Juglans* spp.) in the United States, resulting in an average annual production of >800,000 metric tons of commodity valued at ≈\$2.4 billion (USDA 2007). These three products are also among the top 10 California agricultural exports, bringing into the California economy an average of >\$1.5 billion each year (CDFA 2007). Almonds are currently the leading export for California and were responsible for nearly 20% (\$1.8 billion) of the unprecedented \$9.3 billion 2005 California export market (CDFA 2007).

A major problem in the storage and marketing of these products is infestation by a variety of postharvest insect pests. Of particular concern are field pests of possible phytosanitary importance such as navel orangeworm, *Amyelois transitella* (Walker), which infests all three nut crops, and codling moth, *Cydia pomonella* (L.), which is a major pest of walnuts. To avoid invasion by these pests, importing countries may

require phytosanitary inspections or treatments before allowing California nut products entrance to their markets. Also of concern is the cosmopolitan stored product pest Indianmeal moth, *Plodia interpunctella* (Hübner). Conversations with quality control managers within the tree nut industry in California indicate that Indianmeal moth is the most common reason for customer returns, which reflect the results found within south central U.S. grocery stores (Platt et al. 1998).

Currently, California tree nut processors depend on fumigation with methyl bromide or phosphine to disinfest large volumes of incoming product after harvest and to control infestations during storage. Regulatory actions against methyl bromide (UNEP 2006) as well as insect resistance to hydrogen phosphide (Benhalima et al. 2004), may make these fumigants costly or unavailable to the nut industry. In addition, as the organic industry expands the need for nonchemical postharvest insect control methods increases. These recent concerns over resistance to fumigants, regulatory action, and the pest management needs of the organic industry have generated a renewed interest in developing nonchemical alternative treatments.

One possible nonchemical alternative is the use of low atmospheric pressures (vacuum) to disinfest

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by USDA.

¹ Corresponding author, e-mail: jjohnson@fresno.ars.usda.gov.

² Retired; current address Raleigh, NC.

product. The lethal effects of vacuum were noted as early as the 17th century (Back and Cotton 1925). Numerous researchers have examined the potential of low pressures for insect control, including Back and Cotton (1925), Bare (1948), Calderon et al. (1966), Navarro and Calderon (1979), and Al-Azawi et al. (1983), but the need for sturdy vacuum chambers to treat product limited the utility of the method to relatively small-scale applications. Flexible polyvinyl chloride containers (known as Volcani Cubes or GrainPro Cocoons; GrainPro, Inc., Concord, MA) developed for temporary grain storage (Navarro and Donahaye 1985) also were found to have utility as vacuum treatment enclosures, making the treatment more economical and practical (Navarro et al. 2001). This study investigates the potential of vacuum for disinfecting postharvest tree nut pests by first determining the relative tolerance to low pressures of different life stages of target pests, particularly diapausing larval stages, which are often tolerant of other treatments such as fumigation (Bell 1977a,b; Bond 1984; Cox et al. 1984) and cold storage (Johnson 2007). Field tests were then conducted with almonds treated under vacuum in five MT GrainPro Cocoons.

Materials and Methods

Test Insects. All test insects were from laboratory cultures at the San Joaquin Valley Agricultural Sciences Center (SJVASC), Parlier, CA. The navel orangeworm culture was originally obtained from the University of California, Berkeley, in 1966, and the Indianmeal moth culture was originally obtained from a walnut packinghouse in Modesto, CA, in November 1967. The codling moth culture was obtained from an apple orchard near North Fork, CA, in September 1984.

Indianmeal moth and navel orangeworm were maintained on a wheat bran diet (Tebbetts et al. 1978); codling moth were reared on a lima bean-based agar diet (Tebbetts et al. 1986). Rearing conditions for navel orangeworm and nondiapausing Indianmeal moth were 28°C, 60% RH, and a photoperiod of 14:10 (L:D) h. Rearing conditions for nondiapausing codling moth were 27°C, 60% RH, and a photoperiod of 16:8 (L:D) h. Diapausing Indianmeal moth larvae were obtained by holding rearing jars recently infested with eggs under normal rearing conditions for 1 wk. The jars were then transferred first to an environmental chamber held at 17°C for 1 wk and then to another environmental chamber held at 14°C for at least 4 wk. Both the 17 and 14°C chambers were kept at a photoperiod of 10:14 (L:D) h. Under these conditions, larvae from our Indianmeal moth isolate uniformly entered diapause and were recognized by their color, behavior, and increased size (Tsuji 1958). Diapausing codling moth larvae were reared at 18°C, 60% RH, and a photoperiod of 8:16 (L:D) h (Tebbetts et al. 1986). Codling moth still in the larval stage 50 d after being placed in diapause conditions were assumed to be in diapause.

Eggs, nondiapausing larvae, and diapausing larvae of codling moth, and eggs and nondiapausing larvae of

navel orangeworm were chosen for study. Although there is some evidence that navel orangeworm has some form of diapause (Gal 1978, Legner 1983, Johnson et al. 2002), it is poorly understood; consequently, well-defined diapausing larvae were unavailable for study. Preliminary tests with codling moth and navel orangeworm indicated that pupae were far less tolerant to low pressures than larvae and were not included in our study (J.A.J., unpublished data). Adults of these two species also were excluded, because they are unlikely to be found in treated product. Because the response of most stages of the Indianmeal moth to low pressures has already been examined (Mbata and Phillips 2001), we selected diapausing larvae, which had not been previously tested, and eggs, which had been determined to be the most tolerant life stage for this species.

Laboratory Dose Response. Treatments were done in cylindrical, 45.7-cm-tall by 30.5-cm-diameter stainless steel vacuum chambers (Laco Technologies, Salt Lake City, UT). Chambers were closed with clear acrylic lids and gasket seals and held in an environmental room kept at either 25 or 30°C. Chambers were connected with vacuum hose in series to a vacuum pump (model D25, Precision Scientific, Winchester, VA) and simultaneously pumped down to the target pressure of 50 mmHg. Pressure levels were determined with an absolute pressure capsule gauge (CG-100, Becker Pumps Co., Cuyahoga Falls, OH). Once the chamber pressures reached 50 mmHg (45–60 min), individual chambers were isolated and the treatment was considered to have started. Pressure levels were monitored and readjusted two to three times to 50 mm during the first 2 h of the treatment, after which time pressures remained stable for the remainder of the test.

Test larvae were treated in 18- by 63-mm tubes made from 32 mesh stainless steel screen. Five tubes containing 10–15 fifth instar diapausing or nondiapausing larvae were used for each treatment exposure. Strips of corrugated cardboard were added to tubes with nondiapausing codling moth larvae to give them harborage and reduce cannibalism. Because codling moth was likely to chew into and seek refuge within cork stoppers, tubes containing nondiapausing codling moth were closed with neoprene stoppers. Tubes containing all other test larvae were closed with cork stoppers. Nondiapausing larvae were placed in tubes just before treatment. Because diapausing larvae were shown to be more susceptible to methyl bromide fumigation after being disturbed (Tebbetts et al. 1986), diapausing larvae of both species were placed in tubes and held at 14°C and 10:14 (L:D) h \approx 1–3 wk before treatment.

Codling moth, Indianmeal moth and navel orangeworm eggs were held under rearing conditions (28°C) and treated when 6–30, 6–54, and 30–54 h old, respectively. Test eggs were treated in 9.0-cm plastic petri dishes. Strips of ovipositional substrate containing codling moth or navel orangeworm eggs were attached to the bottom of a petri dish with double stick tape, whereas Indianmeal moth eggs were placed di-

rectly on removable double stick tape attached to card stock which was fixed to the bottom of the petri dish. One dish containing ≈ 100 eggs of each species was used for each treatment exposure.

Preliminary studies showed that eggs and diapausing larvae were more tolerant than nondiapausing larvae, so the latter were treated separately with a different series of exposures. Five exposures were used for all life stages and temperatures. Exposures for nondiapausing larvae were from 6 to 30 h with 6-h intervals at 25°C and from 6 to 14 h with 2-h intervals at 30°C. Exposures for all other life stages were from 12 to 72 h with 12-h intervals at 25°C and 8–24 h with 4-h intervals at 30°C. Preliminary studies also indicated that the presence of product could affect efficacy of vacuum treatments, so treatment chambers contained ≈ 3.5 kg (≈ 7.5 liter) of in-shell walnuts at ≈ 3.3 –4.4% kernel moisture. Tubes, petri dishes, or both with test insects were placed on top of the walnuts during treatment, with one chamber used for each exposure. Control insects were held in a chamber not connected to the vacuum pump and removed after the longest vacuum treatment exposure was completed.

Larvae were removed from tubes and evaluated for mortality ≈ 24 h after treatment. Larvae were considered to be alive if they responded to a light probe. Approximately 3 g of wheat bran diet was added to petri dishes containing eggs immediately after treatment to provide food for newly hatched larvae and prevent cannibalization. Dishes were held for at least 10 d after treatment before examination under a dissecting microscope to count hatched and unhatched eggs. All treatments were replicated three to five times.

Field Trials. Two series of field trials were conducted using five M/T V-HF Cocoons (GrainPro, Inc., Concord, MA), airtight rectangular structures made of UV-resistant polyvinyl chloride. The structures consist of top and bottom pieces joined together with a tongue-and-groove zipper similar to Ziploc bag closures. When filled to capacity, the cocoons were 1.5 m high by 2.95 m long by 1.7 m wide. Vacuum pressures were obtained with a 3-hp rotary, oil-lubricated vacuum pump (U-4-70, Becker Pumps Co.) connected to the Cocoon with a 4.5-m, 44-mm diameter metal reinforced polyvinyl chloride vacuum hose with quick disconnect. A bellows type pressure control switch (Bulletin 836T, Rockwell Automation, Milwaukee, WI) on the pump was available to maintain the pressure level within the treatment Cocoon. Pressures within the Cocoon were monitored with an absolute pressure capsule gauge. In all tests, pressure levels in the Cocoons were below 100 mmHg 10–30 min after evacuation began.

The first series of trials was done using shelled almonds in 1.2- by 1.2- by 1.2-m wooden bins. Two bins of almonds were placed in each of two Cocoons. The bins in one Cocoon were completely filled with almonds (≈ 1 MT) and served as treatments. The bins in the second Cocoon were partially filled (≈ 800 kg) and were used as untreated controls. Test insects and stages used were Indianmeal moth and navel orange-

worm eggs, diapausing Indianmeal moth, and codling moth larvae, and nondiapausing navel orangeworm larvae. Although codling moth is a pest of walnuts and not of almonds, they were included in these field tests because almonds were the only product available. Except for Indianmeal moth eggs, all test insects were placed in 240-ml plastic cups with snap-on lids. A 22-mm-diameter hole was cut into each of the lids and organically cloth was taped over the hole to permit ventilation. Cups were filled with almond kernels and ≈ 50 test larvae of a single species or a strip of paper with 50–100 navel orangeworm eggs were added. Diapausing codling moths were added as cocooned larvae within corrugated cardboard strips. Diapausing larvae of Indianmeal moth and codling moth were added to the cups and held under diapause conditions ≈ 1 wk before treatment. Indianmeal moth eggs (≈ 75 –100) were treated in 18-ml glass vials with screen-centered caps. Four cups (one each for navel orangeworm larvae, navel orangeworm eggs, diapausing Indianmeal moth larvae, and diapausing codling moth larvae,) a vial with Indianmeal moth eggs, and a temperature and humidity data logger (Onset Computer, Bourne, MA) were placed together in plastic mesh bags and buried ≈ 30 cm below the surface of the almonds. Two bags were buried in each of the bins in both treatment and control Cocoons.

Corrugated cardboard was taped around the edges and corners of the bins, and Styrofoam sheeting was placed around the bins to fill out and protect the Cocoon. Both treatment and control Cocoons were sealed, but only the treatment Cocoon was attached to the vacuum pump. Because the tests were conducted during November and December, when maximum outside temperatures in the central valley of California averaged ≈ 17 and 12°C, respectively, lengthy exposures were necessary. Three tests were done with exposures of 168, 216, and 312 h (7, 9, and 13 d). The vacuum pump was allowed to run continuously during the treatments, and the resulting pressure levels, measured by the capsule gauge, were recorded periodically throughout the tests. In all three tests, the average pressure was well below the target of 50 mmHg (see Table 3). After each treatment, the Cocoons were opened and test insects were removed and held at rearing conditions for evaluation. Larvae were evaluated for mortality 1–7 d after treatment. Eggs were placed in Petri dishes containing ≈ 3 g of wheat bran diet and held at least 10 d before being examined under a dissecting microscope to count the numbers of hatched and unhatched eggs.

A second series of trials was done using in-shell almonds in 22.7-kg woven polypropylene bags (polybags). In the treatment Cocoon, 82 polybags ($\approx 1,860$ kg of almonds) were stacked to fill the Cocoon. Because of a limited supply of product, only 30 polybags (680 kg) of almonds were used in the control Cocoon, which was not sealed. Both Cocoons were under portable canopies to provide shade.

Test insects and stages used were Indianmeal moth and navel orangeworm eggs, and diapausing Indianmeal moth and codling moth larvae. All test insects

Table 1. Lethal times (hours) for Indianmeal moth, codling moth, and navel orangeworm life stages exposed to 50 mmHg at 25°C

Stage	n	Slope ± SE	LT ₅₀	95% CI		LT ₉₅	95% CI	
				Lower	Upper		Lower	Upper
Indianmeal moth								
Eggs	3,415	5.27 ± 0.213	16.9 c	15.3	18.5	34.8 de	31.2	40.0
Diapausing larvae	2,171	7.88 ± 0.483	21.9 e	20.7	23.1	35.5 e	33.5	38.2
Codling moth								
Eggs	3,662	6.57 ± 0.443	15.0 b	12.7	17.0	26.7 b	23.8	31.1
Diapausing larvae	2,127	6.30 ± 0.306	15.8 b	14.6	17.0	28.9 bc	26.4	32.4
Nondiapausing larvae	1,774	6.90 ± 0.849	10.4 a	7.9	11.8	18.1 a	16.4	21.8
Navel orangeworm								
Eggs	2,831	11.37 ± 0.994	23.2 f	21.4	24.5	32.4 d	30.0	37.5
Nondiapausing larvae	1,781	9.07 ± 0.425	19.2 d	17.8	20.4	29.1 c	26.7	33.1

Values in the same column with different letters are significantly different ($P < 0.05$; lethal dose ratio test).

were treated in the stainless steel screen tubes described above. Test tubes were placed along with ≈ 0.5 kg of in-shell almonds into plastic mesh bags. Into each mesh bag were placed three tubes with strips of paper containing 50–100 navel orangeworm eggs, three tubes with ≈ 100 Indianmeal moth eggs attached with double-stick tape to strips of card stock, and five tubes each with 10–15 diapausing Indianmeal moth or co-cooned diapausing codling moth larvae in corrugated cardboard strips. Diapausing larvae were placed in the tubes 1–4 wk before treatment. A single mesh bag with test insects were placed in each of two polybags in the treatment cocoon, whereas one mesh bag was placed in a polybag in the control Cocoon. Temperature and relative humidity data loggers were placed within one of the mesh bags in both the control and treatment Cocoon. The polybags were then closed with large binder clips, the treatment Cocoon was sealed and the treatment was begun. Pressures were again monitored periodically using a capsule gauge.

Because California central valley average temperatures in June and July are ≈ 33 and 36°C , respectively, treatment exposures were much shorter than in the first series of tests. Exposures of 48, 30, and 24 h were used. After treatment, test insects were removed and evaluated as described above. In the 48- and 30-h treatments, we attempted to use the pressure controller to maintain the pressure in the treatment Cocoon at ≈ 50 mmHg, but because the differential was very large, pressures ranged from 35 to 120 mmHg, and made it difficult to calculate an average pressure. In the 24-h treatment, a small leak resulted in pressures of only 75 mmHg, with the pump running continuously.

Data Analysis. All laboratory mortality data were analyzed using the probit procedure in PoloPlus 2.0 (Robertson et al. 2003) after a log transformation of exposures. Lethal exposure times for 50 and 95% mortality (LT₅₀ and LT₉₅) were estimated for each species and stage. Estimated exposure times were compared among all life stages and species at each temperature by using the lethal-dose ratio test in PoloPlus 2.0 (Robertson et al. 2003, 2007). For field trials, mortality for each species and life stage was calculated based on the total number of test insects used.

Results

Laboratory Dose Response. Results from the probit analysis at 25°C including lethal dose ratio tests for LT₅₀ and LT₉₅ are given in Table 1. For the purposes of predicting which stages may be most tolerant to vacuum treatments, the results for LT₉₅ may be most useful. LT₉₅ values for diapausing Indianmeal moth larvae and Indianmeal moth eggs (35.5 and 34.8 h, respectively) were similar and were significantly higher than most other stages. The LT₉₅ for navel orangeworm eggs (32.4 h) was similar to Indianmeal moth eggs but significantly less than diapausing Indianmeal moth larvae ($P < 0.05$). Nondiapausing navel orangeworm larvae were also tolerant to vacuum, with an LT₉₅ of 29.1 h, similar to that of diapausing codling moth larvae (28.9 h). The LT₉₅ for codling moth eggs (26.7 h) was similar to that of diapausing codling moth larvae, but significantly less ($P < 0.05$) than nondiapausing navel orangeworm larvae. The stage most susceptible to vacuum was nondiapausing codling moth larvae, with the estimated LT₉₅ (18.1 h) significantly lower than all other stages ($P < 0.05$).

As expected, estimated LT₉₅ values for all life stages were lower at 30°C (Table 2). At the higher temperature, navel orangeworm eggs proved to be the most tolerant stage, with an LT₉₅ (22.7 h) value significantly higher than all other stages (lethal-dose ratio test; $P < 0.05$). Codling moth eggs were the next most tolerant stage at the LT₉₅ response level (19.8 h). LT₉₅ values for Indianmeal moth eggs (17.9 h) and diapausing larvae (17.0 h) were similar but were significantly less ($P < 0.05$) than codling moth eggs. LT₉₅ values for diapausing codling moth larvae and nondiapausing navel orangeworm larvae (14.7 and 15.4 h, respectively) were similar, and the LT₉₅ value for nondiapausing codling moth larvae (12.4 h) was again significantly lower ($P < 0.05$) than all other stages.

Field Trials. In the first series of field trials (Table 3), temperatures within the Cocoons were quite low, averaging 10.5, 8.9, and 6.3°C for the 168-, 216-, and 312-h treatments, respectively. Because ambient temperatures were so low, we extended the treatment exposures to compensate. This also resulted in relatively low treatment pressures (43.1, 38.5, and 17.7

October 2009

JOHNSON AND ZETTLER: VACUUM TREATMENTS FOR NUT PESTS

2007

Table 2. Lethal times (hours) for Indianmeal moth, codling moth, and navel orangeworm life stages exposed to 50 mmHg at 30°C

Stage	n	Slope ± SE	LT ₅₀	95% CI		LT ₉₅	95% CI	
				Lower	Upper		Lower	Upper
Indianmeal moth								
Eggs	2,089	7.40 ± 0.36	10.7 d	10.0	11.4	17.9 c	16.7	19.5
Diapausing larvae	1,322	10.56 ± 0.71	11.8 e	11.2	12.4	17.0 c	16.0	18.4
Codling moth								
Eggs	2,140	8.09 ± 0.74	12.4 e	10.5	13.6	19.8 d	18.2	22.6
Diapausing larvae	1,257	7.53 ± 0.50	8.9 b	7.4	10.0	14.7 b	12.7	19.3
Nondiapausing larvae	1,454	7.23 ± 0.41	7.3 a	6.6	7.9	12.4 a	11.2	14.5
Navel orangeworm								
Eggs	1,894	11.38 ± 0.69	16.3 f	14.8	17.4	22.7 e	21.0	26.0
Nondiapausing larvae	1,770	8.40 ± 0.37	9.8 c	8.9	10.7	15.4 b	13.5	19.7

Values in the same column with different letters are significantly different ($P < 0.05$; lethal dose ratio test).

mmHg) because the pump was run continuously during the treatment.

The effect of the low ambient temperatures can be seen in the high control mortality levels in the egg stages found in the 216- and 312-h exposures. Consequently, the response of eggs to the vacuum treatments could not be determined. The low temperatures had little effect on the larval stages, as shown by the relatively low control mortalities for these stages. Diapausing larvae of both species were more tolerant than nondiapausing navel orangeworm larvae; no survival of navel orangeworm larvae occurred in any of the three tests. Mortality of diapausing Indianmeal moth larvae in the 168- and 216-h treatments (96.8 and 88.3%, respectively) was considerably higher than that for diapausing codling moth (75.1 and 1.5%, respectively). In the 312-h treatment, a single diapausing codling moth larva survived; 100% mortality was observed for all other species and life stages.

Temperatures within the Cocoons were much higher during the second series of field trials (Table 4), averaging 29.5, 27.0, and 25.0°C for the 48-, 30-, and 24-h treatment, respectively. Because of the higher ambient temperatures, treatment exposures were shortened considerably. Control mortality for most stages was acceptable with the exception of the egg stages during the 48-h treatment. We believe temper-

atures were again responsible for this high control mortality, as maximum temperatures reached 40°C during this treatment.

We obtained 100% mortality of all test insects in the 48-h treatment. The 30-h treatment, done at slightly cooler temperatures, resulted in nearly 100% mortality, with only a small number (eight) of navel orangeworm eggs successfully hatching. The 24-h treatment, done at a higher pressure level (≈ 75 mmHg), produced relatively low mortality in diapausing larvae (25.7 and 21.1% for Indianmeal moth and codling moth, respectively) but relatively high mortality for eggs (97.0 and 96.8% for Indianmeal moth and codling moth, respectively).

Discussion

Earlier work on the response of various stored product insects to low pressures has shown that eggs are commonly the most tolerant stage (Bare 1948; Al-Azawi et al. 1983; Mbata and Phillips 2001; Finkelman et al. 2003, 2004). The response to low pressures of diapausing stages, often the most tolerant to fumigants (Bell 1977a,b; Bond 1984; Cox et al. 1984), have not been studied. Results from our laboratory tests show that at the higher mortality level (LT₉₅) the response of diapausing Indianmeal moth larvae is similar to that

Table 3. Mortality of test insects in vacuum-treated shelled almonds held in wooden bins during winter field trials

Temp (°C), mean (max-min.)	Pressure (mmHg)	Exposure (h)	Target insect	Treated		Control	
				n	% mortality	n	% mortality
10.5 (9.0–22.5)	43.1	168	Indianmeal moth eggs	271	97.4	340	17.6
			Indianmeal moth diapausing larvae	187	96.8	165	2.4
			Navel orangeworm eggs	198	99.0	208	46.2
			Navel orangeworm larvae	200	100.0	77	13.0
			Codling moth diapausing larvae	189	75.1	188	1.6
8.9 (8.2–12.2)	38.5	216	Indianmeal moth eggs	325	100.0	360	100.0
			Indianmeal moth diapausing larvae	196	88.3	197	1.0
			Navel orangeworm eggs	399	100.0	434	98.8
			Navel orangeworm larvae	202	100.0	147	0.7
			Codling moth diapausing larvae	196	1.5	210	0.5
6.3 (5.0–8.6)	17.7	312	Indianmeal moth eggs	404	100.0	389	100.0
			Indianmeal moth diapausing larvae	201	100.0	195	2.6
			Navel orangeworm eggs	411	100.0	408	100.0
			Navel orangeworm larvae	210	100.0	201	4.0
			Codling moth diapausing larvae	195	99.5	192	0.5

Table 4. Mortality of test insects in vacuum-treated in-shell almonds held in woven polypropylene bags during summer field trials

Temp. (°C) mean (max-min.)	Pressure (mmHg)	Exposure (h)	Target insect	Treated		Control	
				<i>n</i>	% mortality	<i>n</i>	% mortality
29.5 (19.0–40.0)	50 ^a	48	Indianmeal moth eggs	594	100.0	286	63.3
			Indianmeal moth diapausing larvae	90	100.0	30	3.3
			Navel orangeworm eggs	71	100.0	21	71.4
27.0 (22.0–34.0)	50 ^a	30	Codling moth diapausing larvae	139	100.0	71	0.0
			Indianmeal moth eggs	690	100.0	346	2.3
			Indianmeal moth diapausing larvae	145	100.0	70	0.0
			Navel orangeworm eggs	434	98.2	145	17.9
25.0 (21.0–29.0)	75	24	Codling moth diapausing larvae	132	100.0	70	0.0
			Indianmeal moth eggs	595	97.0	329	2.7
			Indianmeal moth diapausing larvae	140	25.7	70	0.0
			Navel orangeworm eggs	689	96.8	330	16.7
			Codling moth diapausing larvae	139	21.1	65	1.5

^a Average pressures are approximate – pressure ranged from 35 to 120 mmHg.

of Indianmeal moth eggs at 25 and 30°C, whereas codling moth diapausing larvae and eggs are similar at 25°C but eggs are more tolerant at 30°C. Furthermore, our winter field studies indicate that diapausing larvae of both species are tolerant to vacuum treatments at temperatures low enough to be lethal to eggs. Diapausing codling moth larvae in particular were difficult to kill in field trials at low temperatures.

Mbata et al. (2004) showed that younger Indianmeal moth eggs are more tolerant to vacuum than older eggs and suggests that our study may be underestimating the lethal times. However, because egg development is strongly affected by temperature, it is difficult to directly compare egg age between studies. Mbata et al. (2004) ended treatment of Indianmeal moth eggs at 48 h, just before hatch, but under our laboratory conditions, eggs did not hatch until ≈72 h (Johnson and Wofford 1991, Johnson et al. 1995). We also treated all test insects with product (in-shell walnuts), whereas Mbata et al. (2004) did not include product. We have found that the moisture content of product may have an effect on insect mortality during vacuum treatments (Johnson, unpublished data).

The mode of action of low pressure treatments has been shown to be largely due to low oxygen tensions at high humidities (Navarro and Calderon 1979). Diapausing stages are normally characterized as having reduced respiration and oxygen demands and are more tolerant to low oxygen environments (Kukul et al. 1991). As such, diapausing stages also should be more tolerant of the low oxygen environment found in vacuum treatments. Insect mortality under low pressures is increased at low humidities by increasing the moisture loss normally found under reduced oxygen environments (Navarro 1978). Life stages that are tolerant to cold are often tolerant to desiccation as well (Ring and Danks 1994, Appel et al. 1999). This characteristic also may give them tolerance to low pressures. Although Indianmeal moth eggs are less cold tolerant than other stages (Johnson 2007), egg hatch is unaffected by humidity (Morrison and Crawford 1970), indicating that eggs are tolerant of desiccation. This may partially explain the relative tolerance of eggs to low pressures.

Because of the variable treatment pressures and temperatures experienced in the field, it is difficult to compare results from our field tests with our lab studies. Although relative mortality of life stages in field tests was similar to that found in the lab, there were inconsistencies. During the winter tests with shelled almonds in bins, diapausing codling moth survival was consistently higher than diapausing Indianmeal moth larvae, whereas response of diapausing codling moth in the lab was consistently lower than diapausing Indianmeal moth larvae. This suggests that diapausing codling moth may be more tolerant than Indianmeal moth at temperatures lower than those studied in the lab. In the summer treatments of bagged in-shell almonds, the only survival after the 30-h treatment was navel orangeworm eggs, the stage identified as most tolerant in the lab at 30°C. However, mortality of both diapausing codling moth and diapausing Indianmeal moth was considerably lower than either Indianmeal moth or navel orangeworm eggs in the 24 h treatment. This treatment was anomalous in that the pressure never dropped below 75 mmHg and may account for the apparent discrepancy.

The development of inexpensive flexible containers has increased the practicality of vacuum treatments for durable commodities such as tree nuts. Our summer field tests showed good levels of control after 1.5–2 d treatments at temperatures of 27–30°C. This is an improvement over modified atmosphere treatments, also suggested as an alternative to fumigation and similar to vacuum treatments in mode of action, which require exposures of 3–7 d, in addition to initial purging (Kader 1996). Johnson et al. (2002) used a 6-d treatment after a 2-d purge to O₂ levels of 0.4% to control navel orangeworm and raisin moth in almonds and raisins. Summer vacuum treatments also compare favorably to phosphine, where recommended exposures for almonds are 2–3 d plus aeration (Nelson et al. 1980, Kader 1996). Results from our winter field tests show vacuum to be slightly less favorable compared with phosphine at low temperatures. We achieved incomplete control after a 7-d exposure to vacuum at average temperatures of 10°C, whereas a 5-d exposure is recommended for phosphine at 10°C (Bond 1984).

October 2009

JOHNSON AND ZETTLER: VACUUM TREATMENTS FOR NUT PESTS

2009

Successful treatments also may require some experience in loading, sealing, and protecting the Cocoons. We were able to treat shelled nuts in wooden bins only after completely filling the bins to prevent the sides from buckling under the pressure. To avoid rodent damage to the Cocoons, it is recommended that sides of the Cocoons be pulled taut with straps, reducing folds of material at points of contact with the floor (Donahaye et al. 1991). In spite of taking these precautions, we found during our summer tests that rodents were able to travel under the Cocoons down a small groove between the stacked bags, and eventually succeeded in chewing holes in the material.

Acknowledgments

We thank Karen Valero and Richard Gill (USDA-ARS, Parlier, CA) for assistance in conducting this research and David Brandl (USDA-ARS, Parlier, CA) for technical advice. We also thank James Hansen (USDA-ARS Wapato, WA, retired) and Lisa Neven (USDA-ARS Wapato, WA) for reviewing the manuscript.

References Cited

- Al-Azawi, A. F., H. S. El-Haidari, H. M. Al-Saud, and F. M. Aziz. 1983. Effect of reduced atmospheric pressure with different temperatures in *Ephestia cautella*, a pest of stored dates in Iraq. *Date Palm J.* 2: 223-233.
- Appel, A. G., W. J. Moar, and M. J. Tanley. 1999. Water loss and mortality of adult cowpea weevils (Coleoptera: Bruchidae) exposed to desiccants and desiccating environments. *Environ. Entomol.* 28: 979-982.
- Back, E. A., and R. T. Cotton. 1925. The use of vacuum for insect control. *J. Agric. Res.* 31: 1035-1041.
- Bare, C. O. 1948. The effect of prolonged exposure to high vacuum on stored-tobacco insects. *J. Econ. Entomol.* 41: 109-110.
- Bell, C. H. 1977a. Tolerance of the diapausing stages of four species of Lepidoptera to methyl bromide. *J. Stored Prod. Res.* 13: 119-127.
- Bell, C. H. 1977b. Toxicity of phosphine to the diapausing stages of *Ephestia elutella*, *Plodia interpunctella* and other Lepidoptera. *J. Stored Prod. Res.* 13: 149-158.
- Benhalima, H., M. Q. Chaudhry, K. A. Mills, and N. R. Price. 2004. Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. *J. Stored Prod. Res.* 40: 241-249.
- Bond, E. J. 1984. Manual of fumigation for insect control. FAO Plant Production and Protection Paper 54. (<http://www.fao.org/docrep/X5042E/x5042E00.htm#Contents>).
- Calderon, M., S. Navarro, and E. Donahaye. 1966. The effect of low pressures on the mortality of six stored-product insect species. *J. Stored Prod. Res.* 2: 135-140.
- [CDFA] California Department of Food and Agriculture. 2007. California Agricultural Resource Directory 2006. California Department of Food and Agriculture, Sacramento, CA. (<http://www.cdfa.ca.gov/Statistics.html>).
- Cox, P. D., Bell, C. H., J. Pearson, and M. A. Beirne. 1984. The effect of diapause on the tolerance of larvae of *Ephestia kuehniella* to methyl bromide and phosphine. *J. Stored Prod. Res.* 20: 215-219.
- Donahaye, E., S. Navarro, A. Ziv, Y. Blauschild, and D. Weasinghe. 1991. Storage of paddy in hermetically sealed plastic liners in Sri Lanka. *Trop. Sci.* 31: 109-121.
- Finkelman, S., S. Navarro, M. Rindner, R. Dias, and A. Azrieli. 2003. Effect of low pressures on the survival of cocoa pests at 18°C. *J. Stored Prod. Res.* 39: 423-431.
- Finkelman, S., S. Navarro, M. Rindner, R. Dias, and A. Azrieli. 2004. Effect of low pressures on the survival of three cocoa pests at 30°C. *J. Stored Prod. Res.* 40: 499-506.
- Gal, A. 1978. Der Einfluss der Temperatur auf die Fruchtbarkeit, Entwicklungs- und Überlebensrate von *Paramyelois transitella* (Lep.: Pyralidae). *Mitt. Dtsch. Ges. Allg. Angew. Entomol.* 1: 265-269.
- Johnson, J. A. 2007. Survival of Indianmeal moth and navel orangeworm (Lepidoptera: Pyralidae) at low temperatures. *J. Econ. Entomol.* 100: 1482-1488.
- Johnson, J. A., and P. L. Wofford. 1991. Effects of age on response of eggs of Indianmeal moth and navel orangeworm (Lepidoptera: Pyralidae) to subfreezing temperatures. *J. Econ. Entomol.* 84: 202-205.
- Johnson, J. A., P. K. Wofford, and R. F. Gill. 1995. Developmental thresholds and degree-day accumulations of Indianmeal moth (Lepidoptera: Pyralidae) on dried fruits and nuts. *J. Econ. Entomol.* 88: 734-741.
- Johnson, J. A., P. V. Vail, D. G. Brandl, J. S. Tebbets, and K. A. Valero. 2002. Integration of nonchemical treatments for control of postharvest pyralid moths (Lepidoptera: Pyralidae) in almonds and raisins. *J. Econ. Entomol.* 95: 190-199.
- Kader, A. A. 1996. In-plant storage, pp. 274-277. In W. C. Micke [ed.], *Almond production manual*. University of California, Division of Agricultural and Natural Resources, Oakland, CA.
- Kukal, O., D. L. Denlinger, and R. E. Lee. 1991. Developmental and metabolic changes induced by anoxia in diapausing and non-diapausing flesh fly pupae. *J. Comp. Physiol. B* 160: 683-689.
- Legner, E. F. 1983. Patterns of field diapause in the navel orangeworm (Lepidoptera: Phycitidae) and three imported parasites. *Ann. Entomol. Soc. Am.* 76: 503-506.
- Mbata, G. N., and T. W. Phillips. 2001. Effects of temperature and exposure time on mortality of stored-product insects exposed to low pressure. *J. Econ. Entomol.* 94: 1302-1307.
- Mbata, G. N., T. W. Phillips, and M. Payton. 2004. Mortality of eggs of stored-product insects held under vacuum: effects of pressure, temperature, and exposure time. *J. Econ. Entomol.* 97: 695-702.
- Morrison, W. P., and C. S. Crawford. 1970. Effects of relative humidity and parental decapitation on the eggs of *Plodia interpunctella* (Hübner) (Lepidoptera: Phycitidae). *J. Stored Prod. Res.* 6: 39-43.
- Navarro, S. 1978. The effects of low oxygen tensions on three stored-product insect pests. *Phytoparasitica* 6: 51-58.
- Navarro, S., and M. Calderon. 1979. Mode of action of low atmospheric pressures on *Ephestia cautella* (Wlk.) pupae. *Experientia* 35: 620-621.
- Navarro, S., J. E. Donahaye, R. Dias, A. Azrieli, M. Rindner, T. Phillips, R. Noyes, P. Villers, T. Debruin, R. Truby, and R. Rodriguez. 2001. Application of vacuum in a transportable system for insect control, pp. 308-315. In *Proceedings, International Conference of Controlled Atmospheres and Fumigation in Stored Products*, 29 October-3 November 2000, Fresno, CA, Executive Printing Services, Clovis, CA.
- Navarro, S., and E. Donahaye. 1985. Plastic structures for temporary storage of grain, pp. 189-194. In *Proceedings, 8th ASEAN Technical Seminar on Grain Post-Harvest Technology*, 6-9 August 1985, Manila, Philippines, Laguna College, San Pablo City, Philippines.

- Nelson, H. D., W. W. Barnett, and C. A. Ferris. 1980. Fumigation of in-hull almonds on the farm. University of California Cooperative Extension, Fresno, CA.
- Platt, R. R., G. W. Cuperus, M. E. Payton, E. L. Bonjour, and K. N. Pinkston. 1998. Integrated pest management perceptions and practices and insect populations in grocery stores in south-central United States. *J. Stored Prod. Res.* 34: 1–10.
- Ring, R. A., and H. V. Danks. 1994. Desiccation and cryo-protection: overlapping adaptations. *Cryo Lett* 15: 181–190.
- Robertson, J. L., H. K. Preisler, and R. M. Russell. 2003. PoloPlus: probit and logit analysis user's guide. LeOra Software, Petaluma, CA.
- Robertson, J. L., R. M. Russell, H. K. Preisler, and N. E. Savin. 2007. Bioassays with arthropods, 2nd ed. CRC Press, Boca Raton, FL.
- Tebbets, J. S., C. E. Curtis, and R. D. Fries. 1978. Mortality of immature stages of the navel orangeworm stored at 3.5°C. *J. Econ. Entomol.* 71: 875–876.
- Tebbets, J. S., P. V. Vail, P. L. Hartsell, and H. D. Nelson. 1986. Dose/response of codling moth (Lepidoptera: Tortricidae) eggs and nondiapausing and diapausing larvae to fumigation with methyl bromide. *J. Econ. Entomol.* 79: 1039–1043.
- Tsuji, H. 1958. Studies on the diapause of the Indian-meal moth, *Plodia interpunctella* Hübner. I. The influence of temperature on the diapause, and the type of diapause. *Jpn. J. Appl. Entomol. Zool.* 2: 17–23.
- [UNEP] United Nations Environmental Programme. 2006. Handbook for the Montreal Protocol on Substances that Deplete the Ozone Layer. UNEP Ozone Secretariat, Nairobi, Kenya. (http://ozone.unep.org/Publications/Handbooks/MP_Handbook_2006.pdf).
- [USDA] United States Department of Agriculture. 2007. Agricultural statistics. U.S. Dep. Agric., Washington, D.C. (http://www.nass.usda.gov/Publications/Ag_Statistics/index.asp).

Received 1 April 2009; accepted 17 July 2009.

RESEARCH ARTICLE

Bait formulations and longevity of navel orangeworm egg traps tested

by L.P.S. (Bas) Kuenen, Walt Bentley,
Heather C. Rowe and Brian Ribeiro

Standardization of pest monitoring practices and materials to maximize sensitivity to pest populations in the field is a foundation of effective integrated pest management (IPM). In response to changes in the availability of commercial bait material for navel orangeworm (NOW) egg traps, we evaluated potential alternative bait materials for use in monitoring this key pest of almonds, pistachios, walnuts and figs. Navel orangeworm egg traps baited with uninfested nutmeats were as effective as almond meal plus 10% crude almond oil, whereas traps baited with freeze-killed, navel orangeworm-infested nutmeats were less effective. The use of nut mummies (culled during winter orchard sanitation) as trap bait may not produce consistent results since the level of navel orangeworm infestation of these nuts is typically unknown. Three seasons of field tests showed that egg traps baited with almond meal plus 3% or 10% crude almond oil received similar numbers of navel orangeworm eggs, and these traps were equally effective for at least 10 weeks.

When navel orangeworm (NOW) infests nuts and figs, they will contain larvae or pupae and fecal material of the pest. Likewise, navel orangeworm infestation is highly correlated with the infection of nuts by *Aspergillus* spp., which produce carcinogenic aflatoxins. Both result in losses for growers.

Navel orangeworm (*Amyelois transitella*) larvae enter figs or nuts through open ostia (figs) or holes in damaged nut hulls (especially codling moth en-



Top, a pistachio orchard in Kings County. Above left, an adult navel orangeworm pair mating on a pistachio; center, navel orangeworm lay their eggs on mummy nuts in the spring; right, egg traps are used in orchards to monitor navel orangeworm for integrated pest management.

trance wounds in walnuts); they also enter after hull-splitting and drying of almonds, pistachios and walnuts, which occurs normally as these nuts mature. It is believed that navel orangeworm lay eggs on susceptible hosts in response to changes in odors — associated with the physical maturity changes — emitted from the nuts and figs, and possibly in response to altered tactile cues associated with these physical changes. The host odors are attractive to female navel orangeworm, which then lay eggs on the host; mated navel orangeworm females are known to fly upwind to odors from crude almond oil (CAO) (Phelan and Baker 1987).

The ability to monitor pests is a key component of any integrated pest management (IPM) program. The navel orangeworm is a primary pest of about 1.1 million acres of nuts and figs in California, and currently it is monitored by direct counts of eggs or larvae on the host and by navel orangeworm

egg traps (Rice et al. 1976). Trapping data is used to time the early harvest of almonds prior to egg-laying by the third generation of navel orangeworm and for timing insecticide sprays for the third generation in pistachios (Bentley and Surber 1986). The more accurately navel orangeworm populations can be tracked, the better they can be managed, particularly with newer, reduced-risk insecticides that have shorter residual times or require more precise application timing to maximize their effect on navel orangeworm numbers. Although the sex pheromone for this insect has been reported (Coffelt et al. 1979; Leal et al. 2005; Millar and Kuenen 2005), it is ineffective in sticky traps compared to traps baited with unmated females (Kuenen et al. 2001; Millar and Kuenen 2006). Therefore, egg traps will remain important for years to come in the IPM of navel orangeworm.

Current commercial egg traps consist of plastic vials (3.375 inches by

The more accurately navel orangeworm can be monitored, the better it can be managed.

1.625 inches, with three 1.125-inch, screened, round holes in the lower half of the vial) containing a bait attractive to navel orangeworm females, which elicits egg-laying on the surface of the traps. When first introduced, the traps were baited with a mixture of wheat bran, honey, glycerol and water. The traps' efficiency has since been improved by adding ridges around the traps, painting them black and changing the bait from a wheat-bran-based material to almond press cake (an almond-oil processing byproduct) (Van Steenwyk et al. 1986). These black traps, baited with almond press cake plus crude almond oil (10% by weight), have become a de facto standard for navel orangeworm monitoring.

However, in 1997 Liberty Vegetable Co. (Santa Fe Springs, Calif.), the provider of almond press cake, altered its almond-oil processing and now sells almond meal instead of almond press cake as a byproduct. In 2001, we initiated field tests to find the optimum blend of almond meal plus crude almond oil to attract navel orangeworm females, and used red wheat bran as a crude almond oil carrier for comparison. We also investigated the relative attractiveness of infested versus uninfested almond and pistachio nutmeats, because infested almonds are reportedly better attractants for navel orangeworm than uninfested nuts (Andrews and Barnes 1982).

Egg trap tests

Tests were conducted in almond, fig and pistachio orchards in Madera County during the 2001 to 2003 growing seasons. Navel orangeworm egg traps were purchased from Trécé, Inc. (Adair, Okla.) and were filled at least 75% with baits to ensure that the traps' windows remained covered with bait throughout the test periods (see also Van Steenwyk et al. 1986). Traps were suspended on branches about 5 feet above the ground in the outer half of the canopy, and treatments were placed in randomized complete block designs with five or more replicates per test. Each replicate block was laid out along tree rows with at least 65 feet between traps within the replicate blocks

and at least 65 feet between replicate blocks (actual spacing was determined by tree spacings within and between rows). The first trap in each row was at least 165 feet in from the nearest orchard road. All test blocks consisted of areas with no orchard drive rows or any other open spaces within larger orchard blocks.

Typically, egg counts were taken at weekly intervals. After each count, traps were re-randomized by moving them one tree forward within the replicate, and then the last trap in the row was moved to the first trap position in the same row. Trap baits were always formulated in the Kuenen lab, but plot specifics and the choice of orchards were conducted independently by our labs to ensure adequate orchard representation.

Data were analyzed graphically and by ANOVA. No data transformations were necessary as indicated by Bartlett's test for homogeneity of variances (Sokal and Rohlf 1981). Egg counts (eggs/trap/week) were analyzed by 2-way ANOVA using PROC GLM in SAS, and mean separation tests ($\alpha = 0.05$) were conducted with Tukey's HSD test (SAS 2001). No significant block effects were found in any of our studies ($P > 0.05$).

Nutmeats vs. almond meal plus oil

Since some growers and pest control advisors use nutmeats collected from orchard sanitizing procedures in their egg traps, our first test in 2001 compared traps baited with (1) almond pieces, (2) pistachio pieces, (3) navel orangeworm-infested almond pieces, (4) navel orangeworm-infested pistachio pieces, (5) almond meal plus 10% (by weight) crude almond oil or (6) left empty as control traps. For the infested nut pieces, navel orangeworm larvae had been freeze-killed (as they would be by users of culled mummy nuts).

There were no differences in trap catch among uninfested almond, uninfested pistachio or traps baited with crude almond oil ($P > 0.05$) (fig. 1), whereas traps baited with infested almond or infested pistachio pieces were significantly lower ($P < 0.05$) than uninfested almond pieces. The control traps received few eggs. Commercial bait is easier to handle and more easily standardized. Further, since the almond meal containing 10% crude almond oil was as effective a trap bait as the uninfested nutmeats, and since the components are easily manipulated, our subsequent tests focused on assessing the influence of varying amounts of

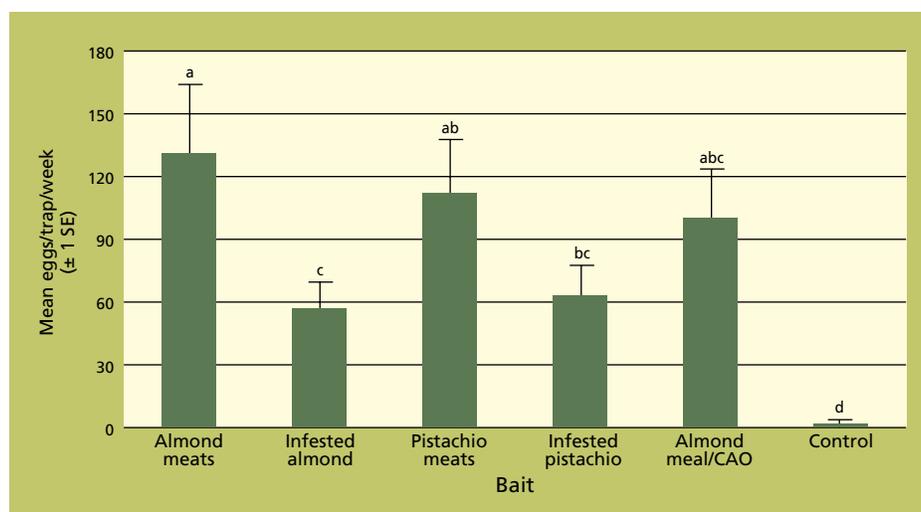


Fig. 1. Mean navel orangeworm (NOW) egg counts from traps containing NOW-infested almond or pistachio nutmeats, uninfested almond or pistachio nutmeats, almond meal plus 10% crude almond oil (CAO) by weight of almond meal, or unbaited controls. Traps were hung in a Madera County fig orchard March 26–April 30, 2001, with one trap per bait type in each of five blocks for a total of 30 traps. Traps were checked weekly. Bars represent \pm one standard error. Columns having no letters in common are significantly different; $P < 0.05$, Tukey's HSD test.

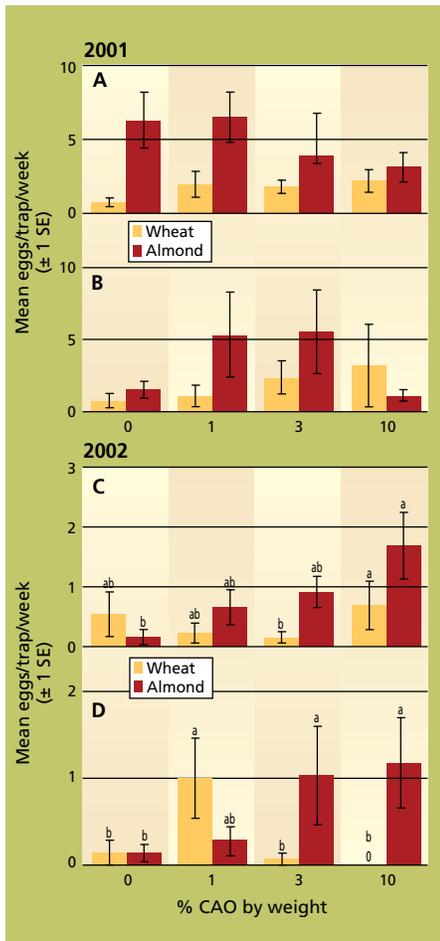


Fig. 2. Mean navel orangeworm (NOW) egg counts from egg traps containing almond meal or wheat bran plus crude almond oil (CAO). In 2001, traps were hung in a Madera County (A) almond orchard May 17–23 and checked daily, and (B) pistachio orchard July 11–Aug. 1 and checked weekly. In both orchards, one trap per bait type was hung in each of five blocks for a total of 40 traps. In 2002, traps were hung in a Madera County (C) pistachio orchard Aug. 8–Sept. 5, and (D) almond orchard Oct. 8–Nov. 5. In both orchards, one trap per bait type was hung in each of seven blocks for a total of 56 traps, and checked weekly. In 2001, there were no significant differences in egg counts within bait types. In 2002, columns having no letters in common are significantly different within bait types; $P < 0.05$, Tukey's HSD test. All bars represent \pm one standard error.

crude almond oil plus almond meal on trap capture.

Standardizing trap baits

Comparisons were made between traps baited with almond meal or red wheat bran mixed with 0%, 1%, 3% or 10% crude almond oil by weight, based on the weight of almond meal. Thus, traps with a given percentage of crude almond oil contained the same amount of crude



Monitoring with egg traps allows growers to better time harvests and more effectively apply lower-risk insecticides. Left, a midseason pistachio cluster and, right, nuts mummifying after harvest.

almond oil whether mixed with almond meal or the less-dense wheat bran.

Our first test with almond meal and wheat bran plus crude almond oil indicated that traps baited with almond meal plus 0% or 1% crude almond oil received more eggs than traps baited with almond meal plus 3% or 10% crude almond oil, and more than all wheat-baited traps ($P < 0.05$) (fig. 2A). In this first test, however, traps were checked daily, whereas in all subsequent tests eggs were counted weekly, which is typical for navel orangeworm monitoring.

A subsequent test in 2001 indicated that traps baited with almond meal plus 1% or 3% crude almond oil received more eggs compared to traps without crude almond oil or traps baited with wheat bran ($P < 0.05$) (fig. 2B). Overall, traps baited with almond meal plus crude almond oil received significantly more eggs than traps baited with wheat bran plus crude almond oil ($P < 0.05$). In 2002, we tested the same treatments but at different times during the growing season and in different orchards (which likely accounts for the lower numbers of eggs per trap compared to 2001). There was a clear trend for higher egg counts on traps with higher amounts of almond meal plus crude almond oil, whereas traps with crude almond oil on wheat bran showed no trend in trap capture in relation to the dosage of

crude almond oil (figs. 2C, D).

In 2003, we conducted two further tests of almond meal plus crude almond oil only, since the wheat bran plus crude almond oil baits typically captured fewer eggs. Trap capture data were combined for the two tests and showed nearly equal trap catch at all doses of crude almond oil tested ($P > 0.05$) (fig. 3).

In this last study and our first with almond meal plus crude almond oil, the treatment without crude almond oil was as good as or better than those with crude almond oil. This is perplexing, since all the almond meal and crude almond oil came from single batches, respectively, from the vendor. It is also important to note that in all our tests, trap capture variability was high and mean trap catch in relation to the dosage of crude almond oil shifted continuously. Even with replicated and repeated tests, consistent significant differences were rare. Nevertheless, over the course of all tests, treatments with almond meal plus 3% crude almond oil typically performed well, so we are compelled to recommend it as the best treatment in this monitoring technique.

Trap bait longevity

In summer 2003, we also examined the longevity of trap baits. Fifteen traps were baited with 3% crude almond oil and 15 with 10% crude almond oil on

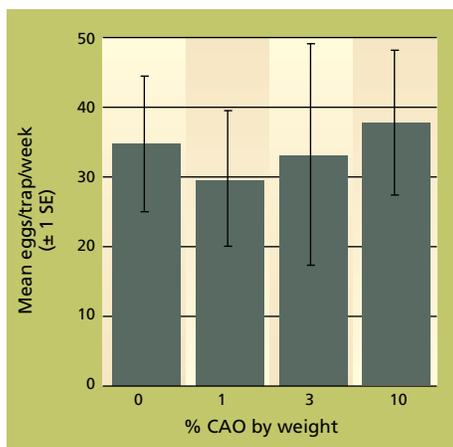


Fig. 3. Mean navel orangeworm (NOW) egg counts from egg traps containing almond meal plus crude almond oil (CAO). Traps were hung concurrently in a fig and pistachio orchard in Madera County May 20–June 26, 2003. In each orchard, one trap per bait type was hung in each of five blocks for a total of 80 traps, and checked weekly. Bars represent \pm one standard error; there were no significant differences in egg counts.

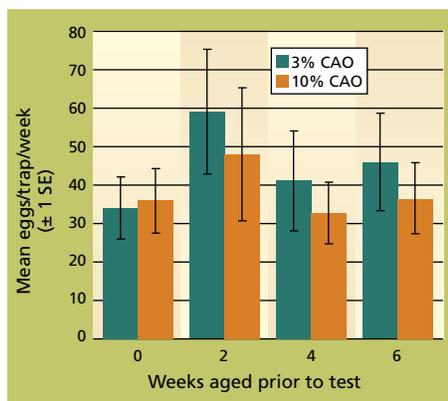


Fig. 4. Mean navel orangeworm (NOW) egg counts from egg traps containing almond meal plus 3% or 10% crude almond oil (CAO) by weight of almond meal; traps with baits had been aged 0, 2, 4 or 6 weeks in a laboratory incubator. Traps were hung in a Madera County pistachio orchard May 2–29, 2003. One trap per bait type was hung in each of five blocks for a total of 40 traps, and checked weekly. Bars represent \pm one standard error; within bait types there were no significant differences in egg counts.

almond meal, and aged (held) in a laboratory incubator at 90°F. Every 2 weeks, five traps of each dosage were removed and held at -4°F until we had traps that were aged at 90°F for 0, 2, 4 and 6 weeks (-4°F is a standard laboratory freezer temperature, at which little or no evaporation of odor compounds occurs). For this test, traps of all age categories were placed in a fig orchard when day-time highs were regularly 90°F to 95°F. Traps were positioned in a randomized complete block design and egg counts were taken weekly for 4 weeks. There were no differences in the capture efficiency of these aged egg traps ($P > 0.05$) (fig. 4) even after aging in the lab for 6 weeks and use in the field for 4 weeks.

Practical implications

Tests over three field seasons and in several orchards demonstrated that almond meal mixed with crude almond oil is an effective trap bait, and traps baited with a near-neutral carrier (wheat bran) plus crude almond oil were not as effective ($P < 0.05$). In addition, traps baited with pistachio or almond nutmeats were as effective as almond meal plus crude almond oil; however, freeze-killed, navel orangeworm larvae-infested nuts captured significantly fewer eggs ($P < 0.05$).

The variability in egg counts on the

traps was always high, with the standard errors typically exceeding the means. Although significant differences in trap counts were rare among the almond meal/crude almond oil baits, traps with almond meal plus 3% or 10% crude almond oil tended to capture the greatest number of eggs, and both traps were equally effective over 10 weeks. We conclude that almond meal plus 3% crude almond oil will be effective in the field, with little or no loss of efficiency for at least 10 weeks.



A new formulation of almond meal mixed with crude almond oil was an effective trap for navel orangeworm eggs. Above, female pistachio flowers.

L.P.S. (Bas) Kuenen is Research Entomologist, U.S. Department of Agriculture Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, Parlier; W. Bentley is IPM Entomologist, UC Statewide IPM Project, UC Kearney Agricultural Center, Parlier; H.C. Rowe is Graduate Student, Department of Plant Sciences, UC Davis; and B. Ribeiro is Staff Research Assistant, UC Statewide IPM Project. This research was supported in part by grants from the California Pistachio Commission. We thank all our cooperating growers for access to their orchards.

References

- Andrews KL, Barnes MM. 1982. Differential attractiveness of infested and uninfested mummy almonds to navel orangeworm moths. *Environ Entomol* 11:280–2.
- Bentley WJ, Surber E. 1986. Chemical control studies on navel orangeworm in pistachio. California Pistachio Commission Production Research Reports, Crop Year 1985–1986. www.acpistachios.org/research.php. p 75–6.
- Coffelt JA, Vick KW, Sonnet PE, Doolittle RE. 1979. Isolation, identification, and synthesis of a female sex pheromone of the navel orangeworm, *Amyelois transitella* (Lepidoptera: Pyralidae). *J Chem Ecol* 5:955–66.
- Kuenen, LPS, Rowe HC, Steffan K, et al. 2001. Incomplete female sex pheromone of the navel orangeworm, *Amyelois transitella*. California Pistachio Commission Production Research Reports, 2001–2002. www.acpistachios.org/research.php. 96 p.
- Leal WS, Parra-Pedrazzoli AL, Kaissling KE, et al. 2005. Unusual pheromone chemistry in the navel orangeworm: Novel sex attractants and a behavioral antagonist. *Naturwissenschaften* 92:139–46.
- Millar JG, Kuenen LPS. 2005. Field and laboratory studies to improve pheromone of navel orangeworm. Proceedings/Annual Report of California Pistachio Commission. www.acpistachios.org/research.php. p 127–40.
- Millar JG, Kuenen LPS. 2006. Field and laboratory studies to improve pheromone of navel orangeworm. Proceedings/Annual Report of California Pistachio Commission. www.acpistachios.org/research.php.
- Phelan PL, Baker TC. 1987. An attracticide for control of *Amyelois transitella* (Lepidoptera: Pyralidae) in almonds. *J Econ Entomol* 80:779–83.
- Rice RE, Sadler LL, Hoffman ML, Jones RA. 1976. Egg traps for the navel orangeworm, *Paramyelois transitella* (Walker). *Environ Entomol* 5:697–700.
- Sokal RR, Rohlf FJ. 1981. *Biometry* (2nd ed.). New York: WH Freeman. 776 p.
- Van Steenwyk RA, Barnett WW, Bentley WJ, et al. 1986. Improved NOW egg traps. *Cal Ag* 40(1):24–5.



Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Determinants of flavor acceptability during the maturation of navel oranges

David Obenland^{a,*}, Sue Collin^b, Bruce Mackey^c, James Sievert^b, Kent Fjeld^b, Mary Lu Arpaia^b^a San Joaquin Valley Agricultural Sciences Center, USDA-ARS, Parlier, CA 93648, United States^b Kearney Agricultural Center, University of California, Parlier, CA 93648, United States^c Western Regional Research Center, USDA/ARS, Albany, CA 94710, United States

ARTICLE INFO

Article history:

Received 19 June 2008

Accepted 2 January 2009

Keywords:

Soluble solids

Acidity

Volatiles

GC olfactometry

BrimA

Flavor

ABSTRACT

Navel oranges of differing maturities were harvested at regular intervals for three successive seasons and evaluated for external color, percent juice, soluble solids concentration (SSC) and titratable acidity (TA). Fruit from harvest dates throughout the season were rated by a sensory panel (12–20 panelists) for flavor likeability (hedonic score), sweetness, tartness and richness (strength of citrus flavor). Gas chromatography/olfactometry was used to identify odor-active volatiles present at each harvest date in the final season. Peel color and BrimA, a parameter calculated by subtracting TA times a constant from SSC, were the most closely related quality parameters to the hedonic score and ratings of sweetness, richness and tartness. A predictive equation for hedonic score was developed using stepwise regression that combined peel color, percent juice and BrimA and accounted for 63% of the variation in the data. Year, location and navel strain had only minor effects on the relationship between the quality parameters and the sensory ratings. Nineteen odor-active compounds were identified, of which six were significantly correlated with changes that occurred in the sensory attributes during navel orange maturation. The SSC/TA ratio, the basis for the current minimum maturity standard in California, was not as closely related to likeability as BrimA. At the minimum maturity standard (SSC/TA) of 8:1, the hedonic score calculated from the overall regression equation was 4.4, a value well into the “dislike” range, indicating that the current standard is likely set at too low of a value to satisfy most consumers.

Published by Elsevier B.V.

1. Introduction

Current maturity standards for California navel oranges require a ratio of soluble solids concentration (SSC) to titratable acidity (TA) of 8:1 and yellow-orange color on at least 25% of the peel surface for a minimum of 90% of the lot (California Department of Food and Agriculture, 2003). The SSC/TA portion of the standard, based upon work done by the United States Department of Agriculture, was utilized by the California citrus industry beginning in 1915 (Chace, 1917). A minimum peel color requirement was later added to deal with immature fruit that were able to pass the standard by virtue of a lack of acidity development. The reliability and usefulness of this standard has been contested from its inception (Chace, 1930), and the basis of exactly how the standard was chosen is not clear in the literature. The idea of the “Pritchett Tongue”, a graphical representation of SSC/TA versus SSC showing the best combinations for good flavor, was advanced in a report in the mid-1950s to lend support to using a 8:1 SSC/TA ratio as a maturity standard (Baier, 1954). The report, however, contains almost no data or descriptions of the methods by which the data

were acquired. In the 1980s, studies that were conducted using sensory panels in California, Texas, Nevada and New York indicated that consumers preferred oranges above the 8:1 ratio and that raising the ratio might lead to increased purchasing (Ivans and Feree, 1987; Pehrson and Ivans, 1988). Although these studies were formally conducted and utilized actual sensory panels, they were of limited scope and did not address the question of whether other orange quality parameters might be useful as indicators of flavor acceptability.

Even though SSC/TA is currently used to determine the minimum maturity standard in California, it has been recognized that this measurement does not always correlate well with the perception of sweetness or tartness in the fruit (Jordan et al., 2001). One difficulty is that the same ratio may be derived from widely differing levels of SSC and TA, leading to different flavor perceptions for the same ratio (Ishii, personal communication). This problem is dealt with by the Florida grapefruit industry by employing different SSC/TA ratios that depend on the SSC levels (USDA, 2002). Jordan et al. (2001), recognizing that sugar and acid have the opposite effect on flavor and that the tongue is more sensitive to acidity, proposed subtracting TA from SSC after multiplying TA by a constant that differs by fruit type. This measurement index, given the name of BrimA, was found by the authors to be more closely related to flavor than SSC/TA.

* Corresponding author. Tel.: +1 559 596 2801; fax: +1 559 596 2803.
E-mail address: david.obenland@fresno.ars.usda.gov (D. Obenland).

Rind color and firmness are nondestructively measured parameters that are associated with and potentially predictive of maturity (Olmo et al., 2000). Rind color is closely linked to SSC (Sites and Reitz, 1949), but color development is greatly affected by climactic conditions, making it unsuitable as a single measure of maturity. Direct measurement of SSC by near infrared spectroscopy is possible in some fruit, but is difficult in thick-skinned fruit like citrus (Nicolai et al., 2007).

Volatile constituents have been identified from orange juices that are very important in determining flavor, including hydrocarbons, alcohols, aldehydes and esters (Hinterholzer and Schieberle, 1998; Nisperos-Carriedo and Shaw, 1990). Although important, use of these volatiles as markers to help determine maturity and flavor acceptability is made difficult by the large numbers of volatiles potentially involved and a lack of understanding regarding which of these volatiles are most linked to changes in flavor during navel orange maturation. Prior studies of orange volatiles have primarily utilized purchased oranges of a single and unknown level of maturity, making it impossible to study this relationship. Purchased oranges are also problematic from the perspective that these fruit have most likely been waxed and therefore may have altered flavor due to fruit-handling practices (Baldwin et al., 1995; Obenland et al., 2008).

The objectives of this research were (1) to conduct a comprehensive experiment over different years and locations, using multiple strains of navel oranges, to fully examine the effectiveness of the current California navel orange maturity standard (SSC/TA) in predicting navel orange acceptability; and (2) to determine if other quality parameters, including aroma volatiles, might be also be useful or even superior predictors of acceptability than SSC/TA.

2. Materials and methods

2.1. Fruit

The experiment was conducted over three seasons, beginning in 2003 and ending in 2006. In the first two seasons, navel oranges (*Citrus sinensis* (L.) Osbeck) were harvested weekly from a navel strain research plot at the University of California Lindcove Research and Extension Center (LREC) near Exeter, CA, from mid-September until mid-November, and then subsequently bi-weekly until early to mid-March. All trees were grafted on Carrizo citrange rootstock. To enable a comparison of the effect of maturation date on quality and sensory characteristics, early-maturing ('Beck Early'), mid-maturing ('Parent Washington') and late-maturing ('Palmer') navel orange strains were harvested at each date. Selection of strains to be used for the experiment was primarily based upon there being an abundance of fruit present on these strains and that they were common strains used by the California citrus industry. In the third season, fruit were harvested from three separate commercial sites and one research site (LREC) in central California (Kern and Tulare Counties): (1) Kern County site 1, strains 'Parent Washington' and 'Atwood'; (2) Kern County site 2, strains 'Beck Early' and 'Thompson Improved'; (3) Tulare County site 1, strain 'Parent Washington'; (4) Tulare County site 2 (LREC); strain 'Parent Washington'. Comparison of mature trees of the same strain ('Parent Washington') across three of the four locations allowed a determination of the effect of location. Harvest sites were visited on a 3-week cycle beginning on September 20 with site 1 and ending on January 17. In all 3 years, fruit was harvested by size from random locations in the tree canopy from multiple trees. After harvest, fruit were transported to the Kearney Agricultural Center in Parlier, CA, where they were held for up to 3 d at 5 °C and 90–95% RH until the fruit was evaluated by the sensory panel.

2.2. Sample preparation

The fruit to be tasted for each day were taken from cold storage and allowed to stand overnight at room temperature. After being washed and dried, the fruit were visually rated for external color using a pictorial color chart and given a rating from 3 (dark green) to 13 (orange). The color chart was developed by researchers at the University of California, Riverside, and a rating of 5 corresponded to the "A" rating, which is part of the California state maturity standards (California Department of Food and Agriculture, 2003). Ratings were carried out by the same person each time except for the very few times when that person was not present. The fruit were then cut lengthwise and the top and bottom third cut away and discarded, leaving a 2.5-cm section from the center of the fruit. One half of the fruit was peeled and then cut into six bite-sized wedges for presentation to the sensory panelists. The other half was used for juicing for quality analysis. After weighing, the fruit were juiced by hand using a commercial table-top juicer (Model 932, Hamilton-Beach, Washington, NC, USA). The juice was weighed and the percent juice calculated by dividing the juice weight by the weight of the unpeeled portion. The juice was then filtered through a screen sieve and placed into a 15-mL centrifuge tube for quality factors determination. The juice samples were either kept at 5 °C until analysis or frozen at –12 °C if it was necessary to store the juice for more than a few days.

In the third year of the experiment, juice samples for volatile analysis were collected from the tasted fruit in a similar manner as for the quality analysis. In this case, the rind was carefully cut away prior to juicing in order to minimize the presence of peel oil in the resulting juice. The juice was placed into 23 mm × 75.5 mm (20 mL) glass vials sealed with a Teflon-coated septum and frozen at –20 °C until analysis. Seven vials, each from an individual fruit, were collected at each harvest from the Parent Washington strain only.

2.3. Quality and sensory analysis

SSC was measured in filtered juice by using a temperature-compensated refractometer (AO Scientific, Model 10423, Buffalo, NY, USA) and TA by titration with 0.1 mol L⁻¹ NaOH to an end point of pH 8.2 using a Radiometer TitraLab 80 Titration System (Lyon, France). Acidity was expressed as percent citric acid. Panelists were served individual fruit wedges to taste in white, 30-mL soufflé cups that were identified with a unique three-digit number. For each test, 12–20 panelists were available. These panelists were mainly employees at the Kearney Agricultural Center and could be considered as being semi-experts due to their familiarity with tasting citrus from numerous prior sensory panel studies with oranges. Samples were presented in random order, with each panelist receiving them in a different order to minimize order effects. Panelists were provided with distilled water and were directed to rinse their palate between samples. Individual, three-sided white booths that had a small doorway through which to receive the sample trays were used for the tasting. Light fixtures with SP30 fluorescent bulbs (General Electric, Fairfield, CT, USA) mounted over the evaluation area to provide standardized lighting. Eight samples were evaluated by each panelist for each tasting session. Each individual fruit was tasted by up to six panelists, with eight fruit being tasted per strain for each harvest date. Fruit from each harvest date was tasted over a 2–3-day period following harvest. Panelists gave each sample a hedonic flavor score ranging from 1 (dislike extremely) to 9 (like extremely). Also, the samples were rated for the degree of sweetness, tartness and richness of flavor by drawing a line on separate 150-mm scales. The measured distance from the 0-point indicated the intensity of the three sensory attributes, with a greater number indicating more sweetness and richness

(desirable flavor characteristic of oranges), but less tartness. Prior to the evaluation, panelists were given instructions regarding definitions of the attributes and how to utilize the line scales.

2.4. Volatile analysis

Six vials (representing six individual fruit) were thawed and pooled from each harvest, with each of the resulting six vials containing 6 mL (final sample volume). No salt was added to the juice, as preliminary experimentation had not found any advantageous benefit of its addition (data not shown). The vials were placed back into storage at -20°C until analysis. Just prior to analysis, the juice from each vial was thawed by partial immersion of the vial for 15 min in a 40°C water bath. Volatiles were then trapped from the headspace of the vial using solid phase microextraction with a $75\text{-}\mu\text{m}$ carboxen/polydimethylsiloxane fiber while maintaining the juice at 40°C . During the 30-min trapping period, the juice was slowly stirred by means of a stir bar. Fiber phase, trapping time and temperature had been previously optimized to provide a large quantity and wide range of odor-active volatiles (Obenland et al., 2008). Analytical conditions for gas chromatography of the volatiles are as detailed in Obenland et al. (2008). Effluent exiting the chromatography column was split between a flame ionization detector (250°C) and a SGE ODO II sniffer port (Austin, TX, USA). Quantification of the FID peaks of interest was performed using standards curves that were generated by the addition of standards to deodorized orange juice, whereas identification was based upon retention times, retention indices and odor of the peak. Mass spectrometry was used to confirm the identifications, using the system described in Obenland et al. (2008). The standard curve for heptanal was used to provide quantification for compounds with an unknown identity.

Sniffing of the column effluent was performed by three panelists that had been extensively trained on detection and identification of different aromas from citrus juice. When an odor was detected, the panelist would slide a lever on a self-made variable potentiometer for which the amount of movement reflected the intensity of the odor. This information, in the form of peaks outputted to the ChemStation software (Agilent, Palo Alto, CA), could be overlaid over the data from the FID detector and used to determine which of the FID peaks were aroma-active and potentially contributing to flavor. Samples from each harvest date were run six times by each of the panelists. For a component to be considered aroma-active, it had to be detected by at least two of the panelists in at least three out of six runs. These were the criteria that we had developed through prior experimentation to ensure that the detected components were valid. Peaks generated by the olfactory potentiometer were normalized by setting the highest value equal to 100 to adjust for differences among the panelists.

2.5. Statistics

Sensory data were analyzed using the hedonic and attribute means across panelists for each fruit. Panelists were considered to be a random effect representing just one panel that differed slightly from time to time but with the same core people and analyses were conducted using different panels as a single group. Stepwise regression with the sensory attributes as the dependent variables was performed using PROC General Linear Model (SAS Institute, Cary, NC) with a significance cutoff of $P \leq 0.15$ for inclusion of variables into the model. Analysis using PROC REG (GLM, SAS) were performed using site, location or year as fixed effects and the quality parameters as continuous explanatory variables, including possible interactions. Transformations were conducted as needed prior to either the regression or GLM analyses. Comparisons of R^2 values between the stepwise regression and GLM analyses were used to determine the influence of site location and year on

the various models. Pearson's correlation coefficients between the sensory attributes and quality parameters were performed using SAS. Regressions and correlations were conducted across all 3 years since analyses had shown no large between-year effects (data not shown). Volatile data were collected from pooled ($n=6$) individual fruit that had been tasted from each harvest date. Analysis was conducted using the GLM (SPSS, Chicago, IL) with harvest date as a fixed effect. Mean separations were performed at the 5% level of significance using the Bonferroni test. Pearson's correlation coefficients between the sensory attributes and volatiles were calculated using SPSS. Equations to best fit the relationships between hedonic score and either SSC/TA or BrimA were determined by using the curve estimation parameter of the SPSS regression procedure.

3. Results

3.1. Relationships between quality and sensory attributes

Pearson's correlation coefficients derived from 3 years of combined data indicated that peel color and BrimA were the quality parameters most closely related to the hedonic flavor score, sweetness, richness and tartness over the course of the entire season (Table 1). In the case of tartness, TA was also an important quality parameter. Percent juice had a very low correlation with hedonic score for any of the sensory attribute ratings. Stepwise linear regression was used to develop equations to predict hedonic score, tartness, sweetness and richness from combinations of the quality attributes (Table 2). Values of R^2 from these predictive equations ranged from 0.53 for richness to 0.68 for sweetness.

3.2. Effect of strain, location and year

During the initial 2003/2004 season, the navel orange strain Beck Early reached the legal harvest maturity standard for California of 8:1 (SSC/TA) by the October 20 harvest, while Parent Washington and Palmer reached this standard on November 3 and November 17, respectively. Statistical analysis were conducted for each of the sensory rating attributes, using quality factors as explanatory variables, with and without strain as a fixed effect in the model, to test whether or not strain had a significant effect in that season (Table 3). Although strain was statistically significant ($P \leq 0.05$) for hedonic score and ratings of sweetness and tartness, the R^2 values for models with and without strain for all four sensory attributes were nearly identical, indicating that strain was not an important factor in describing the relationship of quality and sensory attributes.

Similar results were obtained in experiments designed to test the effect of location. Washington navels were harvested from four separate sites in Tulare and Kern counties in Central California from September 2005 until mid-January 2006 and the same type of analysis performed as was done for strain, except that location

Table 1

Pearson's correlation coefficients between sensory and quality attributes using three seasons of combined data.

	Color	% Juice	SSC	TA	SSC/TA	BrimA
Hedonic score	0.73	0.30	0.59	-0.55	0.61	0.74
Sweetness	0.76	0.28	0.59	-0.61	0.68	0.78
Tartness	0.69	0.20	0.44	-0.70	0.72	0.70
Richness	0.68	0.28	0.60	-0.42	0.52	0.68

Color = external rating of the peel using the 3–13 color scale developed by the University of California, Riverside; % Juice = weight of the juice as a percentage of the total weight of the fruit; SSC = soluble solids concentration; TA = titratable acidity expressed as percent citric acid; BrimA = $\text{SSC} - 3(\text{TA})$. For tartness, a higher rating indicated less tartness.

Table 2

Equations to predict hedonic score, tartness, sweetness or richness from quality attributes obtained using stepwise linear regression from three seasons of sensory and quality data.

Y	Regression equation	R ²
Hedonic score	$Y = 0.142(\text{Color}) - 12.290(\text{BrimA}) + 0.001(\% \text{ Juice}) + 4.283$	0.63
Tartness	$Y = 31.233(\text{SSC}/\text{TA}) + 2.698(\text{Color}) - 190.613(\text{BrimA}) + 3399.051(\text{SSC}) - 16.549$	0.63
Sweetness	$Y = 3.144(\text{Color}) - 239.649(\text{BrimA}) + 0.011(\% \text{ Juice}) + 37.404(\text{SSC}/\text{TA}) + 22.082$	0.68
Richness	$Y = 2.728(\text{Color}) - 97.652(\text{BrimA}) + 0.009(\% \text{ Juice}) - 1537.666(\text{SSC}) + 73.193$	0.53

Color = external rating of peel color using a color chart; BrimA = $\text{SSC} - 3(\text{TA})$; % Juice = weight of the juice as a percentage of the total weight of the fruit; SSC = soluble solids concentration; TA = titratable acidity expressed as percent citric acid.

Table 3

Effect of navel strain, location or year on the relationship between sensory and quality attributes as determined by values of R² calculated from statistical models including or excluding navel strain, location or variety.

Sensory attribute	Fixed effect in model					
	Navel strain ^v		Location ^w		Year ^x	
	Excluded ^y	Included ^z	Excluded	Included	Excluded	Included
Hedonic score	0.69	0.70	0.79	0.81	0.63	0.67
Sweetness	0.75	0.76	0.84	0.87	0.68	0.73
Tartness	0.65	0.66	0.79	0.82	0.63	0.67
Richness	0.60	0.61	0.66	0.70	0.53	0.57

All quality attributes were included.

^v Data from 2003–2004 season.

^w Data from 2005–2006 season using the 'Parent Washington' strain.

^x Data from all three seasons combined.

^y Excluded from model. Stepwise regression analysis used for R² calculation.

^z Included in model. General linear model used for R² calculation.

rather than strain was the fixed effect in the analysis. Location was a significant effect in the analysis with regard to the hedonic score ($P \leq 0.01$), but inclusion of location into the model resulted in only small increase in R² values, indicating that location was not an important effect (Table 3). Similarly, location also had little influence on sweetness, tartness and richness.

The data from all 3 years were combined and also subjected to the same analyses as were performed for strain and location to determine if year was a significant factor in determining hedonic score and the ratings of sweetness, tartness and richness. Although year was statistically significant for hedonic score ($P \leq 0.04$) and

tartness ($P \leq 0.02$), the comparisons of R² values for the different sensory attributes (Table 3) indicated that there was little increase in R² due to the inclusion of year in the model, showing that year had a relatively small impact.

The above-mentioned analyses were conducted using a combined analysis with all of the quality factors together. Additional analyses done for SSC/TA and BrimA separately obtained very similar results as the combined analyses (data not shown) and confirmed that strain, location and year had only a minor impact on the relationship of the sensory attributes with each of the quality factors.

Table 4

Aroma-active volatiles present in 'Parent Washington' navel oranges harvested at time points throughout the 2005/6 season as determined by GC olfactometry.

Compound	Aroma descriptor	Harvest number ^x				
		1	4	7	10	13
Unknown 1 (U1)	Alcohol, sweet	1.76a	1.19a	2.18a	6.16b	6.92b
Unknown 2 (U2)	Metallic	0.21a	0.18a	0.29a	0.91b	1.42c
Pentanal (PEN)	Sour, pungent	31.93a	46.47ab	57.49b	96.69c	52.42ab
Unknown 3 (U3)	Sour	2.31a	2.69a	2.64a	3.66b	3.81b
Hexanal (HEX)	Grassy	7.00a	22.12ab	23.25ab	86.90c	40.55b
Ethyl butanoate (EB)	Fruity	ND	ND	0.63a	8.16b	15.30c
Heptanal (HEP)	Fatty	4.70a	6.21a	8.92b	17.27c	9.33b
α-Pinene (PIN)	Spicy	7.61b	7.63b	8.07b	3.22a	6.07ab
1-Octen-3-one (OCT) ^y	Mushroom	0.36a	0.52ab	0.72bc	1.35d	0.84c
Unknown 4 (U4)	Fatty, lemony	1.17a	1.54a	1.59a	1.64a	2.38b
β-Myrcene (MYR)	Fatty, musty	311.69c	277.69c	264.96bc	79.34a	196.03b
Ethyl hexanoate (EH)	Fruity	0.29a	0.28a	1.11b	3.08c	5.21d
Octanal (OCT)	Fatty, lemony	4.43ab	4.15a	5.48b	9.26c	4.79ab
Limonene (LIM)	Minty	7433.85b	7715.82b	7259.61b	2544.69a	6979.71b
γ-Terpinene (TER)	Citrus	0.85a	1.28b	1.76c	2.55d	1.48bc
Linalool (LIN)	Citrus	44.85a	50.55a	48.55a	71.60b	40.05a
Unknown (U5)	Cereal, fatty	0.67	0.71	0.71	0.67	0.64
(E)-2-Nonenal (NON)	Fatty	2.00a	3.11ab	4.50c	6.64d	3.89bc
Ethyl octanoate (EO)	Fruity, floral	0.75a	1.17cd	1.37d	1.13bc	0.92ab

Fruit were not waxed after harvest and were juiced within 3 d of harvest.

Values presented are in $\mu\text{g L}^{-1}$. Different letters following the values indicate a statistically significant difference ($P \leq 0.05$) among harvests within a compound, $n = 6$. ND = not detectable.

^x Harvest number 1 = September 19; 4 = October 10; 7 = October 31; 10 = November 28; and 13 = January 9.

^y Tentative identification based upon retention index and aroma.

Table 5

Pearson's correlation coefficients between aroma-active compounds and sensory attributes as determined from a series of five harvests throughout the 2005/6 navel orange season.

Compound	Aroma descriptor	Sensory attribute			
		Hedonic	Sweetness	Tartness	Richness
Unknown 1 (U1)	Alcohol, sweet	0.87*	0.83*	0.83*	0.77
Unknown 2 (U2)	Metallic	0.88*	0.89*	0.86*	0.79*
Pentanal (PEN)	Sour, pungent	0.59	0.61	0.45	0.46
Unknown 3 (U3)	Sour	0.89*	0.91*	0.83*	0.76
Hexanal (HEX)	Grassy	0.64	0.67	0.50	0.48
Ethyl butanoate (EB)	Fruity	0.86*	0.87*	0.85*	0.78
Heptanal (HEP)	Fatty	0.66	0.67	0.53	0.53
α -Pinene (PIN)	Spicy	-0.55	-0.57	-0.41	-0.37
1-Octen-3-one (OCT) ^x	Mushroom	0.74	0.75	0.62	0.61
Unknown 4 (U4)	Fatty, lemony	0.87*	0.88*	0.91*	0.83*
β -Myrcene (MYR)	Fatty, musty	-0.71	-0.73	-0.58	-0.55
Ethyl hexanoate (EH)	Fruity	0.91*	0.92*	0.91*	0.85*
Octanal (OCT)	Fatty, lemony	0.45	0.46	0.31	0.34
Limonene (LIM)	Minty	-0.43	-0.45	-0.28	-0.28
γ -Terpinene (TER)	Citrus	0.65	0.66	0.52	0.55
Linalool (LIN)	Citrus	0.13	0.15	-0.04	-0.02
Unknown (U5)	Cereal, fatty	-0.52	-0.52	-0.53	-0.48
(E)-2-Nonenal (NON)	Fatty	0.68	0.69	0.56	0.58
Ethyl octanoate (EO)	Fruity, floral	0.25	0.25	0.22	0.28

A star following a correlation coefficient indicates statistical significance ($P \leq 0.05$).

^x Tentative identification based upon retention index and aroma.

3.3. Aroma volatiles, harvest date and sensory attributes

Using GC-olfactometry, it was possible to consistently smell 19 different odor-active compounds in the orange juice samples (Table 4). All of these compounds produced peaks quantifiable by the FID detector. Fourteen of the compounds were identified by use of retention indices, aroma characteristics, comparison to standards and mass spectrometry. Fatty, fruity and citrus were the most common aroma descriptors noted. Significant changes in amount due to time of harvest were observed in almost all of the compounds. Five of the compounds (U1, U2, U3, EB and EH) increased in amount throughout the season, while six (PEN, HEX, HEP, OCT, TER and NON) increased until harvest 10 (November 28) and then decreased thereafter. Four of the compounds (PIN, LIM, LIN and U5) showed no clear pattern of change. Changes in the hedonic score during the season were significantly correlated with changes in four unknown compounds (U1, U2, U3 and U4), as well as for ethyl butanoate (EB) and ethyl hexanoate (EH) (Table 5). The same pattern with aroma volatiles was observed for the sensory attributes sweetness and tartness. Changes in richness were significantly correlated with changes in U2, U4 and EH (Table 5).

3.4. Relationship of SSC/TA, BrimA and hedonic score

Current minimum maturity standards for California are primarily based upon SSC/TA and so comparisons were made to determine how SSC/TA related to the hedonic flavor scores given by the panelists over the 3-year period of the study. A quadratic function was found to best fit the relationship between SSC/TA and hedonic score (Fig. 1A), while BrimA, a variant of SSC/TA derived from subtracting TA from SSC ($\text{BrimA} = \text{SSC} - k(\text{TA})$), was related in a linear manner to hedonic score (Fig. 1B). We modified the formula for BrimA suggested by Jordan et al. (2001), substituting their recommended constant (k) of 5 with a value of 3 in order to eliminate the generation of negative BrimA values. We found k factors of 3, 4 or 5 to provide nearly identical values of R^2 as calculated from the linear regression of hedonic score versus BrimA, with BrimA calculated using a k of 4 being slightly superior predictor of flavor ($R^2 = 0.5646$) than that calculated from a k of 3 ($R^2 = 0.5555$) or 5 ($R^2 = 0.5604$). Hedonic scores calculated from the quadratic equation for SSC/TA versus hedonic score (Fig. 1A) at various SSC/TA values and the cor-

responding value of BrimA are given in Table 6. At a SSC/TA value of 8.0 (8:1), the current minimum maturity standard in California, the calculated hedonic score was 4.4, which is well into the dislike range. Not until SSC/TA was 13.0, did the hedonic score reach 6.0 (like slightly).

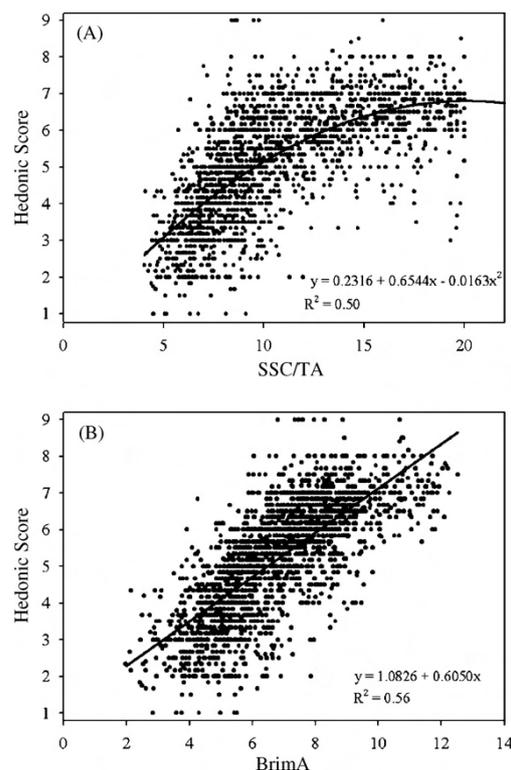


Fig. 1. Relationship between hedonic score and SSC/TA (A) or BrimA (B). $\text{BrimA} = \text{SSC} - 3(\text{TA})$. Points indicate individual fruit ($n = 2124$) that were tasted and measured for SSC and TA over three seasons. Listed equations were those that best fit the data.

Table 6
Hedonic flavor score obtained from a given SSC/TA ratio, the corresponding BrimA, and the average date over three seasons that this SSC/TA ratio occurred on.

SSC/TA	Hedonic score ^x	BrimA ^y	Average date ^z
6	3.6	4.2	September 26
8	4.4	5.5	October 18
10	5.2	6.8	November 8
12	5.7	7.6	November 30
13	6.0	8.1	December 10
14	6.2	8.5	December 21
16	6.5	9.0	January 11

^x Hedonic flavor score calculated from quadratic equation from Fig. 1A.

^y BrimA calculated from linear equation from Fig. 1B.

^z Three-year average calculated from linear regression of SSC/TA and date.

Data were sorted into four classes based on range of TA concentrations (1 = 2.53–1.51; 2 = 1.50–1.11; 3 = 1.10–0.71; 4 = 0.70–0.28) and linear correlations of SSC/TA and BrimA with hedonic score run within each TA class to determine the effect of TA on these relationships (Fig. 2A). Class ranges were derived from an attempt to equally separate the data into four separate classes. Values of R^2 were very similar between the hedonic score and SSC/TA or BrimA for classes 1–3, while R^2 values differed between the two quality factors for class 4 (low acidity). Both SSC/TA and BrimA had low R^2 values in class 4, but BrimA was more closely related to the hedonic score in class 4 than was SSC/TA. The similarity of SSC/TA and BrimA at higher values of acidity (classes 1 and 2) and the lesser similarity

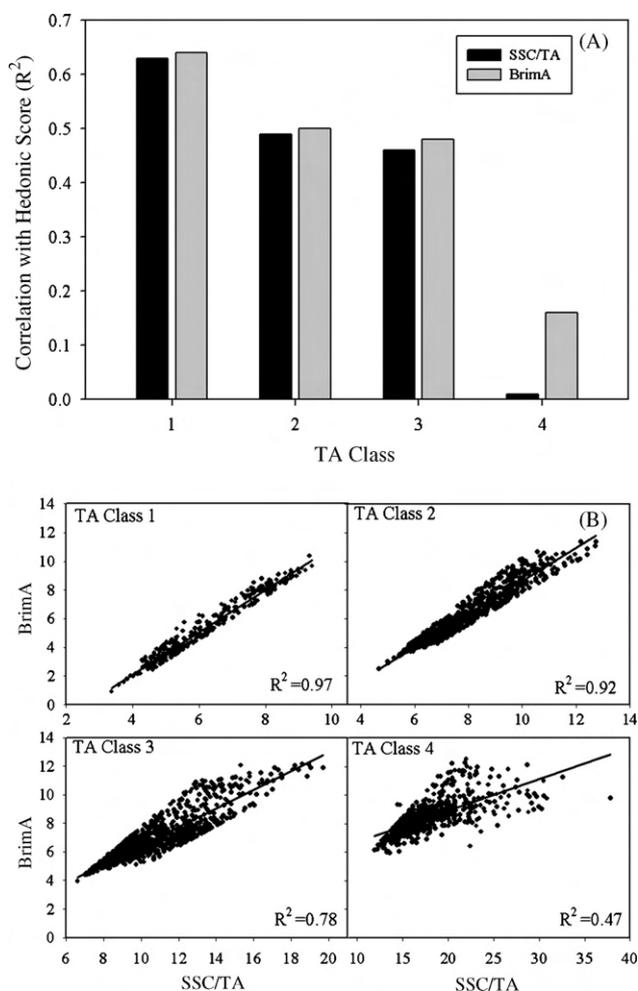


Fig. 2. Linear correlation of SSC/TA and BrimA with hedonic score (A) and correlation of SSC/TA with BrimA by TA class (B). TA classes: 1 = 2.53–1.51; 2 = 1.50–1.11; 3 = 1.10–0.71; 4 = 0.70–0.28.

at low acidity (class 4) were also clearly visible in linear regressions of the two quality factors (Fig. 2B).

4. Discussion

Even though SSC/TA is the current standard in California for determining minimum maturity and legal time of harvest, we found both peel color and BrimA to be more closely related to the flavor of the fruit over the course of the season (Table 1). Sweetness and richness, being components of flavor, were also strongly correlated with these two quality attributes. Although peel color is presently an element of the California maturity standard, it functions more as a means to prevent early-season, low-acid fruit from being certified as mature, rather than a direct measure of maturity (Chace, 1917). Color development is strongly affected by temperature and, as a result, would likely not be a good sole means by which to determine maturity. Our findings support the suggestion by Jordan et al. (2001) that BrimA is a better predictor of flavor than SSC/TA. Recent consumer testing with navel oranges at the University of California, Davis, has also found similar results with regard to SSC/TA and BrimA (Ishii, personal communication). In agreement with the findings of Jordan et al. (2001), who presented data for grapes and grapefruit, our results showed BrimA to have both a higher degree of correlation (Table 1) and a greater linearity in the relationship with flavor (Fig. 1) than SSC/TA. As was also noted by these authors, the advantage of BrimA over SSC/TA is most pronounced in low-acid fruit (Fig. 2). When acidity is low, SSC/TA becomes excessively high relative to BrimA due to SSC/TA being calculated as a ratio, rather than in a subtractive calculation as is BrimA. In our data of three seasons, we observed low acidity to be primarily a phenomenon of late season fruit. Out of 560 low-acid fruit that could be placed into our lowest acidity class (class 4; TA 0.28–0.70), only 14 (2.5%) were harvested during the early part of the season (September and October) that would have had sufficient color development to have met the California maturity standard. This suggests that most of the benefit to be obtained by switching to a standard based on BrimA rather than SSC/TA occurs late in the season at a time when maturity standards are not currently utilized. It cannot be discounted, however, that there are certain lots of navel oranges that have higher proportions of low-acidity fruit during the early season that would be positively impacted by this change in the maturity standard.

Stepwise linear regression analysis identified BrimA, external color and percentage juice as being the combination of quality attributes most predictive of the hedonic flavor score over the entire course of the season (Table 2). It is questionable, however, whether the increase in the R^2 value from 0.56 to 0.63 as a result of the addition of peel color and percentage juice to the selection model would add enough additional precision to warrant the extra effort in data collection. Given that oranges with a very dry texture (low % juice) are unlikely to be acceptable to consumers, however, it is likely that there exists a minimum level of percentage juice that is needed for acceptability.

Our data analysis showed that navel orange strain, location and year have little impact on the relationship between the quality and sensory attributes. Although it cannot be ruled out that there could be changes due to these three parameters under other circumstances, this conclusion indicates that the results are applicable over a wide range of conditions.

The data scatter visible in the relationship between both SSC/TA and BrimA with the hedonic flavor score (Fig. 1A and B) is at least partly due to the difficulty in trying to describe the flavor of fruit solely using TA and SSC, which excludes the important role of volatile compounds. Volatiles, which were determined to be odor-active and potentially have an impact on flavor, were quantified at different stages of maturation to estimate the influence of these compounds on flavor development. The increases in

amount during the progression of the season that occurred with the majority of the volatiles likely incrementally enhanced their overall contribution to flavor (Table 4). Some of the volatiles later declined in abundance, but the amounts still generally exceeded those from the first harvest. The compounds, including aldehydes, esters, hydrocarbons, an alcohol and a ketone, were higher in all but one case in juice concentration than the odor threshold values previously published (Buettner and Schieberle, 2001; Moshonas and Shaw, 1994), indicating a possible role for each in flavor. Use of published odor thresholds, however, must be regarded with caution, since they are generally performed in water and neglect the potential matrix effect (Plotto et al., 2004). Also, the interactive effects of the components on each other undoubtedly alter the impact of each individual volatile component. The amounts of these compounds were often less than had been previously reported (Buettner and Schieberle, 2001; Moshonas and Shaw, 1994), but this could be attributable to differences between the studies such as juice extraction technique, fruit origin, fruit postharvest handling procedures and volatile analytical techniques.

Correlations were conducted between the volatiles and sensory attributes to obtain an estimate of the overall impact of each individual volatile on flavor across all harvests. Two of the six compounds that had a significant correlation with the hedonic flavor score (Table 5) were EB and EH, esters with a fruity, sweet odor. Both have been identified as contributors to orange flavor (Ahmed et al., 1978; Buettner and Schieberle, 2001). EB, due to its low odor threshold, is believed to be especially important (Hinterholzer and Schieberle, 1998). We could not detect any EB until the third harvest at the end of October, after which it greatly increased in amount, indicating that this compound may be especially closely tied to the enhancement of flavor during navel orange maturation. The other four compounds that had significant correlations with the hedonic flavor score could not be conclusively identified even though a measureable peak was present on the FID chromatogram generated from the GC olfactory runs. Aromas of these compounds were described by panelists as being alcoholic, sweet (U1), metallic (U2), sour (unknown 3), and fatty, lemony (U4). Although the odors of these compounds were generally unpleasant on their own, the increasing amounts during the season could be interacting with other volatile as well as nonvolatile flavor components to help give the characteristic orange flavor. Identification of these odor-active compounds would aid in a determination of their importance.

In agreement with prior reports (Ivans and Feree, 1987; Pehrson and Ivans, 1988), we found the current California maturity standard based upon a minimum SSC/TA ratio of 8:1 to be set too low to provide good eating quality navel oranges to consumers. On average, panelists in this study rated fruit with this ratio well into the “dislike” range of the hedonic flavor scale, most likely due to sourness (Pehrson and Ivans, 1988; Ishii, personal communication). This study confirmed this finding in a much more rigorous manner than had previous work, performing sensory evaluations over three separate seasons, using multiple strains and growing locations. A much smaller study that we conducted in the 2004/2005 season using 16 employees of Sunkist Inc., a California citrus cooperative (data not shown), found nearly an identical degree of dislike for fruit at 8:1 SSC/TA as did the large study presented here. Similar results have been found from consumer testing of navel oranges by the University of California, Davis (Ishii, personal communication). We recognize that the KAC panel had shortcomings as a consumer panel due to the relatively small size of our panel and its familiarity with navel oranges, yet these additional studies give reassurance to our findings in terms of their relevance to consumer acceptance.

Another consideration regarding what ratio that the maturity standard should depend on is that commercially the measurements of SSC and TA are done on pooled, randomly selected, 30-fruit sam-

ples (California Department of Food and Agriculture, 2003). Since levels of SSC and TA found in individual fruit can vary within an orchard and even within different locations within the canopy of individual trees (Sites and Reitz, 1949, 1950), this practice can lead to fruit that have ratios lower than the maturity standard reaching the marketplace. Ivans and Feree (1987) reported that in a mid-November sampling of oranges from markets in six different counties in California, 39% of the fruit were below the minimum level, with some being as low as 5:1. In this study, it was observed that in 30-fruit samples that averaged 8:1, there would be individual fruit well below 6:1, and that it was not until the lots reached and exceeded an average of 10:1 that individual fruit with ratios below 8:1 were not found (data not shown). Fruit with very low SSC/TA ratios such as 5 or 6:1 are very sour and were strongly disliked by our panelists (Fig. 1A). Raising the minimum SSC/TA ratio required for harvest would help lessen the number of these low-ratio fruit from entering the marketplace.

Due to the need to taste and determine quality parameters of individual oranges, it was not possible to exactly reproduce in this study the juice extraction and SSC determination methods used by the industry. Preliminary results from our laboratory indicate that SSC determined by using a pressure-actuated citrus press and hygrometer (industry method) is slightly higher than that determined by using a Hamilton-Beach press and refractometer, as was done in this study. This difference is potentially due to the greater inclusion of extraneous soluble solids by use of the citrus press and means that the industry SSC and SSC/TA values are likely somewhat inflated and that the true hedonic score for fruit from the industry at 8:1 is even further into the dislike portion of the hedonic scale than we have indicated.

In conclusion, results from this extensive study indicate that the current California maturity standard of SSC/TA for oranges does not correlate with flavor well when the fruit have low acidity and that BrimA is a superior predictor of flavor under these circumstances. Although low-acidity fruit is primarily a feature of the late season when the maturity standard is not in use, the navel orange industry in California may be better served by using BrimA as a maturity standard rather than the current standard SSC/TA in order to lessen the possibility of low-acid, poor-tasting fruit entering the marketplace. For a flavor quality standard spanning the entire season, BrimA would definitely be recommended over SSC/TA. An additional problem with the current maturity standard that has been highlighted by this research and noted by others (Ivans and Feree, 1987; Pehrson and Ivans, 1988) is that the minimum SSC/TA ratio is set too low for acceptable flavor to be consistently obtained in the early season. The minimum SSC/TA ratio, or BrimA value, needs to be raised to a level that will prevent consumers from purchasing excessively sour fruit. A further point demonstrated by this research is that flavor is not fully described by SSC and TA alone and that aroma volatiles are changing in concert with the observed changes in flavor during navel orange maturation. Further characterization of these aroma volatiles and determination of how to integrate knowledge of the relative levels of these compounds into decisions regarding maturity standards and general fruit quality would be worthy goals of future research.

Acknowledgements

This work was partially funded by a grant from the California Citrus Research Board. The help of Julie Doctor (Sunkist Growers) and the excellent technical assistance of Paul Neipp were much appreciated. We also appreciate the very helpful comments provided to us by members of the California citrus industry, in particular, Mr. Don Roark and Dr. Etienne Rabe. Additional thanks to Dr. Adel Kader for reviewing the manuscript.

References

- Ahmed, E.M., Dennison, R.A., Dougherty, R.H., Shaw, P.E., 1978. Flavor and odor thresholds in water of selected orange juice components. *J. Agric. Food Chem.* 26, 187–191.
- Baier, W.E., 1954. The Pritchett Tongue. *Calif. Citrogr.* 39, 442.
- Baldwin, E.A., Nisperos-Carriedo, M., Shaw, P.E., Burns, J.K., 1995. Effect of coatings and prolonged storage conditions on fresh orange flavor volatiles, degrees brix, and ascorbic acid levels. *J. Agric. Food Chem.* 43, 1321–1331.
- Buettner, A., Schieberle, P., 2001. Evaluation of aroma differences between hand-squeezed juices from Valencia Late and Navel oranges by quantitation of key odorants and flavor reconstitution experiments. *J. Agric. Food Chem.* 49, 2387–2394.
- California Department of Food and Agriculture, 2003. Title 3, Division 3, Chapter 1, Subchapter 4, Article 22, Section 1430.36.
- Chace, E.M., 1917. Maturity standard for Washington navel. *Calif. Citrogr.* 2, 7 and, 17.
- Chace, E.M., 1930. Maturity data on the California Washington navel orange. *Calif. Citrogr.* 15 534, 569.
- Hinterholzer, A., Schieberle, P., 1998. Identification of the most odour-active volatiles in fresh, hand-extracted juice of Valencia late oranges by odour dilution techniques. *Flav. Fragrance J.* 13, 49–55.
- Ivans, E., Feree, M., 1987. Early-season navel oranges may be too sour for consumers. *Calif. Agric.* (January–February), 20–21.
- Jordan, R., Seelye, R., McGlone, A., 2001. A sensory-based alternative to brix/acid ratio. *Food Technol.* 55 (6), 36–44.
- Moshonas, M.G., Shaw, P.E., 1994. Quantitative determination of 46 volatile constituents in fresh, unpasteurized orange juices using dynamic headspace gas chromatography. *J. Agric. Food Chem.* 42, 1525–1528.
- Nicolaï, B.M., Beullens, K., Bobelyn, E., Peirs, A., Saeys, W., Theron, K.I., Lammerly, J., 2007. Nondestructive measurement of fruit and vegetable quality by means of NIR spectroscopy: a review. *Postharvest Biol. Technol.* 46, 99–118.
- Nisperos-Carriedo, M.O., Shaw, P.E., 1990. Comparison of volatile flavor components in fresh and processed orange juices. *J. Agric. Food Chem.* 38, 1048–1052.
- Obenland, D., Collin, S., Sievert, J., Fjeld, K., Doctor, J., Arpaia, M.L., 2008. Commercial packing and storage of navel oranges alters aroma volatiles and reduces flavor quality. *Postharvest Biol. Technol.* 47 (2), 159–167.
- Olmo, M., Nadas, A., García, J.M., 2000. Nondestructive methods to evaluate maturity level of oranges. *J. Food Sci.* 65, 365–369.
- Pehrson, J.E., Ivans, E.M., 1988. Variability in early season navel orange clone maturity and consumer acceptance. In: *Proceedings of the Sixth International Citrus Conference*, Tel Aviv, Israel, pp. 1631–1635.
- Plotto, A., Margaría, C.A., Goodner, K.L., Goodrich, R., Baldwin, E.A., 2004. Odour and flavour thresholds for key aroma components in an orange juice matrix: terpenes and aldehydes. *Flav. Fragrance J.* 19, 491–498.
- Sites, J.W., Reitz, H.J., 1949. The variation in individual valencia oranges from different locations of the tree as a guide to sampling methods and spot-picking for quality. Part I. Soluble solids in the juice. *Proc. Am. Soc. Hort. Sci.* 54, 1–10.
- Sites, J.W., Reitz, H.J., 1950. The variation in individual valencia oranges from different locations of the tree as a guide to sampling methods and spot-picking for quality. Part II. Titratable acid and the soluble solids/titratable acid ratio of the juice. *Proc. Am. Soc. Hort. Sci.* 55, 73–80.
- USDA, 2002. Oranges, grapefruit, tangerines, and tangelos grown in Florida; change in the minimum maturity requirements for fresh grapefruit. *Federal Register*, Vol. 67, No. 232, 7, CFR Part 905.

RESEARCH ARTICLE

New navel orangeworm sanitation standards could reduce almond damage

by Bradley S. Higbee and Joel P. Siegel

The navel orangeworm (NOW), a primary pest of almonds and pistachios in California, is controlled in part by sanitation, with a current threshold of two mummy nuts or fewer per tree. However, almond and pistachio acreage has increased dramatically since the tree mummy threshold was established. This study addresses the impact of this expansion and the possible need for a more stringent standard. Beginning in 2002, the Paramount Farming Company conducted a series of large-scale studies reevaluating the current tree mummy threshold in almond orchards, as well as the impact of ground mummies and proximity to pistachio orchards. The data supports a more stringent threshold of 0.2 mummies per tree. In addition, a new threshold for ground mummies of four per tree for 'Nonpareil' almonds is supported in Kern County, although this needs to be validated in other regions. Proximity to pistachios was an important risk factor for navel orangeworm damage of 2% or less in almonds. Likewise, the influence of pistachios extended 3 miles from the center of the 10-acre almond orchard sections in our experiments to the margin of the nearest pistachio orchard.

Almond and pistachio plantings comprise more than 880,000 acres in the Central Valley (NASS 2006). Almonds account for about 83% and pistachios for about 17% of these plantings (730,000 and 153,000 acres, respectively). 'Nonpareil' is the most popular almond variety, comprising 37.7% of all standing acreage in 2006, and 'Kerman' com-



Sanitation practices in almond orchards can have a significant impact on insect pest damage. Almond "mummies" remaining on the tree after harvest provide overwintering sites for navel orangeworm, which then infests the new crop.

prises almost all pistachio plantings in California (see page 18). From 2003 to 2007, there has been unprecedented expansion in the acreage of both crops: 30% for almond and 31% for pistachio.

In 2005, the combined farm-gate value of almonds and pistachios was approximately \$2.9 billion, according to the Almond Board of California. These crops contribute substantially to the U.S. export balance of trade. Approximately 67% of the almond (ABC 2006) and 49% of the pistachio crop was exported in 2005, according to the California Pistachio Industry Annual Report. Kern County had the single greatest concentration of both crops in 2005, with 20% of total standing almond acres (131,400) and 31.9% of total standing pistachio acres (48,770) (NASS 2006).

Navel orangeworm (NOW), *Amyelois transitella* Walker (Wade 1961), is the major pest of almonds and pistachios in California, and direct damage by this insect can exceed 30% in both crops. During the late 1970s through the early 1980s, navel orangeworm devastated the almond crop, causing average damage of 8.8% in 1978 (F.G.

Zalom, personal communication). By the late 1980s, average damage in almonds was reduced to approximately 4%, due to the efforts of researchers at the U.S. Department of Agriculture (USDA) (Curtis 1979) and the University of California (Engle and Barnes 1983; Zalom et al. 1984).

This reduction in navel orangeworm damage was accomplished via a massive commitment to orchard sanitation, using a threshold of no more than two unharvested (mummy) nuts remaining in each tree, along with early harvest of the 'Nonpareil' crop and on-farm fumigation with insecticides after harvest (UC IPM Online 2007). These practices lowered damage by both reducing navel orangeworm populations and removing nuts before they could become infested by the large populations of navel orangeworm that occur from August through September.

While the 4% damage level was satisfactory for approximately 20 years, both food-quality standards and commodity values are dynamic, and today there is even less tolerance for damage. Since 2002, the almond industry's average



Navel orangeworm control can be achieved in almonds by careful orchard sanitation, early harvest of the 'Nonpareil' variety and postharvest fumigation with insecticides. *Clockwise from top left:* a navel orangeworm adult; a fertile navel orangeworm egg laid on a mummy almond; a hatched egg; and an almond mummy infested with navel orangeworm larvae.

damage goal for navel orangeworm has been 2% or less. Factors contributing to this current threshold include the crop's increased value and the association of kernel damage by navel orangeworm with aflatoxin contamination, a major quality concern (Schatzki and Ong 2001; ABC 2006). In addition, the European Union — the largest market for California almonds — has imposed more-stringent import standards that have lowered the allowable level of aflatoxin B1 to 2 parts per billion (OJEU 2007).

In order to reduce navel orangeworm damage and increase almond quality, the Paramount Farming Company initiated research in Kern County in 2002 to evaluate the complex interactions between current sanitation practices, orchard damage history and proximity to an alternate navel orangeworm host (pistachios). Using

pooled data from 2003 through 2006, we report on how 'Nonpareil' kernel damage is affected by numbers of both tree and ground mummies, as well as proximity to pistachios.

Post-sanitation studies

Between December 2002 and February 2006, a series of long-term, labor-intensive studies on mummy abundance following sanitation was conducted in ranches belonging to all divisions of the Paramount Farming Company in Kern County. More than 50 ranches were divided into 160-acre blocks, which were then subdivided into 40-acre plots, which in turn were quartered into the 10-acre sections comprising our sample units.

Abundance of mummies. Between January and mid-February, 2003 through 2006, we selected four adjacent trees from each of two consecutive

rows (four 'Nonpareil' trees and four pollinizer trees) in each 10-acre section. (Almonds are not self-compatible and in order to achieve maximum yield, 'Nonpareil' must be pollinated by varieties other than itself. As a consequence, any block of almonds contains at least two different varieties.) Separate counts were made of nuts on the ground and those remaining in the trees. All of the fallen nuts from outside the drip line (or berm) between the eight trees were counted, and nuts in the trees were knocked down with bamboo poles (poling) and then counted. The average number of mummies per tree was calculated for both fallen nuts and nuts remaining in the tree for every year of our study. A total of 1,920 sections was used in this analysis, corresponding to 19,200 acres and 15,360 trees. In 2003 and 2004, all these mummies were collected and dissected,

and data for the 2 years were pooled (233,821 ground mummies and 7,371 tree mummies).

Damage to kernels. In August and early September, 2003 through 2006, within 5 days of harvest, samples of 1,500 to 2,000 nuts were collected from these same 10-acre sections by walking a diagonal transect and taking 50 to 100 nuts at intervals of approximately 100 feet. A total of 2,596,008 kernels was obtained by a combination of hand-cracking and a small hulling and shelling machine. All kernels were examined using a lighted 3× magnifier, by personnel trained to identify common insect and cultural defects. On several occasions, subsamples were sent to a Paramount processing plant for independent grading, and the processor grades were in agreement with the laboratory grades.

Damage to kernels was scored and descriptive statistics including mean, standard deviation and pairwise correlations were calculated using JMP software (v. 7.0.1, SAS Institute, Cary, NC). In addition, relative risk, a statistic commonly used in epidemiology to evaluate the likelihood of a dichotomous outcome (one of two outcomes; in this study the outcome of interest was damage of at least 2%) was used to compare damage differences between the tree and ground mummies, and to assess differences in kernel damage by rounding the navel orangeworm damage to the nearest tenth and then contrasting all sections with damage of 2% or more with sections that had damage below this level (Kelsey et al. 1986). Distance in feet was calculated from the center of each almond section to the margin of the nearest pistachio block using ArcMap (ESRI, Redlands, CA) and the Paramount Farming Company GIS mapping database.

Damage higher in tree mummies

The average number of tree mummies was 0.7 (± 5.0 standard devia-

In order to properly sanitize an almond orchard in Kern County, it is essential to remove mummies from the trees and destroy them on the ground.

tion [SD]) and the range was 0 to 69.7 per tree, while the average number of ground mummies was 5.0 (± 5.3 SD) and the range was 0 to 43.7 per tree. In the pooled dataset for 2003 and 2004, 13.64% of tree mummies and 7.91% of ground mummies collected were infested with navel orangeworm. The relative risk for tree-mummy compared to ground-mummy infestation was 1.72 (Chi square = 277, $P < 0.0001$), indicating that tree mummies were 1.72 times as likely to be infested as ground mummies. This infestation disparity is likely due to differential mortality between navel orangeworm in trees and on the ground, but we did not specifically address this in our study. A similar pattern exists in pistachios collected in February (Siegel et al. 2008), but the study did not specifically determine causes of mortality. The average distance from the center of the almond sections to the margin of the closest pistachio block was 8,600 feet (1.6 miles).

In this study, the average kernel damage per sample due to navel orangeworm was 1.6% (± 2.3% SD) and the range was 0 to 20.8%. The standard deviation was greater than the means for mummies and kernel damage due to the inclusion of sections with no navel orangeworm damage and/or no mummies.

The correlations among these variables using the parametric statistic, Pearson product moment coefficient (r), are summarized in table 1. Tree mummies were the most strongly correlated with navel orangeworm damage (0.46, $P < 0.00001$), followed by ground mum-

mies (0.23, $P < 0.00001$). There was a negative correlation between navel orangeworm damage and distance to the pistachio margin (-0.29 , $P < 0.00001$), indicating that damage decreased with distance. Tree and ground mummies were moderately correlated (0.39, $P < 0.00001$), indicating that when tree mummies were high in a section so were ground mummies, but there was considerable variation. Both tree and ground mummies were negatively correlated with distance to the pistachio margin (-0.09 , $P < 0.0001$; -0.06 , $P < 0.005$ respectively). These marginal correlations are statistically significant due to the large sample size, and they indicate that there was a slight tendency for fewer mummies to be recovered closer to the pistachio margins.

Mummies and new crop damage

Tree mummies. Damage in the new crop exceeded the 2% threshold when there were 0.7 mummies or more per tree in the winter (table 2), a reduction of 65% from the current guideline. However, further relative risk analysis supports a more stringent threshold of 0.2 mummies per tree. When sections containing 0.2 or more mummies per tree were compared to sections that had fewer than 0.2 mummies per tree, the relative risk was 2.15 (Chi square = 156, $P < 0.0001$), indicating that they were 2.15 times as likely to have kernel damage equal to or exceeding the 2% threshold. In addition, other factors beside the number of tree mummies

TABLE 1. Correlations among 'Nonpareil' kernel damage by navel orangeworm (NOW), mummies per tree and distance to nearest pistachio margin, 2003–2006

	NOW damage	Tree mummies	Ground mummies	Distance
NOW damage	1.00	0.46	0.23	-0.29
Tree mummies	0.46	1.00	0.39	-0.09
Ground mummies	0.23	0.39	1.00	-0.06
Distance	-0.29	-0.09	-0.06	1.00

TABLE 2. Relationship between average numbers of tree and ground mummies per tree and 'Nonpareil' kernel damage by navel orangeworm, 2003–2006

Tree mummies	Damage	Sections
avg. no./tree	%	no.
0	1.63	605
0.01–0.49	1.22	1,092
0.5–0.69	1.57	91
0.7–0.79	2.32	39
0.8–1.75	3.53	61
≥ 1.76	7.85	44
Ground mummies	Damage	Sections
avg. no./tree	%	no.
0–4.9	1.39	1,272
4.91–7.9	1.57	300
7.91–8.9	1.72	67
8.91–9.0	2.78	44
≥ 9.1	2.72	238

clearly influence navel orangeworm damage, because in the sections that lacked tree mummies, the average kernel damage was 1.6%.

Ground mummies. In this study, the number of ground mummies per tree was also related to damage in the new crop (table 2). We found that kernel damage exceeded the current guideline of 2% when there were 8.9 or more ground mummies per tree. Use of the statistic relative risk indicated that a more stringent threshold of four ground mummies per tree is justified, because sections containing four or more mummies were 1.34 times more likely to have kernel damage exceeding the 2% threshold than sections with fewer than four mummies on the ground (Chi square = 13.6, $P < 0.0001$).

There is currently no established threshold for ground mummies. We suggest using an average of four ground mummies per tree for Kern County. We did not establish causality in this study, and mummies on the ground may harbor the overwintering navel orangeworm population, serve as a host for the first generation of the new crop year, or both. What is clear is that mummies on the ground were more than 36 times as prevalent as mummies in trees in the pooled dataset for 2003-2004, and these ground mummies may contribute to navel orangeworm damage due to their abundance. In order to properly sanitize an almond orchard in Kern County, it is essential to remove mummies from the trees and destroy them on the ground.

Proximity to pistachio

Damage caused by navel orangeworm decreased as distance to the nearest pistachio margin increased (fig. 1). The best fit was obtained using this quadratic equation: % 'Nonpareil' kernel damage = $0.0265156 - 0.00000016 \times \text{distance} + 0.000000000013 \times (\text{distance} - 8,889.8)^2$.

Although this equation is statistically significant (F ratio 112.3, $P < 0.0001$, $r^2 = 0.105$) it does not account for most of the variation, confirming that other factors also play a role in navel orangeworm damage. The relationship between damage and pistachio proximity declined with distance and ceased somewhere between 14,000

and 15,000 feet (table 3). Navel orangeworm damage was highest in the almond sections that were 0.25 mile or less from pistachios; there were 87 sections in this class and 55.2% of them had damage of 2% or more. At a distance of 3 miles or more from pistachios, there were 1,752 almond sections and 26.7% of them had damage of 2% or more. In contrast to sections inside the 3-mile limit, those beyond 3 miles (15,840 feet) were 25% to 50% less likely to have damage that exceeded the 2% threshold (data not shown).

Reducing NOW damage to 2%

Mummy abundance. In order to meet a new threshold of 2% or less kernel damage in Kern County, the average number of mummies should be reduced to 0.2 per tree, and an additional threshold should be established of four ground mummies per tree after mummy destruction by flail mowing. In a 100-tree planting per acre, these new standards correspond to 20 tree mummies and 400 ground mummies per acre, leaving an acceptable total of 420 or more nuts per acre.

Sanitation. Assuming that an average 'Nonpareil' almond tree in a 1-acre planting bears between 12,000 and 18,000 nuts (UC 2006), and that the accompanying pollinizer varieties bear the same number of nuts,

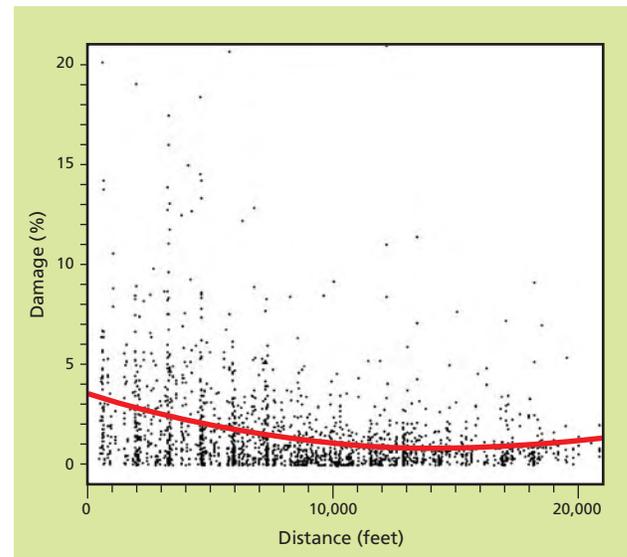


Fig. 1. Relationship between percent navel orangeworm damage and distance (feet) from center of almond block to nearest pistachio margin.

there is a potential load of 1,200,000 to 1,800,000 almonds per acre before harvest. Harvest operations and subsequent sanitation must remove or destroy 99.965% to 99.977% of these nuts in order to successfully meet the challenge of sanitation to ensure 2% or less kernel damage. Using these estimates, our current average sanitation efficiency ranged from 99.953% to 99.969%. Economic analysis is needed to establish a cost-benefit relationship between more stringent sanitation and economic return, in order to enable growers to determine the optimal amount of resources to devote to these practices.

Pistachio proximity. Pistachios as far away as 3 miles from the center of almond blocks may contribute to navel orangeworm damage. Further research

TABLE 3. Relationship between 'Nonpareil' kernel damage by navel orangeworm and distance to nearest pistachio margin, 2004-2006

Distance	Relative risk*	Damage \geq 2%	Sections
<i>miles</i>		%	<i>no.</i>
≤ 0.25	2.27†	55.2	87
≤ 0.50	2.15†	48.5	233
≤ 1.00	2.61†	45.2	577
≤ 1.50	3.29†	39.4	961
≤ 2.00	3.14†	33.6	1,258
≤ 2.50	2.18†	28.6	1,562
≤ 3.00	1.66‡	26.7	1,752

* Relative risk values > 1 indicate increased likelihood of navel orangeworm damage $\geq 2\%$.

† $P < 0.0001$.

‡ $0.005 > P > 0.001$.



is needed to develop a coordinated strategy for managing this pest in both crops, as well as to determine whether additional measures are necessary to manage almonds in proximity to pistachios. Initial studies on the extent of navel orangeworm movement between almonds and pistachios indicate that in pistachios, male navel orangeworm can move up to 1,100 yards in 1 day while females moved up to 100 yards in 1 day (Burks and Higbee 2006).

Conditions vary throughout the growing regions of the Central Valley and there are likely to be differences that influence the factors identified in the Kern County study. Therefore it is essential to validate these findings in other Central Valley areas. Collaborative studies between USDA, UC, UCCE and Paramount Farming Company researchers are under way as part of a newly established areawide program for the control of navel orangeworm in almonds, pistachios and walnuts.

B.S. Higbee is Research Entomologist, Paramount Farming Company, Bakersfield; and J.P. Siegel is Research Entomologist, USDA Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, Parlier. The authors thank the

staff of the Entomology Laboratory at Paramount Farming Company, particularly Lori Smith and Mike Bryant, for technical assistance and gathering the tremendous amount of data required for this study. We also thank Scott Guseman and Lyndi Smith for their assistance with data management and Gary Schengel for his invaluable help in mapping the orchards. We thank Lawrence Lacey and Joseph Smilanick for their constructive comments on the manuscript.

▲ **A large-scale study in Kern County almond orchards found that navel orangeworm damage to nuts can be brought below 2% by reducing the average number of mummies per tree to 0.2 or fewer, and the average number of ground mummies to four or fewer per tree. By the time trees bloom in the spring, sanitation should be complete, since it is difficult to perform once new growth appears.**

References

- [ABC] Almond Board of California. 2006. Orchard sanitation and navel orangeworm control. Fact sheet. Modesto, CA. 1 p. <http://almondboard.com>.
- Burks CS, Higbee BS. 2006. NOW mating disruption, dispersal and damage prediction. Proc 34th Annual Almond Industry Conf. Modesto, CA. p 1–19.
- Curtis CD. 1979. Implementing orchard cleanup. Almond Facts 44:20–1.
- Engle CE, Barnes MM. 1983. Cultural control of navel orangeworm in almond orchards. Cal Ag 37(9-10):19.
- Kelsey JL, Thompson WD, Evans AS. 1986. *Methods in Observational Epidemiology*. New York: Oxford Univ Pr. 366 p.
- [NASS] National Agricultural Statistics Service. 2006. 2005 Almond Acreage Report. US Department of Agriculture. Sacramento, CA. 8 p. www.nass.usda.gov.
- [OJEU] Official Journal of the European Union. 2007. Commission decision on aflatoxin contamination on products originating in the United States. Doc no. C(2007) 3613; Aug. 18, 2007. 3 p.
- Schatzki TF, Ong MS. 2001. Dependence of aflatoxins in almonds on the type and amount of insect damage. J Ag Food Chem 49:4513–9.
- Siegel JP, Kuenen LPS, Higbee BS, et al. 2008. Post-harvest survival of navel orangeworm assessed in pistachios. Cal Ag 62:30–5.
- [UC] University of California. 2006. Regional almond variety trials planted in 1993 progress report. UC Cooperative Extension and UC Davis. 26 p.
- UC IPM Online. 2007. UC Pest Management Guidelines; Almond; Navel Orangeworm. www.ipm.ucdavis.edu/PMG/r3300311.html (accessed October 2007).
- Wade WH. 1961. Biology of the navel orangeworm, *Paramyelois transitella* (Walker), on almonds and walnuts in northern California. Hilgardia 31:129–71.
- Zalom FG, Barnett WW, Weakley CV. 1984. Efficacy of winter sanitation for managing the navel orangeworm, *Paramyelois transitella* (Walker), in California almond orchards. Prot Ecol 7:37–41.

Pre- and Postharvest Treatments to Control Green Mold of Citrus Fruit During Ethylene Degreening

J. L. Smilanick, United States Department of Agriculture–Agricultural Research Service, San Joaquin Agricultural Sciences Center, Parlier, CA 93648; M. F. Mansour, Department of Horticulture, Menofiya University, Shebin El-Kom, Egypt; and D. Sorenson, Fruit Grower's Supply Co., Orange Cove, CA 93646

ABSTRACT

Smilanick, J. L., Mansour, M. F., and Sorenson, D. 2006. Pre- and postharvest treatments to control green mold of citrus fruit during ethylene degreening. *Plant Dis.* 90:89-96.

Two approaches, fungicide applications to trees before harvest and drenching fruit after harvest, were evaluated to minimize postharvest green mold, caused by *Penicillium digitatum*, particularly among fruit subjected to ethylene gas after harvest, a practice termed "degreening" that eliminates green rind color. Preharvest applications of thiophanate methyl (TM) controlled postharvest green mold consistently. In five tests, green mold among degreened orange fruit was 16% when TM was applied 1 week before harvest; whereas, among fruit not treated, the incidence was 89.5%. Thiabendazole (TBZ) applied to harvested fruit in bins before degreening also was very effective. TBZ effectiveness was enhanced by mild heating (41°C), adding sodium bicarbonate, and immersing fruit, rather than drenching them, with the solution. With these measures, an isolate of *P. digitatum* with a high level of TBZ resistance was significantly controlled. In semicommercial tests with naturally inoculated fruit, TBZ and sodium bicarbonate treatment reduced green mold incidence from 11% among untreated orange fruit to 2%. TBZ residues in lemon fruit at 41°C were about twice those treated at 24°C. Neither TM before harvest nor TBZ and sodium bicarbonate applied after harvest influenced green color removal during degreening of orange fruit. Sodium bicarbonate slightly reduced the rate of lemon color change.

Additional keywords: heat treatment

Citrus fruit harvested early in the season are often of acceptable internal maturity but are not of optimum rind color for commercial sale. "Degreening" is a common commercial practice in many parts of the world used to enhance the appeal of the fruit to consumers by the removal of the green color from the peel of orange and lemon fruit by exposure of the fruit immediately after harvest to ethylene gas. Rapid chlorophyll degradation and some carotenoid synthesis occurs during the treatment, which consists of exposure to ethylene at 5 to 10 $\mu\text{l liter}^{-1}$ at 90 to 95% relative humidity for 1 to 5 days at 20 to 22°C in California or 28 to 29°C in Florida (13,18,30). The environment during degreening in California is optimal for the development of green mold, caused by *Penicillium digitatum* (Pers.:Fr.) Sacc. Conversely, in Florida, degreening is conducted at temperatures that inhibit this pathogen, so that green mold incidence is of less importance

there (26). We have observed that losses by *P. digitatum* in California are typically 2 to 4% during degreening, but they may exceed 30% and be a serious problem during disease-conducive years, such as those where heavy rains occur before harvest, or when split fruit or other rind injuries occur on the trees at a high frequency. Protection of the fruit from postharvest decay during degreening is difficult because the fruit have not passed through a packing line, where fungicide applications typically are done, before they are exposed to ethylene gas. Degreening is conducted before the fruit are dumped from the field bins because waxing, cleaning, and other handling can reduce its effectiveness (8,20,23). Drenches of hot water or thiabendazole (TBZ), without other handling, did not influence the degreening rates of Hamlin orange fruit (8).

Approaches employed to minimize losses during degreening include fungicides applied to trees before harvest (4,10,24,31,45), drenching fruit in field bins with fungicides (10), and thermal curing treatments combined with degreening (46). In Florida, the first two approaches commonly are employed but they are not used to specifically control green mold. Some control of postharvest green mold occurs as a consequence of grove fungicide applications, which typically are done not to control green mold after har-

vest but to control melanose, greasy spot, postbloom fruit drop, and other grove diseases common in Florida but not California (42). *Diplodia stem-end rot*, caused by *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl., is the principle postharvest pathogen of concern on degreened citrus fruit in Florida (6), and applications of benomyl and thiophanate methyl in groves before harvest are effective to control it after harvest (31). Bin drenching before degreening in Florida with TBZ is done primarily to control stem-end rot, but some control of anthracnose and green and blue *Penicillium* molds also occurs (10,13). Interest in implementing these practices in California has increased recently because warm rains have occurred early in some harvest seasons and unusually large numbers of navel orange fruit split or cracked before harvest, which together have caused green mold losses during degreening to become unacceptably high in many packinghouses.

We evaluated two approaches to manage green mold, the primary postharvest disease that causes losses during degreening in California. An effective preharvest fungicide with continuing activity that persists to provide postharvest decay control after harvest would be a useful management tool for citrus growers (31,45). Therefore, we applied fungicides to trees one or more weeks before harvest and their effectiveness to control green mold on inoculated and degreened fruit was determined. The second approach was to evaluate drenching bins of harvested fruit with a fungicide before degreening. An effective postharvest fungicide that protects fruit during degreening would be a useful option for packinghouse managers. The fungicide selected for this purpose was TBZ, because of the long, successful use of this material in bin drenchers in Florida. To maximize TBZ effectiveness, we evaluated this material alone or in combination with chlorine, sodium bicarbonate, and heat.

MATERIALS AND METHODS

Pathogen culture. Two *P. digitatum* isolates, TBZ-sensitive (TBZ-s) and TBZ-resistant (TBZ-r) isolates M6R and D201, respectively, were cultured 1 to 2 weeks at 25°C on potato dextrose agar (PDA; Difco Laboratories, Detroit). Spores of each isolate, which originated from a single spore obtained from a lesion on infected fruit, were stored at -70°C on silica gel

Corresponding author: J. L. Smilanick
E-mail: jsmilanick@fresno.ars.usda.gov

Accepted for publication 23 August 2005.

DOI: 10.1094/PD-90-0089

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2006.

and recultured on PDA when needed. Spores of TBZ-s isolate M6R would not germinate on TBZ-amended PDA containing the fungicide at 0.1 µg/ml, whereas those of TBZ-r isolate D201 would germinate on PDA amended with TBZ at 15.0 µg/ml. Growth of an isolate on PDA amended with TBZ at ≥10 µg/ml indicates its resistance is commercially significant (13). Green mold caused by inoculation of TBZ-s isolate M6R on lemon was controlled by TBZ applications of 500 µg/ml, a rate typically applied in California to citrus fruit, whereas this rate did not control TBZ-r isolate D201 (*data not shown*). Spores were collected by adding 5 ml of sterile, deionized water containing 0.05% Triton X-100 to the petri dish colony, rubbing the surface with a sterile glass rod, and passing the suspension through two layers of cheese cloth. The suspension was diluted with water to an absorbance of 0.1 at 425 nm, determined with a spectrophotometer, yielding about 1×10^6 spores/ml (17).

Fruit and inoculation procedures. 'Eureka' lemon (from Ventura County) and navel orange (from the San Joaquin Valley) were used in these experiments. Within 1 or 2 days after harvest, fruit were randomized, blemished fruit were removed, and each was inoculated using *P. digitatum* isolates M6R or D201 by dipping a steel rod with a 1-mm-wide and 2-mm-long tip into a suspension of about 1×10^6 spores/ml, and immediately making a single wound in each fruit with the rod. This is a recommended procedure for evaluating decay control methods for green and blue molds on citrus fruit (17); treatments that control infections made by this method generally work effectively against these diseases on naturally infected fruit.

Preharvest fungicide applications. Fungicides applied as grove sprays to navel orange trees before harvest were (i) azoxystrobin (Abound, 22.9% azoxystrobin; Syngenta Corp., Wilmington, DE) applied at 0.25 kg/ha; (ii) a mixture of cyprodinil and fludioxonil (Switch, 37.5% cyprodinil and 25% fludioxonil; Syngenta Corp., Greenboro, NC) applied at 0.40 and 0.27 kg/ha, respectively; (iii) pyraclostrobin (Headline, 23.6% pyraclostrobin; BASF Corporation, Research Triangle Park, NC) applied at 0.25 kg/ha; (iv) a mixture of boscalid and pyraclostrobin (Pristine, 25.2% boscalid and 12.5% pyraclostrobin; BASF Corporation) applied at 0.29 and 0.14 kg/ha, respectively; and (v) thiophanate methyl (Topsin M WSB, 70% thiophanate methyl; CerexAgri, King of Prussia, PA) applied at 1.58 kg/ha.

Fungicides were applied to navel orange trees with internally mature fruit with a dark green rind in five tests.

Test 1. One of five fungicides at rates previously described or water alone was applied at 2,800 liters/ha to 10 replicate Atwood navel orange trees with a 750-liter-

capacity gasoline-engine-powered handgun sprayer. Fruit were harvested from all treatments once 1 week later.

Test 2. Thiophanate methyl at 1.58 kg/ha or water alone was applied at 933 liters/ha to 10 replicate Washington navel orange trees by 25-liter-capacity gasoline-engine-powered handgun backpack sprayer. Fruit were harvested from the water and fungicide treatments once 1 week later.

Test 3. Thiophanate methyl at 1.58 kg/ha or water alone was applied at 4,665 liters/ha in a commercial grove to 20 Washington navel orange trees by 2,000-liter-capacity commercial air-blast sprayer. Fruit were harvested from the water and fungicide treatments once 1 week later.

Test 4. Thiophanate methyl at 1.58 kg/ha or water alone was applied at 2,800 liters/ha to 20 Washington navel orange trees by a 2,000-liter-capacity commercial air-blast sprayer. Fruit were harvested from the water and fungicide treatments 1, 3, 5, and 7 weeks after treatment.

Test 5. Thiophanate methyl at 1.58 kg/ha or water alone was applied at 2,800 liters/ha to 10 replicate Atwood navel orange trees by a 500-liter-capacity gasoline-engine-powered handgun sprayer. Fruit were harvested from the water and fungicide treatments 1, 4, and 7 weeks after treatment.

Tests 1, 2, and 5 used a randomized complete block design and were conducted at the University of California, Lindcove Research and Extension Center, Tulare County. Tests 3 and 4 were applied to two rows of 10 trees each separated by three untreated rows of trees. Test 3 was conducted in a commercial grove in Kern County, California. Test 4 was conducted at the University of California, Lindcove Research and Extension Center, Tulare County. Rainfall was recorded in California Irrigation Management Information System weather stations 86 or 138 that were located not more than 0.5 km from the groves.

On the day of harvest, an equal number of fruit were clipped by hand from trees and inoculated within 1 or 2 h and degreened for 3 days in ethylene at 5 µl/liter at 20°C when required. After storage for one additional week at 20°C, the number of infected fruit were counted. Each treatment included four replicates of 60 to 75 orange fruit each. In tests 4 and 5, in addition to 1 week after fungicide or water applications, fruit were harvested at later intervals up to 7 weeks after spray application. In test 1, color changes of the fruit during ethylene degreening were recorded. In all tests except test 1, three replicate samples of six fruit each were collected periodically and thiophanate methyl residues were determined using a procedure described later.

Postharvest fungicides applied to fruit. All fruit were inoculated as previously described and stored at 20°C about

24 h before treatments were applied. Unless indicated otherwise, fruit were immersed in 15 liters of each solution contained within 22-liter-capacity stainless steel tanks, where the temperature was maintained by a computer-controlled electric heating element and thermostat, and the solution was stirred continuously. Solution contents included TBZ (98.5% a.i., Fresh Ban 4000; Fresh Mark Corp., Ocoee, FL), sodium hypochlorite (Sigma-Aldrich, Chicago), and sodium bicarbonate (NaHCO₃; Sigma-Aldrich). Sodium hypochlorite content expressed as free chlorine, the sum of OCl⁻ + HOCl, was measured by the DPD method (38) with a colorimeter (Model DR890; Hach, Inc. Loveland, CO).

Temperature influence on postharvest TBZ effectiveness. Light-green lemon fruit were inoculated with TBZ-s isolate M6R as described previously. After 24 h, they were immersed for 60 s in aqueous solutions of TBZ at 0, 25, 50, and 100 µg/ml at temperatures of 16, 27, 38, or 49°C. All solutions contained chlorine (200 µg/ml) and Triton X-100 (0.2 ml/liter). The fruit were not rinsed after treatment. Each treatment included four replicates of 27 lemon fruit each. Green mold incidence on lemon was evaluated after storage for 1 week at 20°C and 95% relative humidity. The experiment was done once.

Temperature, sodium bicarbonate, and chlorine influence on postharvest TBZ effectiveness. Light-green lemon fruit were inoculated using both TBZ-s and TBZ-r isolates as described previously. After 24 h, they were immersed for 60 s in TBZ at 350 µg/ml alone or combined with sodium bicarbonate (NaHCO₃; 3% wt/vol) and chlorine (200 µg/ml). The solution temperatures were 13 or 41°C. The fruit were rinsed briefly with fresh tap water after treatment. Each treatment included three replicates of 75 lemon fruit each. Green mold incidence was evaluated after storage for 3 weeks at 10°C and 95% relative humidity. The experiment was done twice, once with *P. digitatum* TBZ-s isolate M6R and once with *P. digitatum* TBZ-r isolate D201.

Method of postharvest TBZ application and solution temperature on TBZ effectiveness. Light-green lemon fruit were inoculated as described previously. After 24 h, they were immersed or drenched with an aqueous TBZ suspension that contained TBZ at 372 µg/ml alone or combined with sodium bicarbonate (3% wt/vol) and chlorine (200 µg/ml). Fruit were either drenched or immersed for 60 s in the test solutions at 13 or 41°C. Fruit were immersed in 29-liter-capacity jars, passed through a 2,000-liter-capacity tank, or they were drenched with a recirculated, low-pressure, high-volume (33.6 liters/min) spray. The tank (40) and drencher (41) were described in prior publications. Fruit were rinsed for several seconds with fresh water after the immersion treatments but

not after the drench treatments. Each treatment was applied to three replicates of 75 lemon fruit each. Green mold incidence on fruit was evaluated after storage for 5 weeks at 10°C and 95% relative humidity. The experiment was done twice, once with *P. digitatum* TBZ-s isolate M6R and once with *P. digitatum* TBZ-r isolate D201.

Performance of postharvest TBZ, bicarbonate, and chlorine solutions with naturally inoculated orange fruit. Navel orange fruit were drenched in field bins before degreening to reduce decay. Naturally infected orange fruit in field bins were harvested from groves near Sanger, CA, and treated in a packinghouse in Orange Cove, CA. Control fruit were untreated, whereas those treated were drenched for about 15 s with a recirculated solution containing TBZ (350 µg/ml), sodium bicarbonate (3% wt/vol), and chlorine (200 µg/ml) at the ambient temperature (about 15°C), and then dried in air for several hours. All fruit then were placed for 2 days in ethylene at 5 µl/liter at 20°C and 90 to 95% relative humidity and stored for an additional 7 days at 10°C. Afterwards, they were placed on a commercial packing line, where the infected fruit were removed by hand and their number recorded. The test was repeated five times with orange fruit from different groves harvested in November and December 2002. The number of navel orange fruit examined from each treatment from groves 1, 2, 3, 4, and 5 was approximately 37,500, 12,000, 36,000, 45,000, and 36,000, respectively.

Influence of postharvest TBZ concentration and solution temperature on TBZ residues. Uninoculated lemon fruit were immersed for 1 min in one of the test solutions at either 24 or 41°C. TBZ content in the aqueous solutions was 0, 150, 250, 500, or 1,000 µg/ml. Lemon fruit were dried in air before packing in plastic bags or cans. Each treatment consisted of four replicates 12 fruit each. The fruit were stored at 4°C and residues were determined within several days. The test was repeated twice.

Influence of pre- and postharvest treatments on fruit surface color during ethylene degreening. The rate of surface color change of the fruit in test 1, where five fungicides had been applied to navel orange trees 1 week before harvest, was determined during ethylene degreening beginning immediately after harvest. Washington navel orange and Eureka lemon fruit that were dark green in color were either untreated (dry control) or immersed for 60 s in water (wet control), TBZ (200 µg/ml), sodium bicarbonate (3% wt/vol), or a combination of TBZ and sodium bicarbonate. Fruit were not rinsed after treatment. The test was repeated twice with Washington navel orange fruit and once with Eureka lemon fruit. Each treatment included four replicates of 20

fruit each. The fruit were placed in ethylene at 5 µl/l at 20°C and 90 to 95% relative humidity. The rate of color change during ethylene degreening was determined using the Lab system of color notation (27) measured with a colorimeter (model CR200; Minolta Corp., Tokyo). L*, a*, and b* measurements were made initially and every 24 h thereafter and continued until most all green color was absent. Observations of a minimum of 40 fruit were recorded. L* and calculated hue angle values were used for statistical analysis.

Residue analysis. Thiophanate methyl residues in fruit were determined using a method described by Gorbach (19). Fruit (three to five) were placed inside a steel can containing 100 ml of methanol; the container was sealed and rolled slowly at 10 revolutions/min. After 45 min, the container was removed, a portion of the methanol extract was passed through a paper filter (no. 1 Whatman), and 15 µl was injected into a high-performance liquid chromatograph with a mobile phase of acetonitrile:water (40:60) with a UV detector operating at 235 nm. TBZ residues were determined by the "Merck" method as described by Norman et al. (28). Twelve fruit within each replicate were extracted together as described previously, except with ethyl acetate instead of methanol. The surface-stripping solution was passed through a paper filter and the optical density measured with a spectrofluorometer at 360 nm with an excitation wavelength of 302 nm.

Statistical analysis. The incidence of green mold in all experiments, except where the performance of postharvest TBZ, bicarbonate, and chlorine solutions with naturally inoculated orange fruit was assessed in a commercial packinghouse, was evaluated by an analysis of variance applied to the arcsin of the square root of the proportion of infected fruit. The fruit surface L* and hue angle color measurements and fruit residue contents were analyzed by analysis of variance. Fisher's protected least significant difference test ($P \leq 0.05$) was used to separate means. Actual values are shown. A paired *t* test was applied to detect significant differences in the incidence of decay among the

five groups of control and treated orange fruit in the commercial packinghouse test where the performance of postharvest TBZ, sodium bicarbonate, and chlorine solutions applied to naturally inoculated orange fruit was assessed.

RESULTS

Preharvest fungicide treatments. Although all fungicides used in this study significantly decreased postharvest green mold among degreened navel orange fruit, azoxystrobin and thiophanate methyl were the most effective and reduced the disease by 20.1 and 80.6%, respectively (Table 1). Thiophanate methyl as a preharvest spray was the most effective fungicide and was selected for all subsequent grove experiments. None of the fungicides applied to navel orange trees in test 1 influenced the rate of color change (*data not shown*).

In subsequent tests, thiophanate methyl consistently reduced green mold among navel orange fruit by 78.1 to 90.8% compared with the untreated control when the fruit were harvested 1 week after application (Table 2). Residues of thiophanate methyl in fruit harvested 1 week after application were 0.3 to 3.6 µg/g fresh weight depending upon the method of application. In tests 4 and 5, green mold control persisted with some decline in effectiveness up to 7 weeks after application. In test 4, thiophanate methyl residues persisted and increased slightly when measured repeatedly up to 7 weeks after application.

Postharvest fungicide treatments. TBZ effectively controlled green mold on lemon fruit at all concentrations and temperatures tested (Fig. 1). At 49°C, significant control of green mold by the heated-water treatment occurred and TBZ was very effective; treatment with TBZ at 100 µg/ml almost eliminated green mold. Addition of sodium bicarbonate or heat to the TBZ solution significantly improved its effectiveness, even when a TBZ-r isolate of *P. digitatum* was used (Fig. 2). Among lemon fruit immersed for 1 min in TBZ at 350 µg/ml that also contained sodium bicarbonate (3% wt/vol) and chlorine (200 µg/ml), green mold incidence was significantly lower than among those treated with TBZ alone, at both 13 and 41°C. Green

Table 1. Green mold incidence on navel orange fruit as affected by preharvest application of different fungicides³

Product	Fungicide	Rate (kg/ha)	Green mold (%) ²
Control	98.9 a
Switch	Cyprodinil + fludioxonil	0.40 + 0.27	95.8 b
Pristine	Boscalid + pyraclostrobin	0.29 + 0.14	92.0 bc
Headline	Pyraclastrobin	0.25	89.4 c
Abound	Azoxystrobin	0.25	79.0 d
Topsin M WSB	Thiophanate methyl	1.58	19.2 e

³ Fruit were harvested 1 week after application, inoculated with *Penicillium digitatum* isolate M6R, degreened for 3 days in air containing ethylene at 5 µl/liter at 20°C, then stored at 20°C for one additional week.

² Values followed by unlike letters differ significantly by Fisher's protected least significant difference test ($P = 0.05$). An arcsin transform was applied before analysis of variance; actual values are shown.

mold caused by both isolates was particularly reduced by treatment with the combination solution applied at 41°C. Green mold caused by the TBZ-r isolate was reduced by the combination treatment at 41°C to 14%, compared with about 55% with TBZ alone.

The method of application of TBZ significantly influenced its effectiveness. Immersion of lemon fruit for 1 min in a mixture that contained TBZ at 372 µg/ml, sodium bicarbonate (3% wt/vol), and chlorine (200 µg/ml), was more effective than

drenching fruit with this solution for the control of green mold (Fig. 3). When fruit were immersed at the warmer (41°C) temperature, it controlled both the TBZ-s and the TBZ-r isolates effectively. Heating the drencher solution did not improve its effectiveness.

Among naturally inoculated orange fruit treated with TBZ, sodium bicarbonate, and chlorine at 15°C before degreening, the number of decayed fruit was significantly (paired *t* test, $P < 0.001$) reduced among fruit from all five groves compared with

Table 2. Influence of a preharvest application of thiophanate methyl (TM) on postharvest residues and the incidence of green mold^a

Test	Interval ^w	Rainfall ^x	Residue ^y	Green mold incidence (%) ^v		
				TM	Control	Reduction ^z
1	1	1.0	...	17.4	79.4	78.1
2	1	1.0	3.6	17.5	95.1	81.6
3	1	16.5	0.3	8.9	96.9	90.8
4	1	4.1	2.1	13.1	77.0	83.0
	3	28.5	4.8	6.8	80.4	91.5
	5	36.6	4.3	17.0	87.7	80.6
	7	67.6	5.1	26.0	90.5	71.3
5	1	6.1	...	19.2	98.9	80.6
	4	27.5	1.8	41.2	99.1	58.4
	7	51.6	...	31.8	100.0	68.2

^a Navel orange fruit harvested 1 week after TM or water (control) applications were inoculated with spores of *Penicillium digitatum* isolate M6R, degreened immediately for 3 days in ethylene at 5 µg/ml at 20°C, then stored for an additional 4 days at 20°C. Fruit harvested more than 1 week after TM or water treatment were inoculated with *P. digitatum* and stored for 1 week at 20°C.

^v Green mold determined after 7 days of storage at 20°C after degreening or harvest. Each value is the mean of four replicates containing 60 to 75 fruit each.

^w Interval (weeks) between application of treatments to trees and harvest.

^x Rainfall (mm) between application of the treatments and harvest.

^y Thiophanate methyl residues in fruit (µg/g of fresh weight). Each value is the mean of two or three replicates of eight fruit each.

^z Percent reduction in green mold compared with the control.

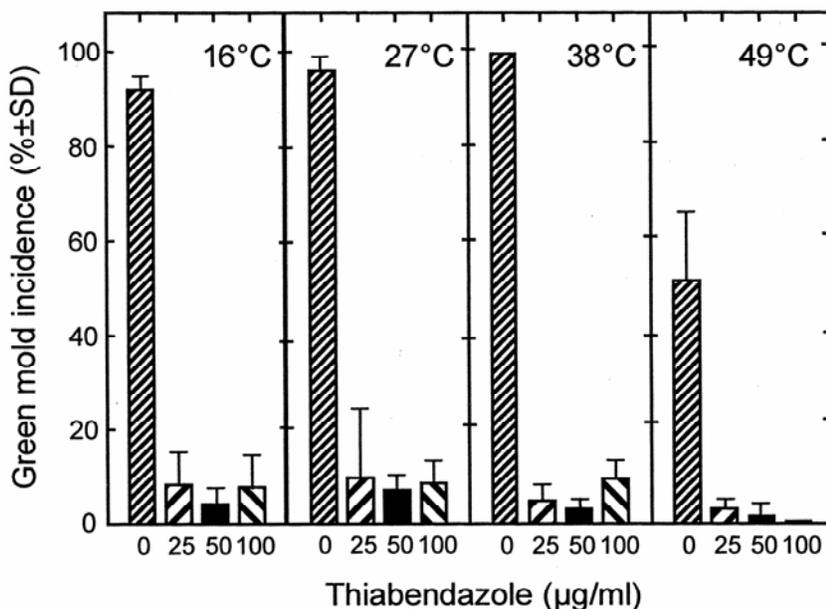


Fig. 1. Influence of thiabendazole (TBZ) solution temperature on its effectiveness to control green mold. Light-green lemon fruit were inoculated with spores of TBZ-sensitive isolate M6R of *Penicillium digitatum*. After 24 h at 20°C, they were immersed for 60 s in aqueous solutions containing TBZ at 0, 25, 50, or 100 µg/ml at temperatures of 16, 27, 38, or 49°C. All solutions contained chlorine (200 µg/ml) and Triton X-100 (0.2 ml/liter), and the fruit were not rinsed after treatment. Each treatment was applied to four replicates of 27 lemon fruit each. Green mold incidence was evaluated after storage for 1 week at 20°C and 95% relative humidity.

untreated fruit (Fig. 4). The average incidence of decayed fruit among untreated fruit was about 11%; whereas, among fruit that were drenched before degreening it was about 2%. Green mold was the most commonly encountered disease.

TBZ residues within the fruit increased linearly when the aqueous TBZ concentration used to treat the lemon fruit increased from 150 to 1,000 µg/ml (Fig. 5). Raising the temperature of the TBZ solution from 24 to 41°C significantly increased the residues about 1.5- to 2-fold.

Change in the rind color expressed as change in hue angle (Fig. 6) was not significantly affected when navel orange fruit were treated with TBZ, sodium bicarbonate, or their combination. However, color change was slightly but significantly delayed in lemon fruit treated with sodium bicarbonate either alone or combined with TBZ.

DISCUSSION

We have observed that the ethylene degreening process, which in California entails exposure of the fruit to ethylene at 5 µl/liter for as long as 5 days, can be plagued by unacceptably high postharvest decay losses on citrus fruit. Green mold is the most important disease during degreening in California for several reasons. In Florida and other subtropical citrus-growing climates, *Diplodia* stem-end rot, caused by *Lasiodiplodia theobromae*, is a common postharvest disease; whereas, in California and other arid growing areas, it is uncommon (18). Losses by *Diplodia* stem-end rot are greatly exacerbated by degreening (2), because ethylene both

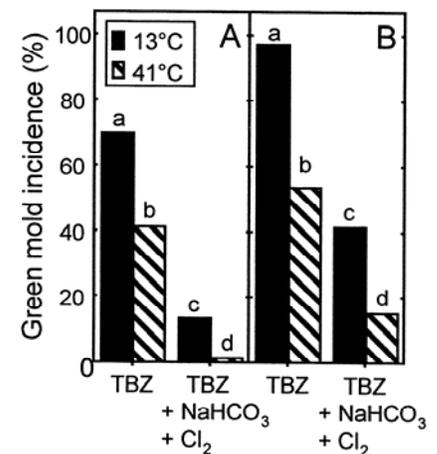


Fig. 2. Influence of the addition of sodium bicarbonate (3% wt/vol) and chlorine (200 µg/ml) and temperature on the effectiveness of immersion for 60 s in thiabendazole (TBZ; 350 µg/ml) to control green mold on lemon fruit inoculated with **A**, TBZ-sensitive isolate M6R or **B**, TBZ-resistant isolate D201 of *Penicillium digitatum*. The fruit were stored for 3 weeks at 10°C before the number of infected fruit was counted. Each column is the mean of three replicates of 75 fruit. Columns within each panel with unlike letters differ significantly ($P \leq 0.05$).

stimulates the growth of this pathogen (11) and accelerates abscission enzymes that predispose fruit to infection (9). Conversely, ethylene does not stimulate *P. digitatum* growth and can even reduce green mold incidence slightly (29). Furthermore, partial control of green mold occurs during the relatively high-temperature (28 to 29°C) degreening conducted in Florida, which does not occur in California because degreening is conducted at cooler temperatures (20 to 21°C) to minimize rind blemishes (20). Ethylene typically is applied to fruit in field bins, and packing line fungicide treatments necessarily are delayed until after the fruit are degreened and washed. However, because degreening can take as long as 5 days, these fungicide applications are too late and their effectiveness for the control of green mold is poor because the pathogen has invaded the rind too deeply by this time to be inhibited by the fungicide. Much better control can be achieved by applying the fungicide before degreening, as either a preharvest spray or postharvest drench, followed after degreening by additional packing line fungicide treatments (10). We found that both of these approaches, commonly employed in Florida, were very effective

under California conditions with few modifications.

Thiophanate methyl applied once as a grove spray before harvest proved to be very effective in controlling green mold, even when the tests were conducted during rainy periods and the fruit were harvested and inoculated as long as 7 weeks following application (Tables 1 and 2). Protection was good, particularly when the fruit were picked 1 week after thiophanate methyl application, even when thiophanate methyl residues were as low as 0.3 µg/g. Green mold did not develop in the rind injuries inoculated with spores of *P. digitatum* because the thiophanate methyl residue

within the rind, remaining from the grove application, had protected the fruit. In other citrus-growing areas, preharvest applications of benzimidazole fungicides consistently have been shown to control postharvest decay effectively when applied before harvest. Grove applications of benomyl (7,12,45), TBZ (3,10,35), carbendazim (24,25), or thiophanate methyl (24,31) all provided good to excellent control of postharvest decay. Presumably, this effectiveness is the result of their systemic activity and persistent residues.

Thiophanate methyl residues within orange fruit were persistent in our work, which explains why this fungicide protected the fruit effectively from infection during and after the degreening process. In test 4, thiophanate methyl residues were significantly lower when measured 1 week after application than when sampled 3, 5, and 7 weeks later. Thiophanate methyl, benomyl, and carbendazim (methyl 2-benzimidazolecarbamate) have the same mode of action (16) and their performance in many citrus applications is similar

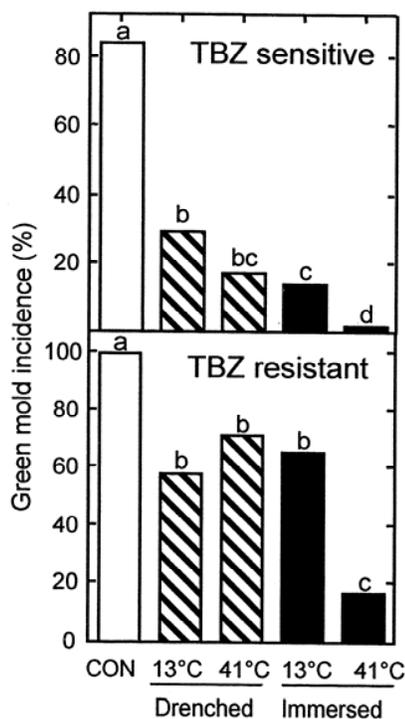


Fig. 3. Green mold incidence among lemon fruit treated with a solution containing sodium bicarbonate (3% wt/vol), thiabendazole (TBZ; 350 µg/ml), and chlorine (200 µg/ml). The fruit were inoculated 24 h before treatment with a TBZ-sensitive (M6R) or a TBZ-resistant (D201) isolate of *Penicillium digitatum*. The solution was drenched over the fruit or they were immersed for 60 s before storage for 5 weeks at 10°C.

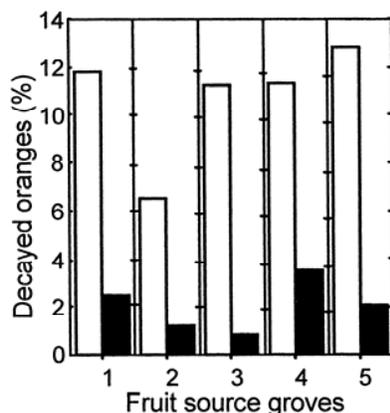


Fig. 4. Natural incidence of decayed orange fruit present after no treatment (white column) or drenching (black column) them in field bins for 15 s at 15°C with a solution containing thiabendazole (350 µg/ml), sodium bicarbonate (3% wt/vol), and chlorine (200 µg/ml) in a commercial packinghouse. All fruit were exposed to ethylene at 5 µl/liter for 2 days at 20°C followed by storage for 7 days in air at 10°C. The numbers of orange fruit examined from each treatment from navel orange groves 1, 2, 3, 4, and 5 were approximately 37,500, 12,000, 36,000, 45,000, and 36,000, respectively. The number of decayed orange fruit was significantly (paired *t* test, $P < 0.001$) lower after the drench treatment.

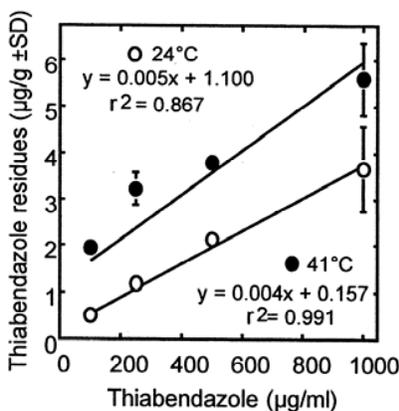


Fig. 5. Influence of thiabendazole solution concentration and temperature on its residues in lemon fruit. The fruit were immersed for 60 s in aqueous solutions of thiabendazole at 24 or 41°C and dried in air. Each value is the mean of four replicates of 12 lemon fruit each.

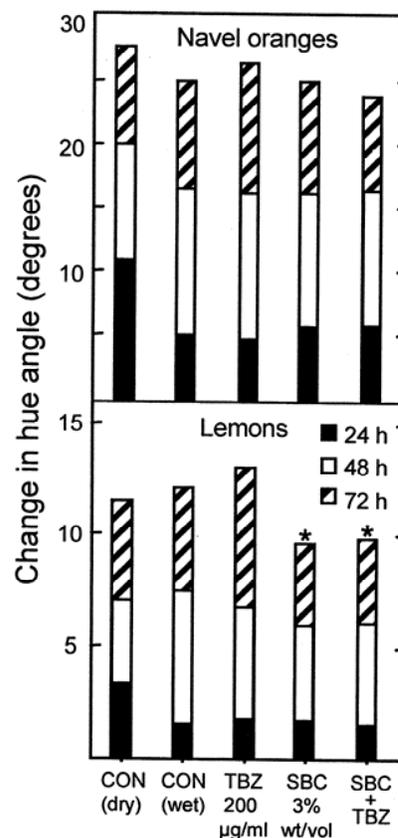


Fig. 6. Change in the rind surface color of navel orange and lemon fruit after immersion for 30 s in water, thiabendazole (TBZ; 200 µg/ml), sodium bicarbonate (SBC; 3% wt/vol), or a combination of SBC and TBZ. All treatments were followed by continuous exposure to ethylene gas at 5 µl/liter at 20°C. The initial hue angle (±standard deviation) of the navel orange and lemon fruit was 106.9 (±1.7) and 109.1 (±1.8), respectively. Asterisks indicate significant differences ($P \leq 0.05$).

(24,31). Carbendazim, occasionally used alone as a fungicide, also is a product of benomyl or thiophanate methyl hydrolysis, although this hydrolysis occurs much more slowly with thiophanate methyl than benomyl (19). We measured carbendazim residues in tests 2 and 3 1 week after thiophanate methyl application and carbendazim residues were less than 0.1 µg/g (*data not shown*). Brown (4) and Brown and Albrigo (7) measured citrus fruit benomyl residues after it was applied to trees. They were able to detect residues, primarily of carbendazim, on Valencia orange fruit up to 70 days after application that were sufficient to reduce the incidence of green mold. They reported that the highest carbendazim residues in the flavedo and albedo were found 14 days after spraying, and that residues on fruit surfaces decreased whereas those within the peel increased for a period of time as carbendazim moved into the fruit. They found that residues of 0.1 to 0.9 µg/g in the orange fruit were measured after application of 1 to 1.5 lbs of benomyl per acre, and residues of 0.14 µg/g were "marginal" to control green mold. In our work, thiophanate methyl residues of 0.3 µg/g were sufficient to give good protection of the fruit from infection. Brown (4) showed that carbendazim residues were relatively resistant to rainfall; 78 and 20% of the residues were removed by simulated rainfall applied immediately and 2 weeks after application, respectively.

When citrus fruit are wounded and inoculated after treatment, such as we did in the grove experiments, effective control of green mold typically requires a persistent systemic fungicide (21). This may explain the poor performance of the other locally systemic and nonsystemic fungicides in our trial (Table 1). Conversely, in packinghouses, both systemic and nonsystemic fungicides can be very effective, because these fruit are treated after wounding and inoculation have occurred as a consequence of harvest operations and subsequent handling before they are treated on packing lines (22). For example, although fludioxonil and azoxystrobin were not effective in our tests, they can very effectively control green mold when applied to inoculated fruit (15,34). It is conceivable that nonsystemic fungicides could be effective treatments in grove applications, if they are able to inactivate sufficient inoculum in the grove to an extent that wounds inflicted during harvest are not inoculated because infective spores are not present.

The use of benzimidazole fungicides in Florida before degreening is a well-studied practice (5,10). Aqueous preharvest sprays with benomyl or TBZ, or a postharvest drench application of TBZ applied before degreening, all effectively controlled decay, which was primarily *Diplodia* stem-end rot. For effective control of *Diplodia* stem-end rot and green mold, a TBZ resi-

due of at least 0.2 µg/g or greater on a whole fruit fresh weight basis was needed, and the residue must be present under the button and within injuries, where most fungal penetration occurs. TBZ bin treatments before degreening were more effective than treatments after degreening, because treatments applied afterward were too late to effectively stop these pathogens.

In our study, a TBZ-sensitive isolate (M6R) of *P. digitatum* was adequately controlled by TBZ at rates lower than 100 µg/ml (*data not shown*). Raising the temperature of this TBZ solution to 49°C and the addition of sodium bicarbonate greatly increased its effectiveness (Fig. 1). The combination of TBZ, a higher solution temperature (41°C), and sodium bicarbonate significantly controlled a TBZ-resistant isolate (D201) of *P. digitatum* (Figs. 2 and 3). Immersing the fruit in the solution was superior to drenching them with it. In the commercial trials, however, the fruit were drenched with an ambient-temperature chlorinated solution of TBZ and sodium bicarbonate and the treatment was effective enough that immersing the fruit in a heated solution was determined to be unnecessary. If TBZ-resistant isolates were present, or if the interval between harvest and treatment was prolonged, these added measures might be cost effective commercially.

The improved performance could be explained by an increase in TBZ residues caused by heat and the inhibition of the fungus by sodium bicarbonate (Fig. 5; 39). Residues of TBZ in citrus fruit are significantly correlated with TBZ concentration of the treatment suspension and solution temperature, but not related to the duration of the treatment (14,33,43). Residues of TBZ in fruit after treatment at 50°C were about two-and-one-half times higher than those after treatment at 20°C (14). The higher residue levels detected following the warmer treatment could be due to an increase in the rate of diffusion of the fungicide across the plant cuticle.

The mechanism of synergy between sodium bicarbonate and TBZ is not known. Similar synergy was observed with imazalil and sodium bicarbonate (39). Sodium bicarbonate raises and buffers the pH of the TBZ solution (which may increase TBZ residues); pH above 8 inhibits *P. digitatum* growth, and sodium bicarbonate inhibits *P. digitatum*, even at neutral pH (39). Wardowski and coworkers (43) found that fruit residues of TBZ increased linearly when the aqueous TBZ solution pH increased; residues after treatment with TBZ solutions at pH 7.9 and 10.8 were about 0.5 and 1.4 µg/g, respectively. The pK_a of TBZ is 5.82 (at 25°C and ionic strength of 0.1 M) (1); therefore, as pH increases, TBZ water solubility decreases. The increase in TBZ effectiveness we observed could be related to the increase in pH caused by the addition of sodium bi-

carbonate. TBZ residues in fruit treated with the flood recovery system were higher than would be expected with the aqueous spray or water wax application systems (43). This supports our findings that, when drenching and immersion methods of TBZ application were compared, the immersed fruit showed much lower green mold incidence than drenched ones. In most of the world, the residue tolerance of TBZ for citrus fruit is 10 mg/kg (whole fruit basis) and fruit in our tests did not exceed this.

In this work, we found that TBZ performance was improved by sodium bicarbonate, so that even a TBZ-resistant isolate of *P. digitatum* was controlled. Recently, we observed similar improvement in imazalil performance where control of imazalil-resistant isolates occurred when it was combined with sodium bicarbonate (39). Addition of sodium bicarbonate to these fungicides is particularly useful in packinghouses, where isolates resistant to both fungicides are common. Although sodium bicarbonate improved fungicide performance, disposal of the used and soiled fungicide solutions is made more difficult by its presence because of its high salt content and pH. Sodium bicarbonate residues increase the rate of fruit water loss, particularly if the fruit are not waxed after treatment and the salt residue remains on them for prolonged periods. Sodium bicarbonate also slightly but significantly delayed the color development of lemon fruit, but not navel orange fruit, during ethylene degreening.

A favorable aspect of both benzimidazole fungicides and postharvest heat treatments is that they can improve the rind quality of citrus fruit during prolonged cold storage by reducing chilling injury. Postharvest treatments with TBZ or benomyl reduced the incidence of chilling injury in grapefruit as expressed by peel pitting (32). Postharvest treatments with TBZ at 1,200 µg/ml at room temperature or at 200 µg/ml at 50°C resulted in similar TBZ residues in Tarocco orange fruit, although TBZ treatments at 50°C more effectively reduced chilling injury symptoms (37). Susceptibility of grapefruit and orange to chilling injury could be reduced by dipping fruit in hot TBZ either before, or in conjunction with, packing line treatments (36,44). The reduction of chilling injury in 'Star Ruby' grapefruit caused by TBZ is not related to its fungicidal properties but may be related to its antioxidant properties, which are stimulated in flavedo tissue by both TBZ and heat treatment (37). Lower doses of TBZ applied with heat were more effective in reducing chilling injury compared with higher doses at room temperature.

In this work, we show two methods, similar to each other in effectiveness, that can minimize postharvest green mold losses that occur during degreening in

California. Preharvest applications of thiophanate methyl did not influence the rate of rind color change during degreening, whereas postharvest treatments containing sodium bicarbonate either alone or with TBZ resulted in detectable but minor delay in the rate of color change of lemon fruit during degreening. The cost of these treatments is not prohibitive when compared with the value of the fruit lost without them when disease incidence is high. Preharvest treatment of trees with thiophanate methyl is a useful option for fruit growers, particularly because only one application was needed and the protection it provided was very persistent. This treatment is best used only in seasons conducive to significant green mold losses because, if used repeatedly, it could lead to the development of thiophanate methyl-resistant isolates in groves, which would also be resistant to TBZ used in packinghouses. Postharvest bin drenching with TBZ and sodium bicarbonate is a practice that would be implemented by packinghouse managers. Sodium bicarbonate and heat enhanced TBZ activity such that even a TBZ-resistant isolate of *P. digitatum* was controlled.

ACKNOWLEDGMENTS

We thank J. Doctor and R. Elliott of Sunkist Growers Fruit Sciences for many useful ideas and encouragement; R. Whitson of Cerexagri for the donation of materials, fungicide residue analysis, and other assistance; S. Wartanessian and M. Sales of Decco Cerexagri for fungicide residue analysis and other advice; J. Maze and W. Stutzman of the University of California Lindcove Citrus Research and Extension Center for the use of their groves and packing line facility; and manager K. Bramer of the Harding and Leggett packinghouse in Orange Cove, CA, for conducting large-scale commercial tests with TBZ and sodium bicarbonate. This work was done with financial assistance supplied by the California Citrus Research Board.

LITERATURE CITED

- Alvarez, J. L. M., Calzon, J. G., and Fonseca, J. M. L. 1997. Determination of pK_a for protonated thiabendazole and the stability constant for the Ni(II)-thiabendazole complex from catalytic polarographic currents. *Bull. Soc. Chim. Belges* 106:733-736.
- Barmore, C. R., and Brown, G. E. 1985. Influence of ethylene on increased susceptibility of oranges to *Diplodia natalensis*. *Plant Dis.* 69:228-230.
- Brown, G. E. 1968. Experimental fungicides applied preharvest for control of postharvest decay in Florida citrus fruit. *Plant Dis. Rep.* 52:844-847.
- Brown, G. E. 1974. Postharvest citrus decay as affected by Benlate applications in the grove. *Proc. Fla. State Hortic. Soc.* 87:237-240.
- Brown, G. E. 1977. Application of benzimidazole fungicides for citrus decay control. *Proc. Int. Soc. Citricult.* 1:273-277.
- Brown, G. E. 1986. *Diplodia* stem-end rot, a decay of citrus fruit increased by ethylene degreening treatment and its control. *Proc. Fla. State Hortic. Soc.* 99:105-108.
- Brown, G. E., and Albrigo, L. G. 1972. Grove application of benomyl and its persistence in orange fruit. *Phytopathology* 62:1434-1438.
- Brown, G. E., and Baraka, M. A. 1996. Effect of washing sequence and heated solutions to degreened Hamlin oranges on *Diplodia* stem-end rot, fruit colour and phytotoxicity. *Proc. Int. Soc. Citricult.* 2:1164-1170.
- Brown, G. E., and Burns, J. K. 1998. Enhanced activity of abscission enzymes predisposes oranges to invasion by *Diplodia natalensis* during ethylene degreening. *Postharvest Biol. Technol.* 14:217-227.
- Brown, G. E., and Craig, J. O. 1989. Effectiveness of aerosol fungicide applications in the degreening room for control of citrus fruit decay. *Proc. Fla. State Hortic. Soc.* 102:181-185.
- Brown, G. E., and Lee, H. S. 1993. Interactions of ethylene with citrus stem-end rot caused by *Diplodia natalensis*. *Phytopathology* 83:1204-1208.
- Brown, G. E., and McCornack, A. A. 1969. Benlate, an experimental preharvest fungicide for control of postharvest citrus fruit decay. *Proc. Fla. State Hortic. Soc.* 81:39-43.
- Brown, G. E., and Miller, W. R. 1999. Maintaining fruit health after harvest. Pages 175-188 in: *Citrus Health Management*. L. W. Timmer and L. W. Duncan, eds. The American Phytopathological Society Press, St. Paul, MN.
- Cabras, P., Schirra, M., Piriei, F. M., Garau, V. L., and Angioni, A. 1999. Factors affecting imazalil and thiabendazole uptake and persistence in citrus fruits following dip treatments. *J. Agric. Food Chem.* 47:3352-3354.
- Cochran, A., Tally, A., and Tedford, E. 2003. Evaluation of tank mixtures of fludioxonil and azoxystrobin for postharvest disease control on citrus. (Abstr.) *Phytopathology* 93:S17.
- Davidse, L. C. 1986. Benzimidazole fungicides: Mechanism of action and biological impact. *Annu. Rev. Phytopathol.* 24:43-65.
- Eckert, J. W., and Brown, G. E. 1986. Evaluation of postharvest treatments for citrus fruits. Pages 92-97 in: *Methods for Evaluating Pesticides for Control of Plant Pathogens*. K. D. Hickey, ed. American Phytopathological Society, St. Paul, MN.
- Eckert, J. W., and Eaks, I. L. 1989. Postharvest disorders and diseases of citrus fruits. Pages 179-260 in: *The Citrus Industry*, vol. V. W. Reuther, E. Calavan, E. C., and G. E. Carman, eds. University of California Press, Riverside.
- Gorbach, S. 1980. A review of methods for the residue analysis of the systemic fungicide benomyl, carbendazim, thiophanate methyl, and thiabendazole. *Pure Appl. Chem.* 52:2569-2590.
- Grierson, W. Cohen, E., and Kitagawa, H. 1986. Degreening. Pages 253-274 in: *Fresh Citrus Fruits*. W. F. Wardowski, S. Nagy, and W. Grierson, eds. AVI Book of Van Nostrand Reinhold Company, New York.
- Gutter, Y. 1969. Comparative effectiveness of benomyl, thiabendazole, and other antifungal compounds for postharvest control of *Penicillium* decay in Shamouti and Valencia oranges. *Plant Dis. Rep.* 53:474-478.
- Gutter, Y. 1969. Effectiveness of preinoculation and postinoculation treatments with sodium orthophenate, thiabendazole, and benomyl for green mold control in artificially inoculated Eureka lemons. *Plant Dis. Rep.* 53:479-482.
- Jahn, O. L., Cubbedge, R. H., and Smoot, J. J. 1970. Effect of washing sequence on the degreening response and decay of some citrus fruits. *Proc. Fla. State Hortic. Soc.* 83:217-221.
- Kuramoto, T. 1976. Resistance to benomyl and thiophanate-methyl in strains of *Penicillium digitatum* and *P. italicum* in Japan. *Plant Dis. Rep.* 60:168-172.
- Ladaniya, M. S., and Singh, S. 1998. Postharvest technology of Nagpur mandarin *Citrus reticulata* Blanco. *Tech. Bull.* 2, NRC Citrus, Nagpur, India.
- McCornack, A. A. 1972. Effect of ethylene degreening on decay of Florida citrus fruit. *Citrus Ind.* 53:14-15.
- McGuire, R. G. 1992. Reporting of objective color measurements. *HortScience* 27:1254-1255.
- Norman, S. M., Fouse, D. C., and Craft, C. C. 1972. Thiabendazole residues on and in citrus fruit. *Agric. Food Chem.* 20:1277-1280.
- Porat, R., Weiss, B., Cohen, L., Daus, A., Goren, R., and Droby, S. 1999. Effects of ethylene and 1-methylcyclopropene on the postharvest qualities of "Shamouti" oranges. *Postharvest Biol. Technol.* 15:155-163.
- Ritenour, M. A., Miller, W. M., and Wardowski, W. W. 2003. Recommendations for Degreening Florida Fresh Citrus Fruits. Circular 1170. Horticultural Sciences Department, Florida Cooperative Extension Service, IFAS, University of Florida, Gainesville.
- Ritenour, M. A., Pelosi, R. R., Burton, M. S., Stover, E. W., Dou, H., and McCollum, T. G. 2004. Assessing the efficacy of preharvest fungicide applications to control postharvest diseases of Florida citrus. *HortTechnology* 14:58-62.
- Schiffmann-Nadel, M., Chalutz, E., Waks J., and Dagan, M. 1975. Reduction of chilling injury in grapefruit by thiabendazole and benomyl during long-term storage. *J. Am. Soc. Hortic. Sci.* 100:270-272.
- Schirra, M., Angioni, A., Ruggiu, R., Minelli, E. V., and Cabras, P. 1998. Thiabendazole uptake and persistence in lemons following postharvest dips at 50°C. *Ital. J. Food Sci.* 10:165-170.
- Schirra, M., Cabras, P., Angioni, A., and Brandolini, V. 2002. Residue levels and storage decay control in cv. Star Ruby grapefruit after dip treatments with azoxystrobin. *J. Agric. Food Chem.* 50:1461-1464.
- Schirra, M., Cabras, P., Angioni, A., D'hallewin, G., and Pala, M. 2002. Residue uptake and storage responses of Tarocco blood oranges after preharvest thiabendazole spray and postharvest heat treatment. *J. Agric. Food Chem.* 50:2293-2296.
- Schirra, M., D'hallewin, G., Cabras, P., Angioni, A., Ben-Yehoshua, S., and Lurie S. 2000. Chilling injury and residue uptake in cold-stored 'Star Ruby' grapefruit following thiabendazole and imazalil dip treatments at 20 and 50°C. *Postharvest Biol. Technol.* 20:91-98.
- Schirra, M., D'hallewin, G., Cabras, P., Angioni, A., and Garau, V. L. 1998. Seasonal susceptibility of Tarocco oranges to chilling injury as affected by hot water and thiabendazole postharvest dip treatments. *J. Agric. Food Chem.* 46:1177-1180.
- Smilanick, J. L., Aiyabei, J., Mlikota Gabler, F., Doctor, J., Sorenson, D., and Mackey, B. 2002. Quantification of the toxicity of aqueous chlorine to spores of *Penicillium digitatum* and *Geotrichum citri-aurantii*. *Plant Dis.* 86:509-514.
- Smilanick, J. L., Mansour, M. F., Margosan, D. A., Mlikota Gabler, F., and Goodwine, W. R. 2005. Influence of pH and NaHCO₃ on the effectiveness of imazalil to inhibit germination of spores of *Penicillium digitatum* and to control postharvest green mold on citrus fruit. *Plant Dis.* 89:640-648.
- Smilanick, J. L., and Sorenson, D. 2001. Control of postharvest decay of citrus fruit with calcium polysulfide. *Postharvest Biol. Technol.* 21:157-168.
- Smilanick, J. L., Sorenson, D., Mansour, M., Aiyabei, J., and Plaza, P. 2003. Impact of a brief postharvest hot water drench on decay, fruit appearance, and microbe populations of California lemons and oranges. *HortTechnology* 13:333-338.
- Timmer, L. W. 1999. Diseases of fruit and foliage. Pages 107-115 in: *Citrus Health Management*. L. W. Timmer and L. W. Duncan, eds. The American Phytopathological Society Press, St. Paul, MN.
- Wardowski, W. F., Hayward, F. W. and Dennis,

- J. D. 1974. A flood-recovery TBZ fungicide treatment system for citrus fruits. Proc. Fla. State Hortic. Soc. 87:241-243.
44. Wild, B. L. 1993. Reduction of chilling injury in grapefruit and oranges stored at 1°C by prestorage hot dip treatments, curing, and wax application. Aust. J. Exp. Agric. 33:495-498.
45. Yildiz, F., Kinay, P., Yildiz, M., Sen, F., and Karacali, I. 2005. Effects of preharvest applications of CaCl₂, 2,4-D and benomyl and postharvest hot water, yeast and fungicide treatments on development of decay on Satsuma mandarins. J. Phytopathol. 153:94-98.
46. Zhang, J., and Swingle, P. P. 2005. Effects of curing on green mold and stem-end-rot of citrus fruit and its potential application under Florida packing system. Plant Dis. 89:834-840.

RESEARCH LETTER

Environmentally regulated abiotic release of volatile pheromones from the sugar-based oral secretions of caribflies

Spencer S. Walse^{a,b*}, Hans T. Alborn^b and Peter E.A. Teal^b

^aUnited States Department of Agriculture, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, 9611 S Riverbend Ave, Parlier, CA, 93648 USA; ^bUnited States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural, and Veterinary Entomology, 1700 SW 23rd Dr, Gainesville, FL, 32604 USA

(Received 15 August 2008; final version received form 19 January 2009)

We report an abiotic mechanism for the emission of volatile insect pheromones that is controlled by environmentally induced change in the physicochemical properties of the sugar-based release matrix. Male *Anastrepha suspensa* (Loew) (caribflies) mark mating sites on leaf surfaces by depositing oral secretions that contain sugar, as well as, γ -hydroxy acid and γ -lactone forms of the diastereomeric aggregation pheromones, epianastrephin and anastrephin. The γ -hydroxy acids extend emission over many days via aqueous equilibrium with the thermodynamically less preferred, but more volatile, γ -lactones ($\sim 100:1$). A kinetic model, which supports a γ -lactone diffusion-limited rate, was generated and tested by measuring the effect of temperature and humidity under fixed and ambient atmospheric inputs, respectively. Results show that pheromone release from the markings occurs with a periodicity that parallels relative humidity and complements the daily pattern of the caribflies' reproductive and aggregative activity. This study provides an example of a physicochemical-based inter-organism communication strategy that has been mechanistically linked to the abiotic environmental processing of volatile chemical signals. The exploitation of this natural connectivity will spur environmentally sustainable chemistries, particularly pheromone-based alternatives to insecticide application.

Keywords: controlled release; pheromones; tephritid fruit flies; environmental processing; γ -lactones

Introduction

One strategy for the development of environmentally sustainable chemical technologies involves the mimicry of processes that mediate natural product interaction with the environment. Elucidating the fate and transport of semiochemicals, natural products that function as signals in inter-organism messages, can be particularly useful in this capacity because their interaction with biotic, as well as abiotic, environmental factors is intrinsic to their evolved utility. Natural biotic mechanisms of semiochemical release, attenuation, and preservation have been studied extensively and are often influenced by environmental parameters (e.g. photoperiod, temperature, and humidity); yet, corresponding literature regarding abiotic mechanisms is lacking. This is curious because understanding natural abiotic processes that influence semiochemical "transmission" will benefit many chemical technologies intended for environmental application.

Pest management practices based on the exploitation of volatile semiochemicals produced by insects provide a poignant example of such technologies. Their utility as sustainable species-specific alternatives to broadcast insecticide application is limited, in many cases, due to an ineffective abiotic release of semiochemicals into the environment. To date, almost all of the semiochemicals employed in this context are naturally released "biologically" by insects directly into air as a function of environmental factors; this type of mechanism is difficult to replicate. In contrast, volatile semiochemicals can also be released "abiotically" from materials deposited by insects for the purpose of marking a location for re-visitation. These natural abiotic mechanisms for semiochemical release, which are just beginning to be characterized, are easier to formulate effectively for use in pest control programs.

For example, in 2002 $\sim 500,000$ lbs. of organophosphorus insecticides were applied to citrus, in part to counteract tephritid fruit fly infestation (*J*) that has

*Corresponding author. Email: spencer.walse@ars.usda.gov

the potential to damage fruit worth ~\$7 billion to the US annually (2). To reduce non-target health risks, naturally produced sexual attractants (i.e. pheromones) can be used for controlling populations of certain tephritid species. In Florida, the Caribbean fruit fly (caribfly) (*Anastrepha suspensa* (Loew); (Diptera: Tephritidae)) represents a serious threat to citrus commodities and nine volatile compounds released by males have been identified that presumably act as pheromones (3–6). Yet, effective lure and trap systems based on these semiochemicals are still lacking due, predominately, to an inability of rubber septa “bleed” based (7–10), capillary-based (11), and membrane-based technologies to mimic the natural ratios and release rates of the pheromone blend over prolonged periods (12–14).

In nature, male *Anastrepha suspensa* (Loew) (caribflies) deposit sugar-based oral secretions (OS) on the underside of plant leaves and these “marked” leaves are frequented day after day by both sexes in the wild (15,16). This suggested that the aggregation of caribflies, the preliminary step in a complicated lek mating system, could result from pheromones that are released abiotically into air for prolonged periods from OS after its deposition (10,15,16). Recent bioassays, in fact, demonstrated that male OS is highly attractive to conspecific males and virgin females (16). Two diastereomeric *trans*-fused γ -lactones, epianastrephin (ES) and anastrephin (AS) (collectively ES/AS) (3,16,17), were identified as aggregation pheromones since they emanated for weeks from male OS and proved to be critical for attraction (Figure 1(a)).

In this manuscript, we describe the mechanism of ES/AS release from OS and how it is affected by abiotic environmental parameters. To uncover its molecular-level underpinnings, the physicochemical phenomenon that influence the volatile emission (i.e. liquid to air transfer) of these pheromones were investigated: the equilibrium distribution of ES/AS between their γ -lactone and γ -hydroxy acid (HA) forms, the kinetics of inter-conversion between the forms, respective Henry's Law constants, and molecular diffusion in OS. A kinetic model, which supports a γ -lactone diffusion-limited rate, was generated by measuring the effect of temperature and humidity, under fixed (i.e. controlled) laboratory inputs; it was then tested using ambient (i.e. uncontrolled) atmospheric inputs within model ecosystems that simulated natural conditions. We also briefly explain how the mimicry of this natural abiotic mechanism for aggregation pheromone release has

significant potential to control caribfly populations since it appears to be an integral component of their reproductive strategy.

Results and discussion

Chemical composition of oral secretions (OS)

The sugar loading and composition in freshly collected male and female OS (pH 5.5 ± 0.3 ; $\bar{x} \pm s$) was determined to be 33.2 ± 2.1 wt.% in solution at a ~ D-glucose:2 D-fructose:sucrose ratio. The concentrations (grand mean \pm SE, $n = 18$) (18) of the lactone and acid forms of ES/AS in pooled samples of male OS were 11 ± 3 and 24 ± 4 ng/ μ L, respectively. The ratio of ES:AS and ES HA:AS HA, ~2.5:1, was also observed in volatile collections and other abiotic studies on ES/AS distribution (19). None of these pheromone components were detected in female OS.

pKa determination

The pKa for ES HA and AS HA were 5.0 ± 0.1 and 5.1 ± 0.3 , respectively. These values were determined as outlined in Harris (20) using potentiometric titration ($n = 3$) of 0.9 mM ES/AS HC (sodium salt) with 1 mM trichloroacetic acid in 10% (v/v) isopropanol and $u \approx 0.01$ M (NaCl) at 25°C.

Inter-conversion of acid and lactone epianastrephin/anastrephin (ES/AS) forms

Three structural variants of intra-molecular esters, β -, γ -, and δ -lactones, are particularly common in semiochemicals. A unique abiotic feature of these lactones, coincident with Baldwin's ring-closure rule (21), is that they exist in equilibria with relatively hydrophilic β -, γ -, and δ -hydroxy acids, respectively, when in aqueous systems. Specifically, the γ -lactone moiety of ES/AS was converted into a γ -hydroxy acid (HA) and corresponding carboxylate under the conditions: $[\text{ES/AS}] < [\text{OH}^-] < [\text{buffer}]$. Although there are rate constants associated with buffer-catalyzed (k_{NaHCO_3}), H_2O -catalyzed ($k_{\text{H}_2\text{O}}$), and acid-catalyzed ($k_{\text{H}_3\text{O}^+}$) hydrolysis, their contributions were minimal in this system as suggested by previous work (22,23). Acid-catalyzed lactonization (k_{LAC}) of γ -hydroxy acids to form *cis*-fused γ -lactones can occur rapidly and often the γ -lactone (i.e. closed ring) form is largely favored at equilibrium (23). However, these are not characteristic features of analogous *trans*-fused systems (24); ES/AS concentrations over 30 days at pH 5.5 comprised only ~1% of solutions fortified with ES/AS HA over the range 20–40°C.

Accordingly, ES/AS loss was dominated by the rate associated with specific base-catalyzed ester hydrolysis (k_{OH^-}) and is expressed by the differential rate equation:

$$-d[ES/AS]_{aq}/dt = k_{HY-ES/AS}[ES/AS]_{aq} \quad (1)$$

where the observable rate constant of hydrolysis, $k_{HY-ES/AS}$ (s^{-1}), is defined as (22,25):

$$\begin{aligned} k_{HY-EA/AS} &= k_{H_3O^+}[H_3O^+] + k_{H_2O}[H_2O] + k_{OH^-}[OH^-] \\ &\quad + k[NaHCO_3] - k_{LAC}[ES/AS \text{ HA}][H_3O^+] \\ &\cong k_{OH^-}[OH^-] \end{aligned} \quad (2)$$

Experimental data support the kinetic model (Supplementary Table 1); plots of $(\ln[ES/AS]_t/[ES/AS]_0)$ versus time were linear, indicating ES/AS hydrolyses followed *pseudo* first-order kinetics. At 40°C, k_{OH^-} (s^{-1}) had values of 0.108 ± 0.013 and 0.127 ± 0.012 for ES and AS, respectively.

Henry's law experiments

Measured values of $K'_{H-ES/AS}^{sat}$ at 25°C agreed well with an estimated value (1.33×10^{-2}) (26). Not surprisingly, the effect of temperature on $K'_{H-ES/AS}^{sat}$ was of similar magnitude to its effect on estimates of ES/AS vapor pressure ($P_{ES/AS}^o$) (26) and reflected minimal variation in calculated ES/AS activity coefficients ($\gamma_{w-ES/AS}^{sat}$) (Supplementary Table 2). Consistent with their acid to lactone equilibrium distribution ($\sim 100:1$), measured values of $K'_{H-ES/AS}$ were approximately a factor of 100 less than $K'_{H-ES/AS}^{sat}$. Previous studies (27), suggest the dependence of air–water partitioning on solute concentration (i.e. within dilute versus saturated solutions) is minimal and provide justification for the direct comparison of $K'_{H-ES/AS}$ and $K'_{H-ES/AS}^{sat}$ in this study. There was good agreement in $K'_{H-ES/AS}^{sat}$ measurements obtained using female OS and synthetic OS (Table 1). $K'_{H-ES/AS}^{sat}$ increased with the temperature of female and synthetic OS; other studies, over comparable solute concentrations and temperature ranges, have reported similar findings (28,29). Although the mechanism(s) is not fully understood, many structure–activity studies have explored the positive correlation between sugar loading and the air to water distribution of esters (28–34); our observations support this trend (Supplementary Figure 1).

Fickian diffusion in oral secretions (OS)

The relationship in OS between molecular diffusivity, viscosity (μ), and temperature can be generalized by the Stokes–Einstein equation:

$$D_{ES/AS} = \frac{k_B T_{OS}}{6 \pi r \mu_{OS}} \quad (3)$$

where $D_{ES/AS}$ is ES/AS's translational diffusion coefficient ($cm^2 s^{-1}$), k_B is the Boltzmann constant ($1.38 \times 10^{-23} \text{ kg m}^2 \text{ s}^{-2} \text{ K}^{-1}$), and r is the hydrodynamic radius of “spherical” ES/AS ($\sim 4.47 \text{ \AA}$) (35). It effectively describes the diffusion of ester volatiles in D-glucose, D-fructose, and sucrose solutions over the ranges examined in this study (36–40). Citing similarities in viscosity, which is directly proportional to the sugar loading, Chandrasekaran and King (30) reported that ethyl acetate diffusion in ~ 1 D-glucose:2 D-fructose:1 sucrose solutions reasonably approximated its diffusion in sucrose (or D-glucose, or D-fructose) solutions alone. Likewise, we observed good agreement ($<10\%$ variation) between viscosity estimates based solely on sucrose (μ_{SU}) (41) and viscosity measurements of OS (μ_{OS}) and its synthetic analogs (μ_{SA}) at a given sugar loading and temperature (Supplementary Table 3). Therefore, predicted translational diffusion coefficients for ES/AS in OS ($D'_{ES/AS}$) were obtained by substituting μ_{SU} for μ_{OS} in Equation 3.

Volatile pheromone emission: fixed conditions

The following kinetic model is based on an assimilation of the physicochemical properties described above and was developed to explain measurements of airborne ES/AS, released from OS, within VCCs that permitted tunable temperature and absolute humidity.

OS functioned as a humectant due to its sugar content; the concentration of water in air (C_{a-H_2O}) relative to that in OS (C_{OS-H_2O}) at equilibrium (i.e. $K = C_{a-H_2O}/C_{OS-H_2O}$) was affected, as indicated by changes in OS volume (from 10 μL), by temperature and absolute humidity (Table 2). OS volume, and consequently, OS sugar loading were proportional to the corresponding relative humidity (%) (Table 2, Supplementary Figures 2–5). The equilibrium between C_{a-H_2O} and C_{OS-H_2O} was established within 2 h (Supplementary Figure 6); accordingly, ES/AS concentrations measured >2 h after deposition were utilized in model development. There was minimal difference in the volatile emission of ES/AS from either supplemented female or male OS at air flows of 140, 315, 480, or 660 cm^3/min .

The flux ($mol \text{ cm}^{-2} \text{ s}^{-1}$) of ES/AS from female OS supplemented with ES/AS could be described by a stagnant boundary model under liquid-film control and the partial differential equation (42,43,44):

Table 1. Air to water equilibrium distribution of ES/AS, reported as $K_{H-ES/AS}^{sat}$ ($\times 10^2$), was affected by sugar loading and temperature.

Temperature (°C)	Sugar loading (wt.%) at ~ D-glucose:2 D-fructose:sucrose ratio					
	Female OS	Synthetic OS (0.01 M NaHCO ₃ , pH 5.5)				
	~ 33	25	33	45	55	65
20	0.8±0.3	0.7±0.4	0.9±0.2	1.2±0.4	1.4±0.2	1.8±0.4
25	1.5±0.3	1.4±0.5	1.8±0.4	2.0±0.3	2.5±0.5	3.1±0.3
30	2.0±0.4	1.8±0.4	2.2±0.3	3.4±0.2	3.5±0.5	5.4±0.5
35	2.9±0.6	2.8±0.6	3.2±0.3	4.5±0.5	5.0±0.4	6.6±0.6
40	4.8±0.5	4.5±0.3	5.0±0.4	5.7±0.5	6.2±0.6	7.3±0.4

Table 2. OS functioned as a humectant due to its sugar content, that is, the equilibrium distribution of water between air and OS was affected, as indicated by changes in OS volume (from 10 µL), by temperature and absolute humidity over the ranges of this study.

Temperature (°C)		Absolute humidity, C_{a-H_2O} (mM)			
		0.67±0.2	0.77±0.2	0.85±0.2	0.92±0.3
20	Relative humidity (%) ^a	70	81	89	96
	OS volume (µL)	7.0±0.4	9.2±0.3	11.8±0.4	16.1±0.6
	OS-SA (cm ²) ^b	0.33	0.34	0.45	0.58
	wt. (%) ^c	41.5	35.1	29.6	23.6
	χ_{w-OS}	0.94 ^d	0.94 ^d	0.95 ^d	0.95 ^d
	α ($\times 10^5$ cm s ⁻¹) ^e	5.8±0.5	8.1±0.8	13.3±0.8	18.3±0.9
25	Relative humidity	52	60	67	75
	OS volume	5.5±0.4	6.6±0.5	8.7±0.4	12.1±0.4
	OS-SA	0.24	0.28	0.43	0.56
	wt. (%)	47.5	43.0	36.4	29.1
	χ_{w-OS}	0.93	0.94	0.94	0.95
	α ($\times 10^5$ cm s ⁻¹)	3.6±0.3	5.6±0.6	9.2±0.6	15.3±0.7
30	Relative humidity	39	45	51	58
	OS volume	3.0±0.2	4.2±0.2	6.4±0.4	9.5±0.5
	OS-SA	0.19	0.23	0.28	0.44
	wt. (%)	62.4	54.7	43.7	34.3
	χ_{w-OS}	0.92	0.93	0.94	0.95
	α ($\times 10^5$ cm s ⁻¹)	1.9±0.2	3.1±0.4	5.1±0.3	12.5±0.6
35	Relative humidity	30	35	39	42
	OS volume	2.0±0.5 ^f	3.0±0.3	3.5±0.3	4.5±0.2
	OS-SA	0.18	0.19	0.19	0.26
	wt. (%)	70.6	62.4	58.7	52.5
	χ_{w-OS}	0.90	0.92	0.92	0.94
	α ($\times 10^5$ cm s ⁻¹)	1.9±0.2	2.2±0.2	2.5±0.2	3.3±0.3
40	Relative humidity	23	28	30	34
	OS volume	1.0±0.5 ^f	1.8±0.5 ^f	2.0±0.5 ^f	2.8±0.4
	OS-SA	0.17	0.17	0.18	0.18
	wt. (%)	83.5	73.8	66.5	64.4
	χ_{w-OS}	0.86	0.90	0.91	0.92
	α ($\times 10^5$ cm s ⁻¹)	0.7±0.2	1.4±0.2	1.9±0.1	2.5±0.2

^a $100 \times C_{a-H_2O} / C_{a-H_2O}^{sat}$, where $C_{a-H_2O}^{sat}$ varies with temperature.

^bError = ±0.07 cm² (see Supplementary Figure 4).

^cSugar loading (wt.%) in OS at a ~ D-glucose:2 D-fructose: sucrose ratio.

^dMoles water/moles water+moles total carbohydrates+moles ES/AS.

^eCorresponds to release from ES/AS supplemented female OS (10 µL deposit).

^fDetermined by extrapolation of linear least-squares analysis of data with OS volume 5.5–2.75 uL versus relative humidity (%) (see Supplementary Figure 5).

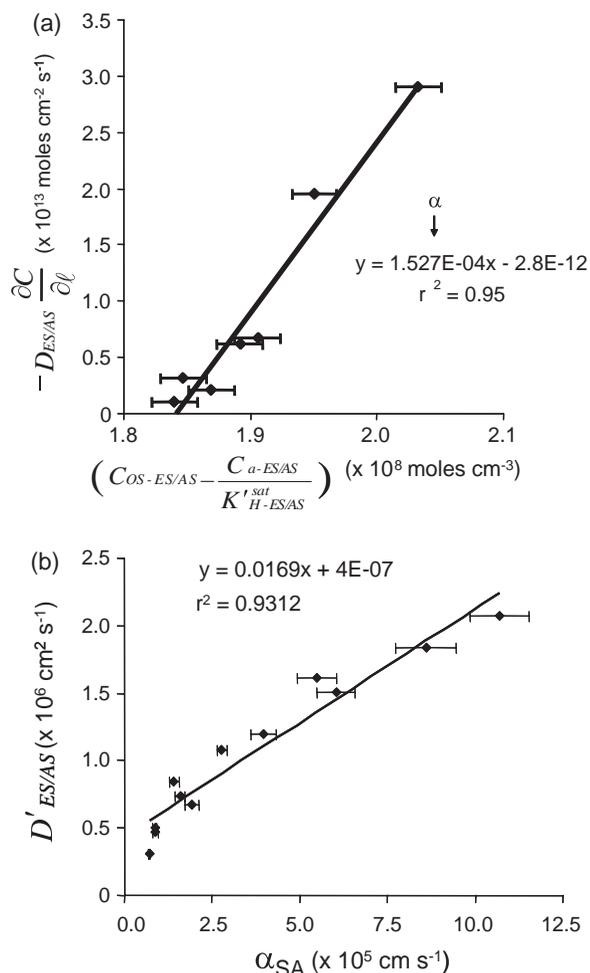


Figure 2. (a) The linear relationship (correlation coefficient: $r^2=0.95$) between the flux of ES/AS and $(C_{OS-ES/AS} - \frac{C_{a-ES/AS}}{K'_{H-ES/AS}})$ at 25°C and absolute humidity of 0.92 mM. A similar relationship, the slope of which is termed α , was obtained over the investigated ranges of temperature and humidity and supports a diffusion-controlled liquid to air partitioning (i.e. volatilization) of ES/AS (44). (b) The linear relationship (correlation coefficient: $r^2=0.92$) between α_{SA} and $D'_{ES/AS}$ in this study; the slope obtained by linear least-squares analysis of the data represent ℓ/L .

$$\text{Flux (F)} = -D_{ES/AS} \frac{\partial C}{\partial \ell} = \alpha \left(C_{OS-ES/AS} - \frac{C_{a-ES/AS}}{K'_{H-ES/AS}} \right) \quad (4)$$

where ℓ (cm) is the thickness of the OS stagnant film, $C_{OS-ES/AS}$ and $C_{a-ES/AS}$ are the respective ES/AS concentrations (mol cm^{-3}) in OS and air, and α is a proportionality constant (cm s^{-1}) that is defined as (44):

$$\alpha = \frac{L D_{ES/AS}}{\ell} \quad (5)$$

Note that under the conditions where L , a dimensionless parameter, and ℓ are established, α represents the transfer velocity of ES/AS through the stagnant layer, v_{OS} (cm s^{-1}).

Experimental data support this kinetic model and suggests the release rate of ES/AS from supplemented female OS results from diffusion-limited liquid to air transfer that is fast relative to ES/AS hydrolysis at pH 5.5 ($t_{1/2}@40^\circ\text{C} \cong 12-20 \times 10^3$ d); plots of $(C_{OS-ES/AS} - \frac{C_{a-ES/AS}}{K'_{H-ES/AS}})$ versus $(-D_{ES/AS} \frac{\partial C}{\partial \ell})$ were linear in all cases (Figure 2(a)). Relative humidity affected the slope (α) obtained by a linear least-squares analysis, independent of its effect on $K'_{H-ES/AS}$ (Table 1 and 2, Supplementary Table 2, Supplementary Figures 1 and 7).

To further isolate the role of diffusion in the relationship between α and relative humidity, we normalized measured fluxes with the corresponding OS surface area (SA) (see Table 2) to obtain a SA-corrected description of the release rate of ES/AS from OS (mol s^{-1} or ng h^{-1}):

$$\begin{aligned} &\text{release rate/OS SA} \\ &= \alpha_{SA} \left(C_{OS-ES/AS} - \frac{C_{a-ES/AS}}{K'_{H-ES/AS}} \right) \end{aligned} \quad (6)$$

Figure 2(b) shows the direct linear relationship (correlation coefficient: $r^2=0.92$) between α_{SA} and $D'_{ES/AS}$ for data above 40% relative humidity, since relative humidity lower than this are atypical within the geographic distribution of caribflies (45). According to Equation 5, the slope obtained by linear least-squares analysis of the data represent ℓ/L . Evaluation of equations developed in Crank (44) to describe diffusion in a cylinder and (hemi)sphere under conditions of “surface evaporation,” yield L approximations of two and three, respectively, for this system. These L values correspond to an overall OS stagnant film thickness (ℓ) of ~ 0.04 cm, a reasonable estimate based on values for water ($\ell_{H_2O} \approx 0.05-0.005$ cm) (42).

Our data indicate the diffusion-limited release rate of ES/AS from OS is sensitive to changes in temperature and humidity to the extent that their daily fluctuation elicits a marked effect. However, even when “dry” air at 40% relative humidity was maintained, there was $>98\%$ recovery within 15 h after deposition from 10 μL of female OS supplemented with ES/AS at concentrations naturally found in male OS. Since ES/AS aggregation pheromone release from natural male OS occurs over many days, or weeks, we investigated whether the

extension of volatile emission was attributable to the γ -hydroxy acid forms of ES/AS, which occur at nearly twice the concentration of the γ -lactone forms in fresh OS.

When ES/AS release from female OS supplemented with ES/AS HA was experimentally determined and measured values of $K'_{H-ES/AS}$ were substituted into Equation 4 for $K'^{sat}_{H-ES/AS}$, α (or α_{SA}) was reduced ~ 100 -fold in comparison to identical conditions with ES/AS supplemented OS (Supplementary Figure 8). This reduction corresponded with ~ 100 -fold increase in the duration of ES/AS volatile emission from OS. At relative humidity levels $< 75\%$, these studies were impeded by the time course required to collect seven data points for the kinetic model; predicted release rates indicate sampling would be required for $\sim 12,000$ – $120,000$ h in these instances, well over ($> 10 \times$) the average caribfly lifespan.

The reflection ($\sim 1:1$) of $K'_{H-ES/AS}$ on α_{SA} provides additional evidence for this kinetic description and is consistent with a ES/AS release mechanism driven by aqueous equilibrium between γ -hydroxy acids, corresponding carboxylates, and their thermodynamically less preferred ($\sim 100:1$), but relatively volatile, γ -lactone equivalents (Figure 1(b)). In OS, this speciation was apparently only minimally influenced by humidity-induced changes in the relative concentrations of involved species ($\chi_{w-OS} > 0.95$) (see Table 2).

We were able to mimic this natural abiotic mechanism by supplementing female OS with γ -hydroxy acid and γ -lactone forms of ES/AS at concentrations matching those in males, as the release from $10 \mu\text{L}$ of the “composite” and male OS were consistent (Figure 3). When the first data points (< 24 h), which represent release derived primarily from the γ -lactone forms of ES/AS initially present in OS, are omitted from plot (b), note the ~ 100 -fold reductions in α values compared to OS supplemented with ES/AS only at 75% relative humidity (see Figure 2(a)).

Volatile pheromone emission: simulated natural conditions

The predictive kinetic description of volatile pheromone emission was tested in model ecosystems that were designed to simulate natural conditions, particularly with respect to temperature and humidity inputs. A 24 h time course was chosen to isolate the release of volatile ES/AS that occurs as a result of “fresh” OS deposits. Consequently, impacts on the ES/AS pheromone emission resulting from ES/AS HA and the colonization of OS by phyloplane microbes were negligible.

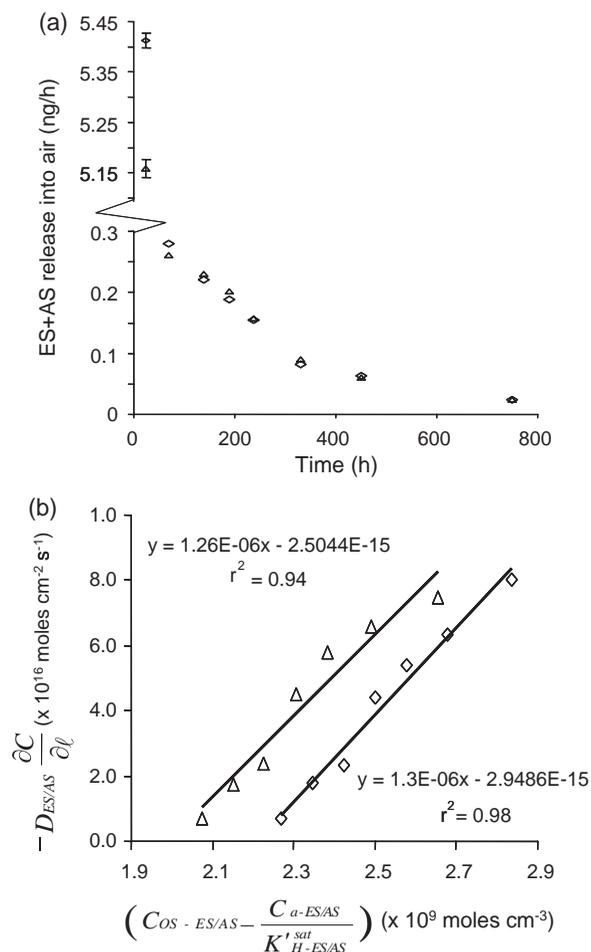


Figure 3. (a) When female OS ($10 \mu\text{L}$) was supplemented with γ -lactone ($11 \text{ ng}/\mu\text{L}$) and γ -hydroxy acid ($24 \text{ ng}/\mu\text{L}$) forms of ES/AS at concentrations matching those males, release at 75% relative humidity from the “composite” (\diamond) and male (\triangle) OS were consistent. (b) Note the ~ 100 -fold reductions in α relative to that in Figure 2(a), when OS was supplemented under identical conditions with just the γ -lactone forms of ES/AS.

Within the model ecosystems, predicted ES/AS diffusion in OS ($D'_{ES/AS}$) paralleled relative humidity and was inversely related to temperature (Figure 4(a)). ES/AS emission from OS (ng/h) was measured and also predicted by solving Equation 6 with the average temperature and relative humidity values over the respective dates (A: 31°C , 58%; B: 19°C , 68%); between comparisons, initial ES/AS concentrations were similar (A: $\sim 3 \mu\text{g}$; B: $\sim 0.5 \mu\text{g}$) and OS SA was assumed to be equal. The deviation between observed and predicted release fluctuated with the periodicity of $D'_{ES/AS}$ (Figure 4(b)). We have interpreted the results to mean that under ambient atmospheric inputs of temperature and humidity, just as under fixed inputs, ES/AS volatile emission from OS occurs with a diffusion-limited rate.

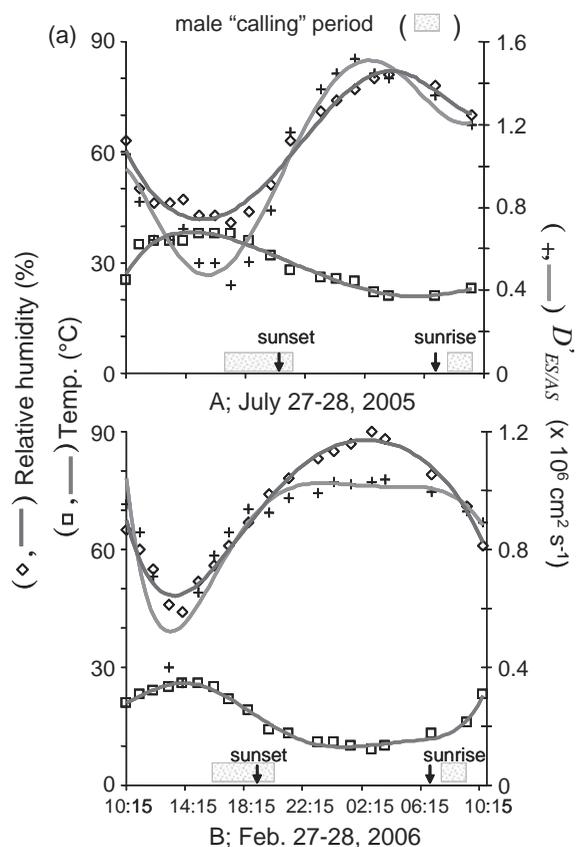


Figure 4(a). The predicted diffusion coefficients of ES/AS in OS ($D'_{ES/AS}$) relative to environmental parameters within the model ecosystems. Males biologically synthesize and release aggregation pheromones during “calling” periods (■).

It is interesting to note that within model ecosystem B there appears to be a nocturnal enhancement of ES/AS pheromone release from OS deposited on the underside of “natural” leaf surfaces relative to glass. Given the topographically heterogeneous microstructure of the loquat leaf epidermis relative to the surface of glass, OS would be expected to have a larger effective SA when deposited on leaves, facilitating additional mass transport of atmospheric moisture and ES/AS through the leaf-OS interfacial region. Non-atmospheric biological water supplies to OS not applicable to glass, such as transpiration and/or guttation (46), could also function to decrease OS sugar loading and increase ES/AS emission from OS on a leaf surface. However, they are not likely because data on gas exchange and leaf-water content at mid-day relative to pre-dawn indicate that loquat stomata are closed at night (47).

The pattern of caribfly reproductive activity must be examined to appreciate the ecological utility of this environmentally regulated abiotic strategy for aggre-

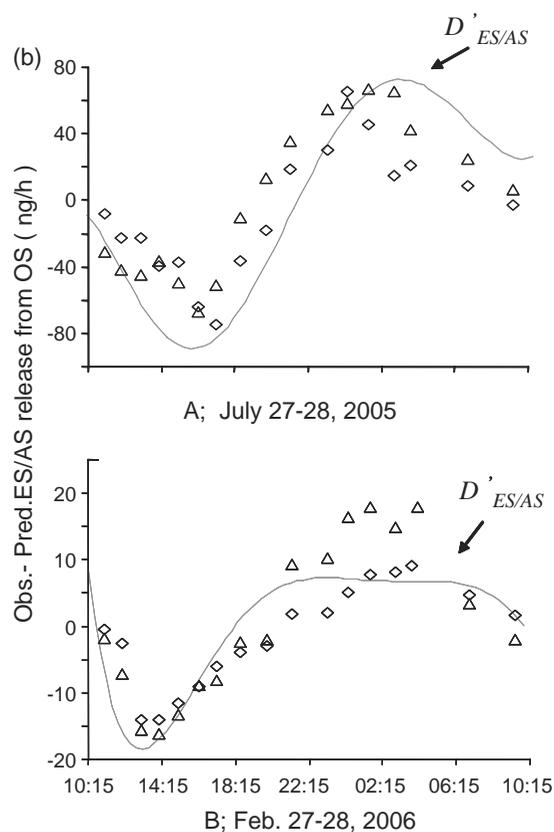


Figure 4(b). OS deposition from 100 male caribflies occurred “naturally” onto glass in A (Δ , rep. 1; \diamond , rep. 2) and male OS was mechanically deposited ($10 \times 10 \mu\text{L}$) onto the underside of loquat leaf (Δ) or glass-slide (\diamond) surfaces within B. The deviation between observed and predicted release of volatile ES/AS from OS, based on solving Equation 6 with time-course average temperature and relative humidity values, fluctuated with the periodicity of $D'_{ES/AS}$.

gation pheromone release. Mature male “calling” behavior occurs during two periods each day, beginning ~ 30 min after sunrise and ~ 3 h prior to sunset (see Figure 4(a)) (10,46–48). ES/AS biological synthesis and release, OS deposition, and female attraction to lek sites is known to occur during these periods, although they are more pronounced in the afternoon when mating occurs. ES/AS release from a single male (~ 1000 ng/h) is considerably larger than the release from OS deposited by 100 males (~ 300 ng/h); thus, there is a strong potential for periods of biological release to overwhelm abiotic release from OS, particularly that originating from remote locations. Our data indicate, however, that the maximum in predicted diffusion ($D'_{ES/AS}$) occurs between “calling” periods to provide release of ES/AS from deposited OS markings at night and in the early morning when relative humidity is high. Interestingly,

the diel periodicity of this abiotic release coincides with male attraction to lek sites, which is known to occur around sunrise in the field (49,50).

Time-resolved bioassays in flight tunnel model ecosystems, which would function to bridge the mechanistic data presented here and the field observations, are needed to confirm that male attraction to male OS occurs in the early morning, before they begin biological ES/AS release to presumably establish mating territories. Nevertheless, our results strongly suggest that the environmentally regulated abiotic release of aggregation pheromones is a critical aspect of the inter-caribfly communication strategy because the abiotic environmental processing of aqueous equilibria is what that regulates the volatilization of ES/AS from male OS.

Experimental

General

The chemical characterization, isolation, and synthesis of ES, AS (3), and their respective γ -hydroxy acids (ES HA and AS HA) were as reported in Walse et al. (17). Barnstead E-pure™ water (18 M Ω cm) was used for solutions. All other chemicals were obtained from commercial sources unless otherwise noted. Gas chromatography – ion trap mass spectrometry (GC–MS) and high performance liquid chromatography – electrospray ionization mass spectrometry (HPLC–ESIMS) retention times and spectra were used for chemical verification (Supplementary Table 1). Specifics of the analytical methodology, reported previously (17), are briefly described in supporting online material.

Inter-conversion of acid and lactone epianastrephin/anastrephin (ES/AS) forms

A series of 0.01 M buffers, set to ionic strengths of $u = 0.1$ M with NaCl, were adjusted with 0.01 M HCl and NaOH to pH 3 (H₃PO₄), 5.5 (NaHCO₃), 8 (NaH₂PO₄), 10 (Na₂CO₃), or 11.1 (Na₂CO₃). For ES/AS hydrolyses, the buffers (20 mL) were transferred to 20-mL amber glass vials (Fisher®, Pittsburgh, PA) and 50 μ L of ES or AS in acetonitrile (ACN) was added to afford initial concentrations of 10 μ M, below their estimated solubility of 0.2 mM at 25°C (26). Temperatures were maintained and mixing was controlled (170 rpm) with a Lab-Line® Environ-Shaker. Samples (1.0 mL), acquired as a function of time, were transferred to 4-mL amber glass vials pre-charged with 1.0 mL of hexane containing tetradecane internal standard at 0.8 μ L/L hexane. Hexane-extractable analytes were removed from the

buffer solutions by mixing for 2 min with a vortex Genie®. Emulsions were broken with ~ 100 mg NaCl and the hexane layer was analyzed with GC–MS. With a 25- μ L syringe, duplicate 10 μ L aliquots of the aqueous layer were removed, combined with 2 μ L of ACN containing 6 μ g of (+)-sclareolide (Sigma®, St. Louis, MO) external standard, and analyzed via HPLC–MS. For lactonizations, ES/AS HA aqueous stocks (pH 7) were added to 1 mL of 0.01 M buffer to afford initial concentrations of 0.9 mM in 2-mL glass vials; aqueous sampling was as described above.

Insects and oral secretions (OS)

Caribflies were cultured as described previously (16). Briefly, adult flies were separated by sex within two days after emergence from pupae. Sexes were kept in separate cages (25 \times 25 \times 25 cm³) and rooms within a greenhouse. Each cage contained a water source and food, a 3:1 mixture by mass of table sugar to hydrolyzed yeast. After squeezing abdomens with fingertips until regurgitation, OS from 11–14-day-old sexually mature adults (14) were harvested with a glass capillary (1 mm i.d.) that penetrated a vial under slight negative pressure at 4°C. Collections were made 2 \pm 0.5 h prior to sunset, pooled until ~ 0.6 mL was accumulated, and stored at -70°C .

Physicochemical characterization of oral secretions (OS)

An enzyme-coupled colorimetric assay was used to selectively determine concentrations of glucose, fructose, and sucrose in OS (51). A BEE-CAL™ microprobe was used to measure the pH of OS. Viscosity, μ (mPa \cdot s), was measured using a Cannon–Manning Semi-Micro Viscometer (No. 75 and 350).

Pooled collections of OS (0.5 mL) were diluted with water to 1 mL. These samples were extracted with hexane and analyzed for ES/AS by GC–MS, or they were transferred to DSC-18 1-mL solid-phase extraction cartridges (Supelco®) that had been pre-conditioned and cleaned with sequential ACN (2 \times 1 mL), methanol (2 \times 1 mL), and water (3 \times 1 mL) rinses. The cartridges were flushed with water (3 \times 1 mL) to remove polar OS components such as salts and sugars. The analytes were then eluted into 4-mL tubes with rinses (3 \times 1 mL) of 0.05% formic acid in 50% ACN. Eluants were concentrated via Speed Vac® to 0.5 mL and 120 μ L of ACN containing 360 μ g of external standard was added prior to HPLC–MS analysis.

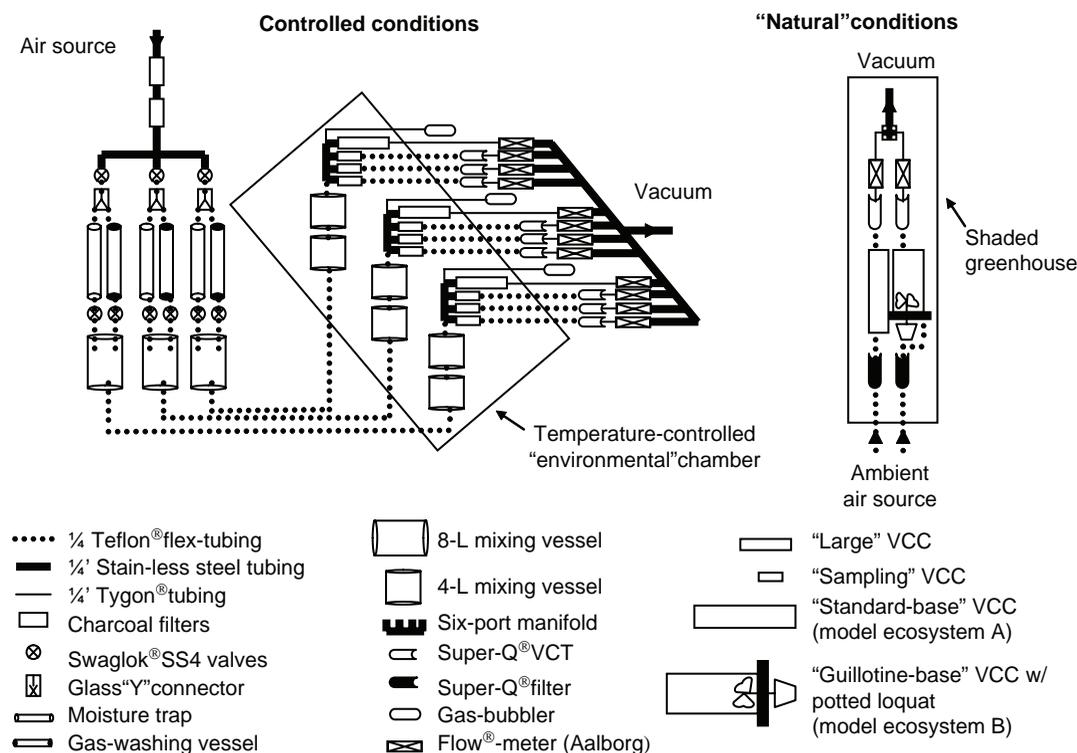


Figure 5. Schematic of volatile collection systems, modified from Heath and Manukian (52) that were used to determine the amount of airborne ES/AS pheromone, released from OS, under controlled and “natural” ambient atmospheric inputs of humidity and temperature.

Collection of volatile pheromones: fixed conditions

A modified volatile collection system (VCS) of Heath and Manukian (52) was used (Figure 5). A compressor pushed air (60 psi) through two Altech® L21 charcoal filters in series. Valves metered diversion into three streams that were subsequently split through a water-filled 500-mL gas washing bottle and a Drierite® filled moisture trap in parallel. The air streams of each pairing were metered to allow tunable absolute humidity (i.e. the concentration of water in air; C_{a-H_2O}) upon recombination in an 8-L glass-mixing vessel. The “conditioned” air supplies (three total) passed into two 4-L glass equilibration reservoirs in series and then a six-port manifold, all located within a temperature-controlled chamber. One port of each manifold was connected to a gas bubbler, located outside the chamber, which was used to verify a slight excess in airflow was maintained. Three Analytical Research Systems® (ARS) RV-A3 “sampling” volatile collection chambers (VCC) and a “large” 4CHB12R5 VCC, containing only a digital thermo-hygrometer, were connected to the remaining ports. Pheromones released from samples were captured on ARS glass tube (4 cm long \times 4 mm i.d.) volatile collector traps (VCT) containing 20 mg

Altech® Super-Q adsorbent. A 560 mmHg vacuum was metered to allow equivalent airflows (140–660 cm^3/min) between the VCCs.

Chamber temperature (20–40°C) and the absolute humidity of conditioned-air supplies, C_{a-H_2O} (0.67–0.92 mM), were maintained for 6 h prior to substrate introduction. These conditions encompass those typical to caribflies’ endemic range, the Greater Antilles and Florida (45). OS substrate was deposited (10 μL) onto 1”-square glass slides that were inserted into the “sampling” VCCs. Substrate consisted of either male OS, female OS, female OS supplemented with pheromone components, or 0.01 M NaHCO_3 buffer at pH 5.5 containing pheromone components and the same sugar concentrations present in OS (i.e. synthetic OS). VCTs were removed as a function of time and flushed with methyl *tert*-butyl ether (MTBE) (3 mL) into a volumetric glass vial pre-charged with 0.5 mL of MTBE containing tetradecane internal standard at 0.8 $\mu\text{L}/\text{L}$ MTBE. The eluant was reduced to 0.5 mL with a gentle N_2 stream and analyzed by GC–MS. ES/AS collection efficiencies from synthetic OS were determined to be >98% over the range 5000–0.5 ng. Potential residual inputs of ES/AS from VCS components were below detection limits ($\sim 3.4 \times 10^{-9}$ M) in 30-day control

collections. After sampling was concluded, substrate humectant properties were examined; the diameter of the OS deposit was measured and, if possible, the volume was estimated by drawing it into a 25- μ L syringe.

Collection of volatile pheromones: simulated natural conditions

Duplicate VCCs (ARS # 6CHB18R52) equipped with a “standard-base” (model ecosystem A) or a “guillotine-base” (model ecosystem B), which accommodated a young loquat tree (*Eriobotrya japonica*), were located within a shaded greenhouse. A vacuum was metered to provide airflow of 660 cm³/min through each chamber as previously reported (53). Both VCC ends of each model ecosystem were fitted with VCTs; one functioned downwind to immobilize pheromones, the other (upwind) filtered a supply of ambient air from outside the greenhouse (Figure 5). After recording the temperature and humidity using a digital thermo-hygrometer housed within each model ecosystem, downwind VCTs were removed as a function of time, processed, and analyzed as described above.

For type A model ecosystems, VCC glass end/connecting fixtures were removed and replaced with nylon screen before 100 sexually mature male caribflies were introduced (with food and water sources (16)) and allowed to deposit OS “naturally” onto the glass from 10:00 PM to 9:00 AM the following morning. Prior to sampling ES/AS emitted from the OS, flies were removed and the fixtures were reincorporated. For type B model ecosystems, 10 \times 10 μ L of male OS was deposited onto either a 3” \times 1” glass slide or the underside of loquat leaves at 9:00 AM.

Henry’s law experiments

Dimensionless Henry’s Law constants, equilibrium descriptors of air–water partitioning (42), are defined as:

$$K'_{\text{H-ES/AS}} = C_{\text{a-ES/AS}} / C_{\text{s-ES/AS HA}} \quad (7)$$

$$K'_{\text{H-ES/AS}}^{\text{sat}} = C_{\text{a-ES/AS}} / C_{\text{s-ES/AS}}^{\text{sat}} \quad (8)$$

and represent concentration (mol cm⁻³) fractions of ES/AS in air ($C_{\text{a-ES/AS}}$) relative to ES/AS HA in solution ($C_{\text{s-ES/AS HA}}$) or ES/AS in solution under saturated conditions ($C_{\text{s-ES/AS}}^{\text{sat}}$). They were measured ($n = 8$) at 20, 25, 30, 35, and 40°C for 0.01M NaHCO₃ ($u = 0.1M$) at pH 5.5, female OS, and synthetic OS. The solution (1 mL) and an appropriate volume of pH 7 ES/AS HA aqueous stock (for $K'_{\text{H-ES/AS}}$) or ES/AS in ACN (for $K'_{\text{H-ES/AS}}^{\text{sat}}$), to afford concentrations of 0.9 mM, were encapsulated in a 2-mL septum-

capped glass vial for 5 days. Prior to aqueous sampling (or hexane extraction) as described above, a needle fitted to a VCT was used to access vial headspace (~ 1 mL). Another needle, passing a N₂ stream at ~ 0.5 mL/min, was subsequently introduced to flush it. The VCT was removed after 10 min, processed, and analyzed as in the volatile pheromone collections.

Conclusion

Decades of research on using semiochemical attractants in pest management practices as “green” alternatives to broadcast insecticide application have outlined the importance of replicating the natural semiochemical release mechanism(s) utilized by a species and the marked influence that environmental factors can have on semiochemical release. In light of this knowledge and the example provided above, natural abiotic mechanisms for semiochemical release appear particularly well-suited for exploitation due to the intrinsic connectivity that exists between physicochemical-based inter-organism communication strategies and the abiotic environmental processing of the chemical signal. Specifically, this study points to the use of sugar-based solutions as media for the diffusion-controlled release of volatile insect aggregation pheromones. Unique features of this natural system, which are highly coveted when targeting the population control of flying insects, include release of volatile pheromones that is inversely related to temperature and directly related to relative humidity, as well as the ability to incorporate relatively hydrophilic pheromone precursors, linked to the volatile pheromone form through aqueous equilibria, into the media for the purpose of attenuating volatilization. Although the potential of sugar-based semiochemical release systems to serve sustainable agriculture is difficult to gauge presently, it is interesting to note the link between sugar-solutions and the ecology of many Diptera, Hemiptera, and Hymenoptera insect pests.

Acknowledgements

The United States Department of Agriculture financed this work. The colorimetric sugar assay was done by Dr. Sherry LeClere (USDA-ARS-CMAVE). We extend gratitude to James H. Tumlinson III (Pennsylvania State University and USDA-ARS-CMAVE) for his input on this research.

Electronic supplementary information

Additional methods, results, tables, and figures. This material is available free of charge via the online article page, from the multimedia tab.

References

- (1) USGS. NAWQ Pesticide National Synthesis Project. <http://water.usgs.gov/nawqa/pnsp> (accessed Feb 14, 2009).
- (2) USDA. APHIS. 2006. Exotic Fruit Fly Strategic Plan. http://www.aphis.usda.gov/plant_health/ea/downloads/ffeis.pdf (accessed Jan 6, 2009).
- (3) Battiste, M.A.; Streckowski, L.; Vanderbilt, D.P.; Visnik, M.; King, R.; Nation, J.L. *Tetrahedron Lett.* **1983**, *24* (26), 2611–2614.
- (4) Chuman, T.; Sivinski, J.; Heath, R.R.; Calkins, C.O.; Tumlinson, J.H. *Tetrahedron Lett.* **1988**, *29*(50), 6561–6564.
- (5) Nation, J.L. *Environ. Entomol.* **1975**, *4* (1), 27–30.
- (6) Rocca, J.R.; Nation, J.L.; Streckowski, L.; Battiste, M.A. *J. Chem. Ecol.* **1992**, *18*(2), 223–243.
- (7) Butler, L.I.; McDonough, L.M. *J. Chem. Ecol.* **1979**, *5*, 825–837.
- (8) Heath, R.R.; Landolt, P.J.; Tumlinson, J.H.; Chambers, D.L.; Murphy, R.E.; Doolittle, R.E.; Dueben, B.D.; Sivinski, J.; Calkins, C.O. *J. Chem. Ecol.* **1991**, *17* (9), 1925–1940.
- (9) Heath, R.R.; Teal, P.E.A.; Tumlinson, J.H.; Mengelkoch, L.J. *J. Chem. Ecol.* **1986**, *12* (12), 2133–2143.
- (10) Nation, J.L. *J. Chem. Ecol.* **1990**, *16* (2), 553–572.
- (11) Weatherston, I.; Miller, D.; Dohse, L. *J. Chem. Ecol.* **1985**, *11* (8), 953–978.
- (12) Heath, R.R.; Epsky, N.D.; Landolt, P.J.; Sivinski, J. *Fla. Entomol.* **1993**, *76* (2), 233–244.
- (13) Landolt, P.J.; Heath, R.R. *Pest Management in the Subtropics, Integrated Pest Management-A Florida Perspective*; Intercept Ltd: Andover, Hants, UK, 1996; pp 197–207.
- (14) Nation, J.L. In *Fruit Flies their Biology, Natural Enemies, and Control*; Robinson, A.S.; Hooper, G., Eds.; Elsevier: Amsterdam, 1989; Vol. 3A; pp 189–205.
- (15) Sivinski, J.; Epsky, N.D.; Heath, R.R. *J. Insect Behav.* **1994**, *7* (1), 43–51.
- (16) Teal, P.E.A.; Lu, F. *Arch. Insect Biochem. Physiol.* **2001**, *48* (3), 144–154.
- (17) Walse, S.S.; Lu, F.; Teal, P.E.A. *J. Nat. Prod.* **2008**, *71*, 1726–1731.
- (18) Skoog, D.A.; West, D.M.; James Holler, F. *Fundamentals of Analytical Chemistry*, 7th ed.; 1996.
- (19) Battiste, M.A.; Streckowski, L.; Coxon, J.M.; Wydra, R.L.; Harden, D.B. *Tetrahedron Lett.* **1991**, *32* (39), 5303–5304.
- (20) Harris, D.C. *Quantitative Chemical Analysis*, 4th ed.; 1995, pp. 282–283.
- (21) Baldwin, J.E. *J. Chem. Soc., Chem. Commun.* **1976**, *18*, 734–736.
- (22) Perdue, E.M.; Wolfe, N.L. *Environ. Sci. Technol.* **1983**, *17*, 635–643.
- (23) (a) Storm, D.R.; Koshland, D.E. *J. Am. Chem. Soc.* **1972**, *94* (16), 5805–5815; (b) Storm, D.R.; Koshland, D.E. *J. Am. Chem. Soc.* **1972**, *94* (16), 5815–5825.
- (24) Streckowski, L.; Visnik, M.; Battiste, M.A. *Synthesis* **1983**, *6*, 493–494.
- (25) Mabey, W.; Mill, T. *J. Phys. Chem. Ref. Data.* **1978**, *7*, 383–415.
- (26) United States Environmental Protection Agency, EPI-Suite software [Online]. 2002. (a) WATERNT v1.00., (b) HENRYWIN v1.90., (c) MPVPWIN v1.41. <http://www.epa.gov/opptintr/exposure/pubs/episuite.htm> (accessed 14 Feb, 2009).
- (27) (a) Prausnitz, J.M. *Molecular Thermodynamics of Fluid-Phase Equilibria*; Prentice Hall: Englewood Cliffs, NJ, 1969; (b) Przyjazny, A.; Janicki, W.; Chrzanowski, W.; Staszewki, R. *J. Chromatogr.* **1983**, *280*, 249–260.
- (28) Covarrubias-Cervantes, M.; Champion, D.; Debeaufort, F.; Voilley, A. *J. Agric. Food Chem.* **2004**, *52*, 7064–7069.
- (29) Kieckbusch, T.G.; King, C.J. *J. Agric. Food Chem.* **1979**, *27* (3), 504–507.
- (30) Chandrasekaran, S.K.; King, C.J. *AIChE.* **1972**, *18* (3), 513–526.
- (31) Friel, E.N.; Linforth, R.S.T.; Taylor, A.J. *Food Chemistry* **2000**, *71*, 309–317.
- (32) Nawar, W.W. *J. Agric. Food Chem.* **1971**, *19* (6), 1057–1059.
- (33) Lakshmi, T.S.; Nandi, P.K. *J. Phys. Chem.* **1976**, *80* (3), 249–252.
- (34) Nahon, D.F.; Harrison, M.; Roozen, J.P. *J. Agric. Food Chem.* **2000**, *48*, 1278–1284.
- (35) Fuller, E.N.; Schettler, P.D.; Giddings, J.C. *Ind. Eng. Chem.* **1966**, *58*, 19–27.
- (36) Dey, P.C.; Motin, M.A.; Biswas, T.K.; Huque, E.M. *Monatshefte fur Chemie.* **2003**, *134*, 797–809.
- (37) Contreras-Lopez, E.; Champion, D.; Hervert, H.; Blond, G.; Le Meste, M. *J. Agric. Food Chem.* **2000**, *48*, 1009–1015.
- (38) Covarrubias-Cervantes, M.; Champion, D.; Debeaufort, F.; Voilley, A. *J. Agric. Food Chem.* **2005**, *53*, 6771–6776.
- (39) Hikita, H.; Asai, S.; Azuma, Y. *CJChE.* **1978**, *56*, 371–374.
- (40) Yamamoto, S.; Saeki, T.; Inoshita, T. *Chem. Eng. J.* **2002**, *86*, 179–184.
- (41) Gilli, R. 1997. Momento on sugar. AvH Association. <http://www.seas.upenn.edu/courses/belab/be309/SucroseCalculator.html>.
- (42) Schwarzenbach, R.P.; Gschwend, P.M.; Imboden, D.M. *Environmental Organic Chemistry*; John Wiley & Sons: New York, 1993.
- (43) Carslaw, H.S.; Jaeger, J.C. *Conduction of Heat in Solids*; 2nd ed.; Oxford University Press: London, 1959.
- (44) Crank, J. *The Mathematics of Diffusion*; Oxford University Press: London, 1975.
- (45) SERCC. 2005. Historical Climate Summaries for Puerto Rico and the U.S. Virgin Islands. Columbia, SC. <http://www.sercc.com/climateinfo/historical/historical.html> (accessed Jan 6, 2009).
- (46) Hughes, R.N.; Brimblecombe, P. *Agric. For. Meteorol.* **1994**, *67* (3, 4), 173–190.

Green Chemistry Letters and Reviews 217

- (47) García-Legaz, F.M.; López-Gómez, E.; Beneyto, J.M.; Navarro, A.; Sánchez-Blanco, M.J. *J. Plant Phys.* **2008**, *165* (10), 1049–1060.
- (48) Epsky, N.D.; Heath, R.R. *Environ. Entomol.* **1993**, *22* (2), 464–469.
- (49) Burk, T. *Florida Entomol.* **1983**, *66* (3), 330–344.
- (50) Hendrichs, J.P. PhD Thesis, University of Florida, Gainesville, 1986.
- (51) (a) Hendrix, D.L. *Crop Science* **1993**, *33*, 1306–1311;
(b) Tarpley, L.; Dahlberg, D.M.V.; Miller, FR. *Crop Science* **1993**, *33*, 338–341.
- (52) Heath, R.R.; Manukian, A. *J. Chem. Ecol.* **1992**, *18* (7), 1209–1226.
- (53) Heath, R.R.; Manukian, A. *J. Chem. Ecol.* **1994**, *20* (3), 503–604.

BIOLOGICAL CONTROL—MICROBIALS

***Psytalia* cf. *concolor* (Hymenoptera: Braconidae) for Biological Control of Olive Fruit Fly (Diptera: Tephritidae) in California**VICTORIA Y. YOKOYAMA,¹ PEDRO A. RENDÓN,² AND JOHN SIVINSKI³

USDA-ARS, San Joaquin Valley Agricultural Sciences Center, 9611 South Riverbend Ave., Parlier, CA 93648

Environ. Entomol. 37(3): 764–773 (2008)

ABSTRACT The larval parasitoid, *Psytalia* cf. *concolor* (Szépligeti), reared on Mediterranean fruit fly, *Ceratitis capitata* (Weidemann), by the USDA-APHIS-PPQ, Guatemala City, Guatemala, was imported into California for biological control of olive fruit fly, *Bactrocera oleae* (Gmelin), in olives, *Olea europaea* L. Mean percentage parasitism of olive fruit fly third instars infesting fruit in field cages ranged from 7.0 in Grapevine to 59.7 in Santa Barbara and in free releases ranged from 0 in Grapevine to 10.6 in Santa Barbara after 4- to 6-d exposures. In the laboratory, more parasitoids developed to adults in olive fruit fly larvae that were 11–13 d old than in larvae 8–10 d old. Adult parasitoids lived significantly longer when provided with water than adults without water in environmental chambers at 5°C, 85% RH; 15°C, 65% RH; 25°C, 25% RH; and 35°C, 25% RH. Adult parasitoids lived for 48 d with honey for food and water and 32 d with food and sugar solution at 15°C and 65% RH. Survival of adult parasitoids without food and water in greenhouse tests was ≈4 d in a simulated coastal climate and 1 d in a simulated inland valley climate and was significantly increased by providing food and water. The parasitoid did not develop in the beneficial seedhead fly, *Chaetorellia succinea* (Costa), in yellow star thistle. The rate of parasitism of walnut husk fly, *Rhagoletis completa* Cresson, larvae in green walnut husks was 28.4% in laboratory no-choice tests. In choice tests, the rate of parasitism of walnut husk fly versus olive fruit fly larvae in olives was 11.5 and 24.2%, respectively.

KEY WORDS larval parasitoid, *Bactrocera oleae* (Gmelin), *Olea europaea* L.

Olive fruit fly, *Bactrocera oleae* (Gmelin), was first detected in California in 1998 (Rice 2000). The introduction and distribution of the pest throughout the state has created a serious economic threat to the olive industry. High populations of olive fruit fly occur in the coastal areas, and low numbers of the pest are found in the San Joaquin Valley of California, where canning olives, *Olea europaea* L., are produced (Yokoyama et al. 2006). Quarantine strategies to mitigate pest populations in harvested fruit transported to processing plants were developed by Yokoyama and Miller (2004). Other methods to detect and control the pest were also studied (Yokoyama et al. 2006). However, biological control was found to have the greatest potential for reducing olive fruit fly populations in heavily infested areas (Yokoyama et al. 2004).

European countries have used parasitoids for biological control of olive fruit fly in commercial olives for many decades. A braconid, *Opius concolor* Szépligeti,

from Tunisia was released in Greece (Kapatos et al. 1977, Neuenschwander et al. 1983) and was later found to be the most abundant parasitoid of olive fruit fly in southern Crete (Michelakis 1990).

The larval parasitoid used in our studies was collected from tephritids infesting coffee in Kenya (Wharton et al. 2000) and shipped to Guatemala for rearing in 1999, where it has been carefully maintained in a pure colony. This parasitoid was originally described as an *Opius* sp. and briefly considered a synonym of *Psytalia humilis* (Silvestri) (Kimani-Njogu et al. 2001). Wharton et al. (2000) later described it as *Psytalia* cf. *concolor* from Kenya. Based on DNA work, *P. cf. concolor* in our study differs by a single base pair from the *P. concolor* maintained in colonies in California and Hawaii that are labeled Kenya but originate from Italy (Wharton et al. 2006), including the *P. concolor* studied by Sime et al. (2006) for olive fruit fly control in California.

We reared *P. cf. concolor* on Mediterranean fruit fly, *Ceratitis capitata* Weidemann, at the Medfly Parasitoids Rearing Facility La Aurora, Programa La Mosca del Mediterráneo (MOSCAMED), Guatemala, and imported it into California to determine its potential for biological control of olive fruit fly (Yokoyama et al. 2004, 2008). We selected *P. cf. concolor* for further study because the parasitoid could be mass reared, was

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by the USDA-ARS or USDA-APHIS-PPQ.

¹ Corresponding author, e-mail: vyokoyama@fresno.ars.usda.gov.

² USDA-APHIS-PPQ, Center for Plant Health Science and Technology, 4a. Avenida 12-62, Zona 10, Guatemala City, Guatemala.

³ USDA-ARS, Center for Medical, Agricultural, and Veterinary Entomology, 1700 SW 23 Dr., Gainesville, FL 32604.

found to develop in olive fruit fly, and its origins could be determined.

Factors that would affect the effectiveness of *P. cf. concolor* to control olive fruit fly include the ability of the parasitoid to adapt to different climatic conditions in California, the availability of food for the adult stage, the capacity to parasitize different life stages of olive fruit fly, and the potential to attack nontarget fruit flies. The objectives of our study were to determine the ability of *P. cf. concolor* to survive on olive fruit fly under laboratory, greenhouse, and field conditions that represent the diverse climatic regions of California and elucidate the susceptibility of other tephritids as hosts.

Materials and Methods

Production and Shipment of Parasitoids. *Psytalia cf. concolor* adults were reared from early third instars of the Antigua strain of Mediterranean fruit fly in the quarantine facility at MOSCAMED, San Miguel Petapa, Guatemala. Larval exposure cages were made from an inner plastic ring (10 cm inner diameter by 1.5 cm high by 0.5 cm wide) that was cut (0.2 cm wide) for flexibility, an outer plastic ring (10.8 cm inner diameter by 1.5 cm high by 0.3 cm wide), and nylon chiffon cloth (30 cm wide by 40 cm long). Naked Mediterranean fruit fly third instars were placed in the center of one side of the cloth. The cloth was folded in half to cover the larvae. The covered larvae were placed into the outer ring and secured in place with the inner ring. The larval exposure cage was moistened with water and placed on the screened top of a Plexiglas cage (30 cm wide by 30 cm long by 30 cm high) that contained *P. cf. concolor* females. Approximately 2,600 Mediterranean fruit fly larvae were exposed for 1.5 h to female parasitoids at a ratio of 1 female per 6.5–19.5 larvae. The parasitized larvae were placed on sawdust in Plexiglas cages for pupation, and the parasitoid adults that emerged were collected with glass aspirators.

The parasitoids were transported in paraffin-coated paper cups (11.0 cm diameter by 14.9 cm tall). A thin sponge (8.0 cm diameter by 2.5 cm high) was glued to the bottom of the cup and saturated with water. Newspaper (\approx 45 cm wide by 10 cm long) was pleated (8–10 folds) and glued to the inside of the cup. The top of the cup was covered with organdy cloth that was glued to the rim and fastened with a rubber band. Parasitoids (500 females and 500 males) that were 3 d old were placed inside the cup through a small opening in the cloth. The opening was sealed with cloth and glue. Honey was spread on the cloth for food. The cups were placed in a cardboard box with crushed paper for support, sealed with tape, and shipped by air freight.

On arrival at the USDA–ARS, Parlier, CA after 2 d in transit, the adults were placed into two wooden sleeve cages (72 cm wide by 42 cm deep by 50 cm tall) with Plexiglas tops. Mortality was calculated by counting the number of dead parasitoids in the bottom of the cups and reported as a percentage of the total number of parasitoids in the shipment. Honey was placed on

the inside of the top of each cage for food, and deionized water was provided with a cellulose sponge (5 cm wide by 7 cm long by 2 cm high) placed through a slit in the lid of a plastic bowl (11.5 cm diameter by 4 cm high). The sleeve cages were maintained in an isolated holding room at a daily $24.0 \pm 1.5^\circ\text{C}$ and $63 \pm 3\%$ RH (\pm SEM), and a photoperiod of 12:12 (L:D) h with simulated dawn and dusk conditions (model HLT Dawn/Dusk Simulator; Hughes Lighting Technologies, Lake Hopatcong, NJ). The parasitoids were observed and allowed to mate for at least 2 d. Parasitoids (163–303) were retained in each of three sleeve cages to determine the maximum life span under these conditions, and reported as the mean \pm SEM days parasitoids were alive after arrival in Parlier, CA.

Voucher specimens of *P. cf. concolor* were placed in the Entomology Laboratory, Plant Pest Diagnostics Center, California Department of Food and Agriculture, Sacramento, CA, and the Systematic Entomology Laboratory, National Museum of Natural History, Smithsonian Institution, Washington DC.

Field Cages and Releases. Parasitoids were collected from the sleeve cages with cylindrical paper cartons (9.5 cm diameter by 10 cm high; model HD16; Solo Cup, Urbana, IL) by brushing the adults inside and closing the opening with organdy fabric held in place with the collar of the lid. Honey and a sponge (3 cm wide by 4 cm long by 2 cm high) saturated with deionized water were placed on top of the fabric and covered with plastic food wrap held in place with a rubber band. The cartons of parasitoids were transported in insulated coolers by automobile or airplane for field studies.

Cylindrical cages were sewn from polyethylene screen (48.3 cm diameter by 61.0 cm long; no. C32A, 12 openings per cm; Synthetic Industries, Gainesville, GA). The base of the cage was finished with a cotton muslin fabric sleeve (48.3 cm diameter by 53.3 cm long) that had a cord sewn into the hem. Boning was sewn into the seam around the diameter of the cage between the screen and muslin sleeve to help maintain the cylindrical shape of the cage.

Each cage was placed over olive branches that had a large amount of fruit (290–498) infested with olive fruit fly in the canopy. Parasitoids were introduced into the cage from the opened paper cartons. The cage was closed at the base by pulling the cord to tighten the fabric sleeve around the branches and by fastening the sleeve with nylon tie wraps. The cages were removed from the trees by cutting off the branch above the ties after each exposure period.

Parasitoids (450–2,000) were released in trees without cages by attaching open cartons with vinyl flagging tape (2.8 cm wide) to an olive branch near fruit infested with olive fruit fly. The number of parasitoids used in cage tests and releases were based primarily on quantities received in each shipment and the number allocated for other tests.

Fruit collected for pretest controls to determine the number of olive fruit fly larvae were sampled at random from the same trees used for cage tests and releases. Postrelease samples of fruit were collected at

random in the immediate vicinity of the same trees in which parasitoids were released. Each fruit sample was considered a replicate.

Temperature loggers (model XTI08-5 + 37; Intermountain Environmental, Logan, UT) with external thermistors (model TMC6-1T; Intermountain Environmental) on extension cables (1.8 m long) were used to determine the temperature, and humidity loggers (model SRHA08; Intermountain Environmental) were used to determine relative humidity. A temperature and a humidity logger was placed inside each of three cages and on branches outside of each of the three cages in the canopy of trees at each location and programmed to record 720 determinations/d.

Four yellow panel Pherocon AM traps (Trécé, Adair, OK) each with a clear plastic packet (10.5 cm wide by 10.5 cm high) of ammonium bicarbonate bait (15–20 g) and a plastic dispenser (1.7 cm wide by 4.8 cm long) containing pheromone (1,7-dioxaspiro[5,5]undecane, 80 mg) were used to trap olive fruit fly adults at each location. Traps were placed in trees with cages, and in general, one trap was used per tree spaced one to several trees apart. The traps were suspended at mid-canopy ≈ 2.4 m high in a shaded area near fruit (Yokoyama et al. 2006) that was close to the cage. Each trap was considered a replicate.

Test Locations. Field cage and release studies were conducted in California in the following locations from September to November 2002: Aborn Road and Ruby Avenue, San Jose; Mission Canyon Road and Las Canoas Road, Santa Barbara; Paseo del Verano Norte and Cumana Terrace, San Diego; and Interstate Highway 5 and Grapevine Road, Grapevine. Mature olive trees, primarily of the Mission type, with canopies topped for hand harvesting and bearing fruit infested with olive fruit fly were selected in the following arrangements at each site: San Jose, a single windrow of 9 trees; Santa Barbara, a staggered border row of 7 trees along a commercial orchard; San Diego, a windrow of 11 trees; and, Grapevine, 9 trees in a commercial, landscaped area.

On 18 October 2002, in San Jose, three replicates of 563–585 olive fruit infested with olive fruit fly were collected for controls. Six replicate cages were placed in each of two trees with 60 parasitoids in each cage. Approximately 1,950 parasitoids were released in a tree without cages. Two traps were placed in each of two trees with cages. After a 5-d exposure, all cages, loggers, traps, and five replicates of 431–543 postrelease fruit weighing 958–1,275 g were collected.

On 9 October 2002, in Santa Barbara, three replicates of 630–731 infested olive fruit were collected for controls. Six replicate cages were placed in each of four trees with 50 parasitoids in each cage. Approximately 450 parasitoids were released in a tree without cages. Four traps were placed in three trees with cages. After a 6-d exposure, all cages, loggers, traps, and three replicates of 432–614 postrelease fruit weighing 807–1,141 g were collected.

On 2 October 2002, in San Diego, three replicates of 293–311 infested olive fruit were collected for controls. Six replicate cages were placed in each of five

trees with 50 parasitoids in each cage. Approximately 1,500 parasitoids were released in a tree without cages. Four traps were placed in each of four trees with cages. After a 5-d exposure, all cages, loggers, traps, and five replicates of 509–755 postrelease fruit weighing 761–1,146 g were collected.

On 30 September 2002, in Grapevine, three replicates of 157–237 infested olive fruit were collected for controls. Six replicate cages were placed in each of four trees with 30 parasitoids in each cage. Approximately 500 parasitoids were released in one tree. Four traps had been placed on 19 September in three trees adjacent to trees with cages and in one tree with a cage. After a 4-d exposure, all cages, loggers, traps, and three replicates of 383–452 postrelease fruit weighing 1,162–1,463 g were collected.

All materials were returned to the laboratory for evaluation. Fruit from controls, inside cages, or near parasitoid releases were removed from the branches, counted, and placed in plastic containers (22 cm wide by 32 cm long by 13 cm high) covered with organdy cloth and held in the laboratory at 23°C. The total number of fruit was reported as the mean \pm SEM of the replicates. The weight of fruit in controls was reported as the mean \pm SEM of the replicates. Olive fruit fly pupae and adults and parasitoid adults that emerged from fruit collected from cages and near releases were counted for ≥ 56 d from the beginning of the exposure period. Temperature and humidity data were reported as the daily mean \pm SEM inside and outside of three replicate cages. Mean daily temperature and humidity were compared inside and outside of cages with a two-tailed paired *t*-test, α level at 0.05, and both inside and outside temperature and humidity were compared among locations using a one-way analysis of variance (ANOVA) and Tukey's test (GraphPad Software 2007). Percentage females and the total number of olive fruit fly adults captured per day were reported as the mean \pm SEM of four replicate traps and compared among the locations using a one-way ANOVA and Tukey's test (GraphPad Software 2007).

Parasitoid-induced mortality of olive fruit fly in the field cage and release tests was calculated by $1 -$ the number of all life stages that emerged from fruit in each test divided by the expected number of all life stages to emerge. The latter value was based on the number of all life stages that emerged per fruit in the controls. Percentage parasitoid induced mortality was reported as the mean \pm SEM of the replicates.

Calculation of Parasitism. The number of olive fruit fly larvae and pupae that emerged in controls for 4 d after the fruit was collected was reported as the mean \pm SEM third instars and third instars per fruit. The number of third instars per fruit was multiplied times the number of fruit in each test replicate to estimate the number of third instars exposed to the parasitoids and reported as the mean \pm SEM. Percentage parasitism was calculated by dividing the number of parasitoid adults that emerged in the exposed fruit by the estimated number of third instars and reported as the mean \pm SEM of the replicates.

Larval Susceptibility to Parasitism. Olives were exposed to olive fruit fly adults for oviposition for 1 d to obtain 8- or 10-d-old larvae and for 2 d to obtain 11- to 13-d-old larvae. After exposure to oviposition, three replicates of 42, 36, and 39 olives were held at 23°C for 8, 10, and 11 d, respectively. Each replicate of postinfested fruit was placed in an aluminum frame cage (30.5 cm wide by 30.5 cm long by 30.5 cm high; model 1450B; BioQuip Products, Rancho Dominguez, CA) with polyethylene screening. Forty parasitoids were placed for 4 d in each cage. One cage each of 8- or 10-d postinfested fruit and one cage of 11- to 13-d postinfested fruit were used for nonexposed controls.

The number of olive fruit fly pupae and adults per fruit that emerged in the nonexposed controls was reported as the combined mean \pm SEM of 8- and 10-d postinfested fruit and the mean of 11- to 13-d postinfested fruit. This value was multiplied times the number of fruit in each replicate to estimate the number of larvae that were exposed to the parasitoids and reported as the mean \pm SEM of the replicates. Percentage parasitism was calculated by dividing the number of parasitoid adults that emerged from the exposed fruit by the estimated number of larvae, reported as the mean \pm SEM of the replicates. The results for the exposed 8- and 10-d postinfested fruit and respective nonexposed controls were combined, and reported as values for 8–10 d. Percentage parasitism was arcsine transformed and compared between 8- to 10- and 11- to 13-d postinfested fruit with a two-tailed unpaired *t*-test (GraphPad Software 2007).

Parasitoid Survival in Laboratory Tests. Cages were constructed with cylindrical paper cartons (9.5 cm diameter by 10 cm high; model HD16; Solo Cup, Urbana, IL). A plastic cup lid (6.5 cm diameter) was glued to the side of the carton and used as a platform when the cage was placed on its side. A hole (1.3 cm diameter) was cut into the top of the cage near the opening. The neck of an inverted glass vial (1.6 cm diameter by 6 cm tall) was filled with deionized water or 5% sucrose in deionized water, plugged with cotton, and placed through the hole in tests in which liquid was provided. Parasitoids (22–60) were placed in each cage, and the opening was covered with organdy fabric held in place with the lid collar. Replicate cages (4–15) were prepared with parasitoids that were provided with honey for food on the fabric cover and no water, water, or 5% sucrose in water. The cages were placed in laboratory environmental chambers (model E32560; Lab-Line, Melrose Park, IL), and parasitoid survival was evaluated every 1–5 d for the following constant temperature and relative humidity combinations, 5°C, 85%; 15°C, 65%; 25°C, 25%; and 35°C, 25%, until all adults had died. Results were reported as the mean \pm SEM number of days adults were observed alive in each replicate cage. Survival was compared with or without water or 5% sucrose in water using a one-way ANOVA and Tukey's test (GraphPad Software 2007).

Parasitoid Survival in Greenhouse Tests. Temperature and relative humidity were maintained in a par-

tioned glass greenhouse (5.6 m wide by 9.3 m long) with swamp coolers and heaters and monitored with a hygrothermograph (model CT485; Omega Engineering, Stamford, CT).

Cages were sewn from polyethylene screen (35 cm wide by 35 cm long by 56 cm high). An opening (12 cm wide by 12 cm high) was cut into one side of the cage for access and covered with a flap (30 cm wide by 30 cm high). The cage was suspended inside a polyvinyl chloride pipe frame (2.7 cm diameter; 42 cm wide by 42 cm long by 65 cm high).

The greenhouse was maintained to simulate a coastal climate on the cooler side and an inland valley climate on the warmer side and reported as the daily mean \pm SEM of the diurnal and nocturnal temperatures and relative humidities. Newly eclosed parasitoid adults (35–58) were placed in a cage, and their survival tested with either no food and no water or honey for food and water. Deionized water was provided through a cellulose sponge (5 cm wide by 7 cm long by 2 cm deep) placed through the lid of a plastic bowl (11.5 cm diameter by 4 cm deep). Six replicate cages were used on each side of the greenhouse. Survival in each cage was determined every 1–4 d until all adults had died. Results were reported as the mean \pm SEM number of days adults were observed alive in each replicate cage. Parasitoid survival with and without food and water and between the cool and warm side of the greenhouse was compared with a two-tailed paired *t*-test (GraphPad Software 2007).

Seedhead Fly as a Host. Whole plants of yellow star thistle, *Centaurea solstitialis* L., with floral buds (fruit) infested with the larval stage of a tephritid seedhead fly, *Chaetorellia succinea* (Costa), were collected from fields in Hercules, CA, on 10 October 2004. Infested bouquets of floral buds with 25-cm stem lengths were cut at random from different plants. The bouquets were placed in 250-ml Erlenmeyer flasks filled with deionized water in aluminum frame cages (30.5 cm wide by 30.5 cm long by 30.5 cm high; model 1450B; BioQuip Products) with polyethylene screening.

Fifty females and one to three male parasitoids were placed in each cage and held in a room at a daily $24.0 \pm 1.5^\circ\text{C}$ and $63 \pm 3\%$ RH (mean \pm SEM) and a photoperiod of 12:12 (L:D) h. The infested yellow star thistle was exposed to the parasitoids for 2 d in three replicate cages and for 7 d in six replicate cages in no-choice tests. At the end of the exposure period, the bouquets were removed from each cage, and all parasitoids were removed. The bouquet was placed in a new cage and observed every 1–3 d for 67–75 and 56–67 d in tests with 2- and 7-d exposures, respectively. The number of seedhead fly adults and parasitoids that emerged from the bouquets was counted. The floral buds were removed from the stems, counted, and dissected, and all life stages of the seedhead fly and parasitoids were counted.

A control to estimate the number of seedhead fly larvae in the yellow star thistle buds at the time of exposure to the parasitoids in no-choice tests was made by collecting five replicates of a random sample of yellow star thistle floral buds (50–51) from all har-

Table 1. Mean \pm SEM trap captures of olive fruit fly adults, temperatures, and relative humidities over 4–6 d with parasitoid cages in olive trees with fruit infested with olive fruit fly in 2002

Location	Trap captures ^a		Cage daily temperature (°C)		Cage daily %RH	
	Percent females	No. per day	Interior	Exterior	Interior	Exterior
San Jose ^b	44.1 \pm 3.0ab	27.2 \pm 3.4a	15.2 \pm 0.1a	15.0 \pm 0.2a	78.1 \pm 2.6	73.8 \pm 0.8a
Santa Barbara ^c	42.5 \pm 5.5ab	7.6 \pm 0.6b	15.3 \pm 0.5a	14.8 \pm 0.3a	84.2 \pm 1.7	85.4 \pm 0.8b
San Diego ^d	47.1 \pm 1.3a	36.6 \pm 2.7a	16.6 \pm 0.3ab	17.0 \pm 0.2b	70.0 \pm 0.3	66.3 \pm 0.4c
Grapevine ^e	30.9 \pm 3.4b	10.0 \pm 2.0b	16.9 \pm 0.2b	17.0 \pm 0.1b	38.8 \pm 2.0	35.8 \pm 2.1d

Means within a column followed by the same letter are not significantly different ($P > 0.05$, Tukey's test; GraphPad Software 2007).

^a Four replicates.

^b 18–23 Oct.

^c 9–15 Oct.

^d 2–7 Oct.

^e 30 Sept. to 4 Oct.

vested plants. The floral buds were dissected and inspected for the larval stages of the seedhead fly. The length of each larva was measured and determined as first, second, and third instars according to lengths that ranged from <2.5, 2.5–3.5, and 4.0–5.0 mm, respectively. Each instar was reported as the percentage of all larvae that were dissected from the floral buds in the control. The total number of larvae were counted and reported as the mean \pm SEM per floral bud. The number of seedhead fly larvae exposed to the parasitoids was calculated by multiplying the number of larvae per floral bud in the control times the number of fruit in each test replicate and reported as the mean \pm SEM.

Female parasitoid behavior in the presence of seedhead fly larvae in the host was studied by dissecting five green, open floral buds with yellow or dried petals, which indicated larval feeding, and counting the number of third instars in each bud. Based on the number of third instars per bud, four bouquets of infested floral buds (61–74) were each placed in a separate cage with mated female parasitoids (46–56) at the ratio of 15 per 20 floral buds. The females were observed for searching or probing behavior on the infested floral buds for \approx 1 h two to four times each day between 0700 and 1500 hours for 2.5 d. The percentage of live females per cage (4) that showed searching or probing behavior was reported as the mean \pm SEM for all observation periods (9).

Walnut Husk Fly Compared with Olive Fruit Fly as a Host. The susceptibility of walnut husk fly to parasitism was determined in a laboratory no-choice test and a comparative choice test between walnut husk fly and olive fruit fly. English walnuts, *Juglans regia* L., infested with walnut husk fly larvae were collected from trees in Fresno and Hanford, CA, and placed in aluminum frame cages. In the no-choice test, 20 infested walnuts were placed in each of three replicate cages with 30 female and 15 male parasitoids per cage for 7 d. Twenty walnuts were used for nonexposed controls and placed over sand in a plastic container to collect emerging third instars as described by Yokoyama et al. (1992). To evaluate mortality caused by exposure to parasitoids, the total number of walnut husk fly pupae that emerged in the control and the total number of walnut husk fly pupae and parasitoid

adults that emerged in each no-choice test was compared for significance by a two-tailed paired *t*-test (GraphPad Software 2007) and reported as the mean (\pm SEM) of the replicates.

In choice tests, 16 walnuts infested with walnut husk fly larvae and 32 olive fruit infested with olive fruit fly second to third instars in the laboratory were placed in each of three replicate cages with 11–12 females and 6 male parasitoids for 7 d. Sixteen infested walnuts and 32 infested olive fruit were used for nonexposed controls and placed in plastic containers to collect the emerging larvae. These larvae were used to determine the relative density of the host in the fruit as described below.

The no-choice and choice tests were held at 24.0 \pm 1.5°C and 63 \pm 3% RH (mean \pm SEM) and a photoperiod of 12:12 (L:D) h. The total number of pupae that emerged in controls 26–27 d after the start of each test was counted in walnuts in no-choice tests and in walnuts and olive fruit in choice tests. This number was divided by the number of fruit in each control to calculate the number of larvae per fruit and multiplied by the number of fruit in each no-choice and choice test replicate to estimate the number of larvae that were exposed to the parasitoid and reported as the mean \pm SEM of the replicates.

The number of adult parasitoids that emerged from fruit in no-choice and choice tests was counted 43–44 d after the beginning of the test when parasitoids ceased to emerge. The number of parasitoids was divided by the estimated number of larvae in each test to calculate percentage parasitism and reported as the mean \pm SEM of the replicates. Percentage parasitism between walnut husk fly larvae in walnuts and olive fruit fly larvae in olive fruit was compared with a two-tailed unpaired *t*-test (GraphPad Software 2007).

Results

Shipment of Parasitoids. Mortality of *P. cf. concolor* adults imported from MOSCAMED, Guatemala City, Guatemala, was \approx 1% on arrival in Fresno, CA, after 2 d of transport by air and ground. The parasitoids were 3 d old when collected and packaged in Guatemala, 5 d old when they were received, and \geq 7 d old when used in laboratory and field tests. The maximum life span of

Table 2. Mean \pm SEM no. of fruit, estimated olive fruit fly third instars exposed 4–6 d to parasitoids, and percentage parasitism of olive fruit fly third instars in four locations in California in 2002

Location	Test	No. parasitoids released	No. fruit	No. third instars	Percent parasitism
San Jose	Control	0	572.7 \pm 6.5	129.0 \pm 16.6	
	Cage	60	370.8 \pm 54.1	83.4 \pm 12.2	24.1 \pm 5.2
	Release	2,000	489.4 \pm 22.5	110.1 \pm 5.0	1.6 \pm 0.5
Santa Barbara	Control	0	689.0 \pm 30.4	90.7 \pm 0.9	
	Cage	50	497.8 \pm 75.0	65.7 \pm 9.9	59.7 \pm 9.4
	Release	450	548.0 \pm 58.2	72.3 \pm 7.7	10.6 \pm 0.3
San Diego	Control	0	301.3 \pm 5.2	267.7 \pm 43.4	
	Cage	50	397.8 \pm 67.1	352.9 \pm 59.6	13.1 \pm 2.1
	Release	1,500	630.4 \pm 40.0	559.2 \pm 35.52	4.1 \pm 0.8
Grapevine	Control	0	196.0 \pm 23.1	3.3 \pm 0.9	
	Cage	30	289.5 \pm 25.2	5.2 \pm 0.5	7.0 \pm 4.5
	Release	500	410.7 \pm 21.0	7.4 \pm 0.4	0

the parasitoids that were maintained in sleeve cages was 69.3 ± 6.7 (mean \pm SEM) from the day the shipment was received.

Evaluation of Parasitism in Test Locations. The mean percentage of females of olive fruit fly adults collected in yellow panel traps was $<50\%$ for all locations and significantly different ($F = 3.77$; $df = 3,12$; $P = 0.041$) among the locations (Table 1). Trap captures ranged from a mean of 7.6 in Santa Barbara to 36.6 adults per day in San Diego and were significantly different ($F = 33.5$; $df = 3,12$; $P < 0.0001$) among the locations. Mean \pm SEM fruit size measured by weight in controls was 2.02 ± 0.01 , 1.26 ± 0.04 , 1.77 ± 0.02 , and 2.94 ± 0.18 g in San Jose, Santa Barbara, San Diego, and Grapevine, respectively. Mean temperatures inside cages ranged from 15.2°C in San Jose to 16.9°C in Grapevine and outside of cages ranged from 14.8°C in Santa Barbara to 17.0°C in San Diego and Grapevine. Mean relative humidity inside cages ranged from 38.8% in Grapevine to 84.2% in Santa Barbara and outside of cages ranged from 35.8% in Grapevine to 85.4% in Santa Barbara. Temperatures inside and outside of cages were not significantly different in each location, but the relative humidity was significantly higher inside ($t = 8.38$, $df = 2$, $P = 0.014$) than outside the cage in San Diego. Temperatures on the outside of cages were significantly different ($F = 36.35$; $df = 3,8$; $P < 0.0001$) among locations. The San Diego and Grapevine location temperatures were significantly

higher ($P < 0.001$) than the Santa Barbara and San Jose locations. The mean relative humidity on the outside of the cage was significantly different ($F = 426.4$; $df = 3,7$; $P < 0.0001$) among the locations.

The mean \pm SEM number of third instars per fruit in controls for cage and release tests were 0.2 ± 0.0 in San Jose, 0.1 ± 0.0 in Santa Barbara, 0.9 ± 0.1 in San Diego, and 0.02 ± 0.01 in Grapevine. Based on controls, the mean number of third instars in cage tests ranged from 5.2 in Grapevine to 352.9 in San Diego and in release tests ranged from 7.4 in Grapevine to 559.2 in San Diego (Table 2). Mean percentage parasitism in cages ranged from 7.0 in Grapevine to 59.7 in Santa Barbara and in releases ranged from 0 in Grapevine to 10.6 in Santa Barbara. Mean percentage parasitism was highest in cages versus releases in all sites.

Mean percentage of parasitoid-induced mortality of olive fruit fly larvae after exposure to parasitoids in cages ranged from 20.2 in Santa Barbara to 55.6 in San Diego and in releases ranged from 0 in Santa Barbara and Grapevine to 42.0 in San Diego (Table 3).

Larval Susceptibility to Parasitism. The mean \pm SEM number of 8- to 10-d-old and 11- to 13-d-old olive fruit fly pupae and adults per fruit that emerged in controls was 0.9 ± 0.4 (two cages of 36–42 fruit) and 1.1 (one cage of 39 fruit), respectively. Percentage parasitism was significantly higher ($t = 2.946$, $df = 7$, $P = 0.0215$) in olive fruit fly larvae that were 11–13 d old than in larvae 8–10 d old (Table 4).

Parasitoid Survival in Laboratory Tests. Survival of adult parasitoids at different temperatures and humidities in laboratory environmental chambers with food and no water, with water, or with a 5% sugar water solution are shown in Table 5. Adults lived significantly longer when provided with water than adults

Table 3. Mean \pm SEM percentage parasitoid induced mortality of all olive fruit fly larvae after 4- to 6-d exposure to *P. cf. concolor* in four locations in California

Location	Test	Expected no. adults ^a	Percent mortality
San Jose	Cage	404.2 \pm 59.0	38.9 \pm 9.6
	Release	533.4 \pm 24.5	1.1 \pm 0.8
Santa Barbara	Cage	348.5 \pm 52.5	20.2 \pm 9.2
	Release	383.6 \pm 40.7	0
San Diego	Cage	823.5 \pm 139.0	55.6 \pm 6.6
	Release	1,304.9 \pm 82.9	42.0 \pm 7.7
Grapevine	Cage	37.6 \pm 3.3	22.2 \pm 13.4
	Release	53.4 \pm 2.7	0

^a Number of fruit collected in each test multiplied by the no. of all stages of olive fruit fly per fruit collected in controls.

Table 4. Mean \pm SEM percentage parasitism of olive fruit fly larvae exposed 4 d to *P. cf. concolor*

Larval age (d)	No. parasitoids	No. fruit	No. larvae	Percent parasitism
8–10	40	39 \pm 1.3	35.1 \pm 1.2	24.4 \pm 7.2a
11–13	40	39.0 \pm 0.0	42.9 \pm 0.0	73.7 \pm 13.4b

Means within a column followed by the same letter are not significantly different ($P > 0.05$, *t*-test; GraphPad Software 2007).

Table 5. Mean \pm SEM survival of *P. cf. concolor* adults at constant temperature and humidity in laboratory environmental chambers with or without water or a sugar water solution and with food (honey)

Temperature (°C)	Percent relative humidity	Survival (d)		
		No water + food ^a	Water + food ^b	5% sugar water + food ^c
5	85	7.2 \pm 0.2a	15.1 \pm 1.3b	9.8 \pm 0.2ab
15	65	4.0 \pm 0.0a	47.6 \pm 5.0b	32.5 \pm 3.8ab
25	25	1.0 \pm 0.0a	22.6 \pm 2.1b	13.8 \pm 1.5b
35	25	0.0 \pm 0.0a	11.7 \pm 1.4b	3.0 \pm 0.0a

Means within a row followed by the same letter are not significantly different ($P > 0.05$, Tukey's test; GraphPad Software 2007).

^a Four replicates of 22–34 adults.

^b Fifteen replicates of 22–40 adults.

^c Four replicates of 32–60 adults.

without water at 5°C and 85% RH ($F = 6.98$; $df = 2, 20$; $P = 0.0050$, Tukey's test, $P < 0.01$); 15°C and 65% RH ($F = 11.41$; $df = 2, 20$; $P = 0.0005$, Tukey's test, $P < 0.001$); 25°C and 25% RH ($F = 16.53$; $df = 2, 20$; $P = 0.0001$, Tukey's test, $P < 0.001$); and 35°C and 25% RH ($F = 12.86$; $df = 2, 20$; $P = 0.0003$, Tukey's test, $P < 0.001$). Survival of adults provided with a sugar solution was longer than those without water but not significantly so at 5 and 15°C. At 25°C, survival of adults provisioned with either water or a sugar solution was similar, but at 35°C, adults receiving the sugar solution lived only 3 d longer than adults without water. Adults that received no water lived for 7 d at 5°C and 85% RH, and the length of survival decreased with an increase in temperature and corresponding decrease in humidity. Adult parasitoids lived the longest at 15°C and 65% RH when provided with food and water (48 d) or food and sugar solution (32 d).

Parasitoid Survival in Greenhouse Tests. In the cooler side of the greenhouse, the daily mean \pm SEM diurnal temperature and humidity were 26.5 \pm 0.2°C and 62.7 \pm 0.9%, and the daily mean nocturnal temperature and humidity were 24.7 \pm 0.2°C and 58.5 \pm 1.5%. In the warmer side, the daily mean diurnal temperature and humidity were 36.2 \pm 0.3°C and 31.4 \pm 1.4%, and the daily mean nocturnal temperature and humidity were 25.6 \pm 0.3°C and 47.5 \pm 2.8%. Survival of adult parasitoids was \approx 4 d on the cool side and 1 d on the warm side of the greenhouse without water and food and was significantly increased ($t = 5.71$, $df = 5$, $P = 0.0023$) on the cool side and on the warm side ($t = 10.25$, $df = 5$, $P = 0.0002$) when provided with water and food (Table 6). Survival was significantly higher with ($t = 9.50$, $df = 5$, $P = 0.0002$) and without ($t =$

Table 6. Mean \pm SEM survival of *P. cf. concolor* adults in a greenhouse with or without water and food (honey)

Daily temperature (°C)	Daily percent relative humidity	Survival (d)	
		No water, no food ^a	Water + food ^a
25.9 \pm 0.2	61.1 \pm 1.1	4.3 \pm 1.3	21.3 \pm 2.2
32.2 \pm 0.2	37.4 \pm 2.2	\leq 1.0 \pm 0.0	3.5 \pm 0.3

^a Six replicates of 35–58 adults.

3.25, $df = 5$, $P = 0.0227$) water and food on the cool side versus the warm side of the greenhouse. Survival with food and water at mean daily temperatures of 26 and 32°C in greenhouse tests was similar to survival at constant temperatures of 25 and 35°C in environmental chambers.

Seedhead Fly, Walnut Husk Fly, and Olive Fruit Fly as Hosts. The mean \pm SEM number of seedhead fly larvae per floral bud in no-choice test controls was 0.15 \pm 0.06. The percentage of first, second, and third instars in the control was 8.1, 62.2, and 29.7%, respectively. The parasitoid did not develop in seedhead fly larvae in yellow star thistle, which was confirmed by dissections (Table 7). Yellow star thistle floral buds that were used to observe female parasitoid searching or probing behavior had a mean of 1.6 \pm 0.4 (SEM), and a range of one to three seedhead fly third instars per floral bud. A mean of 1.6 \pm 0.6% (SEM) of the females showed searching behavior on the infested floral buds per observation period. The mean percentage of females that searched was highest (5.65%) when the parasitoids were first introduced into the cages with the bouquets. No females were observed to probe or insert the ovipositor into the infested floral buds.

The mean number of walnut husk fly larvae per fruit was 12.5 (20 infested walnut fruit) in the no-choice test control and 23.5 (16 infested walnut fruit) in the choice test control. The mean number of olive fruit fly second and third instars per fruit was 3.7 (32 infested olive fruit) in the choice test control. The parasitoid reproduced in walnut husk fly larvae in the parasitoid and olive fruit fly larvae in olives (Table 7). Parasitism of walnut husk fly was higher in no-choice tests than in choice tests with olive fruit fly. Parasitism of olive fruit fly larvae in choice tests was not significantly different ($t = 1.36$, $df = 4$, $P = 0.2450$) than walnut husk fly larvae. The total number of walnut husk fly pupae that emerged in the control was not significantly different ($t = 3.69$, $df = 2$, $P = 0.0662$) than the mean (\pm SEM) total number of walnut husk fly pupae and parasitoid adults (286.7 \pm 77.7) that emerged in no-choice tests, indicating that no additional host mortality occurred from exposure to parasitoids

Discussion

The parasitoid, *P. cf. concolor*, was successfully imported into California from Guatemala City, Guatemala, with a high rate of survival after shipment. On arrival, the adults were long lived under laboratory conditions and easily maintained until used in experiments or released into olive trees infested with olive fruit fly. Four locations in California (Fig. 1) were selected to study field interactions between parasitoids and olive fruit fly. The study areas included San Jose, in the central coast; Santa Barbara, in the southern coast; San Diego, in the southern coast just north of Mexico; and Grapevine, at the southern end of the San Joaquin Valley where olives are grown primarily for canning. These locations represented regional habitats where olive fruit fly occurred as a newly intro-

Table 7. Mean \pm SEM percentage parasitism in no-choice tests with seedhead fly in yellow star thistle, walnut husk fly in green walnut husks, and in a choice test with walnut husk fly in walnuts versus olive fruit fly in olives

Cage test	Species	No. fruit	No. larvae	Exposure (d)	Percent parasitism
No-choice	Seedhead fly	141.0 \pm 13.0	21.2 \pm 2.0	2	0
		270.8 \pm 18.7	40.6 \pm 2.8	7	0
No-choice	Walnut husk fly	20.0 \pm 0.0	250.0 \pm 0.0	7	28.4 \pm 9.3
Choice	Walnut husk fly	16.0 \pm 0.0	376.0 \pm 0.0	7	11.5 \pm 2.2
	Olive fruit fly	32.0 \pm 0.0	118.4 \pm 0.0	7	24.2 \pm 9.1

duced pest on olives and the effectiveness of the parasitoid could be evaluated.

Numbers of olive fruit fly adults were monitored in the study locations with yellow panel traps that had been used in previous studies (Yokoyama et al. 1992, Yokoyama and Miller 2007) and were found to be more effective in capturing adults than ChamP traps (Yokoyama et al. 2006). Two traps per 2–4 ha are recommended for monitoring olive fruit fly adults, and use of more than one trap in our small test locations provided greater accuracy in determining adult numbers (Johnson et al. 2006). More males than females were captured in these traps in all locations (Table 1), which is similar to previous findings (Yokoyama et al. 2006). The highest number of adults per day were captured in San Diego, and the lowest number was in Santa Barbara, which represents the adult population of olive fruit fly in these locations.

The physical attributes of the cage designed for this study was acceptable to evaluate parasitism of olive fruit fly and did not cause detrimental internal conditions. Temperature and humidity were found to be similar inside and outside of cages in all locations except San Diego, where the humidity was higher inside the cage. The humidity in Grapevine was lower than the other locations, reflecting the arid conditions of the lower San Joaquin Valley.

The method we used to calculate percentage parasitism was similar to those used by Neuenschwander

et al. (1983). In our study, olive fruit fly third instars were used to determine the rate of parasitism because development of the parasitoid to the adult stage was more successful in thirds than in earlier instars. Fruit samples were collected for laboratory controls at each test location to determine the number of olive fruit fly larvae in each instar during the period of exposure to parasitoids. The laboratory controls were used instead of field cage controls to eliminate monitoring at distant locations and to allow daily observations of olive fruit fly pupal emergence. Variability in the number of parasitoids in field cage tests and releases resulted from the number of parasitoids shipped and divided among laboratory and greenhouse tests, whereas variability in exposure to olive fruit fly in the field resulted from travel schedules to distant test locations.

Tests in Santa Barbara resulted in the highest rate of parasitism of olive fruit fly in cages and releases (Table 2) compared with other locations, although higher numbers of olive fruit fly adults were captured (Table 1), and older larvae were more abundant in San Diego than in Santa Barbara. The lower temperature and higher humidity in Santa Barbara versus San Diego may have enhanced parasitism. These conditions were found by Yokoyama and Miller (2007) and Yokoyama et al. (2006) to support olive fruit fly growth and development. Olive fruit fly larvae were least abundant and rates of parasitism were lowest in Grapevine, where temperatures tended to be higher and the humidity lower than in the other locations. Weather conditions or low host density alone may be related to the small number of parasitoid progeny recovered from fruit infested with olive fruit fly. Based on the results of these tests, biological control of olive fruit fly by the parasitoid would be more effective in cool, coastal climates where the host is abundant.

Olive fruit fly larvae exposed to parasitoids in cage tests in all locations and in the San Diego release test showed high levels of parasitoid induced mortality (Table 3). Mortality was based on the number of all life stages that emerged in the control fruit and in most cases was higher than expected from parasitism of third instars alone (Tables 2 and 4). Yokoyama et al. (2004) attributed this effect to mortality among first and second olive fruit fly instars caused by the parasitoid. This observation was also reported by Calvitti et al. (2002) in olive fruit fly by the egg parasitoid, *Fopius arisanus* (Sonan). He reported that a proportion of dead eggs are always associated with parasitism even if percentage parasitism is low.

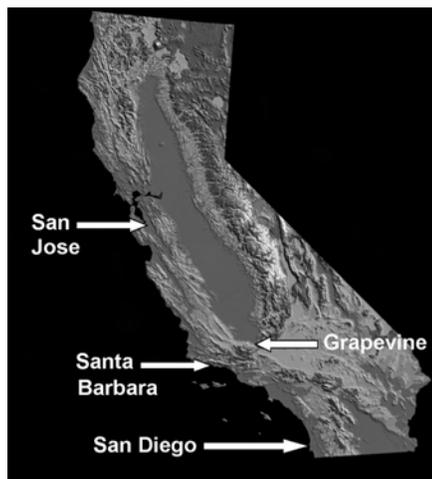


Fig. 1. Parasitoid and olive fruit fly study locations in California.

A greater number of *P. cf. concolor* completed development to the adult stage in olive fruit fly third instars that were 11–13 d old than in younger larvae (Table 4). In a similar study with two cultures of *P. concolor*, Sime et al. (2006) also found the parasitoids to reproduce most successfully in third instars. Canale and Loni (2006) found that *P. concolor* was more successful in locating third instars than second instars of Mediterranean fruit fly in laboratory dishes. Development of *P. cf. concolor* in olive fruit fly second instars (Yokoyama et al. 2004) would result in higher larval mortality. Based on our findings, *P. cf. concolor* developed more successfully in 11- to 13-d-old olive fruit fly larvae than in 8- to 10-d-old larvae.

In laboratory tests, parasitoid longevity was ≈ 7 wk at 15°C and 65% RH when food and water were provided (Table 5). These conditions were similar to the test environments of the coastal locations of San Jose and Santa Barbara (Table 1), where parasitism was highest in cage tests and in release tests in Santa Barbara (Table 2).

Parasitoid longevity when provided with food and water was $\approx 2, 3,$ and 1.5 wk at 5 (85%), 25 (25%), and 35°C (25% RH), respectively (Table 5). The dry environment of Grapevine (Table 1) may help explain the low level of parasitism in this location (Table 2). Sime et al. (2006) showed *P. concolor* also had higher survival at a constant temperature of 15°C than at higher temperatures when provided with food and water. To ensure accuracy in our study in relation to the different regional climates in California, more replicates were used to determine optimal parasitoid survival in the presence of water and food than without water or with sugar water in the four combinations of temperature and humidity.

The controlled environment in greenhouse tests was more similar to natural conditions than laboratory tests at constant temperature and humidity because the diurnal and nocturnal temperatures and humidities fluctuated during the day. Parasitoids lived longer in the cool and humid side of the greenhouse than in the warm and dry side (Table 6). The presence of food and water enhanced survival in both greenhouse climates and would help the parasitoid to survive under less than optimum temperature conditions in the field. The maximum length of survival in both laboratory and greenhouse tests was based on the days in which live individuals were last seen in each test because observations were not done on a daily basis. Therefore, actual survival may have been longer than we reported. These studies have shown that biological control of olive fruit fly by *P. cf. concolor* would be most effective in regions with mild climates and a source of food and water for adults.

Host specificity is a primary consideration for potential release of parasitoids for olive fruit fly control (Nadel et al. 2005). Before a large-scale release program for *P. cf. concolor* could be implemented, the status of a beneficial tephritid, a seedhead fly, in yellow star thistle and a walnut pest, walnut husk fly, were evaluated (Table 7). The results of laboratory no-choice tests showed that the parasitoid did not attack

the seedhead fly. This observation is important because yellow star thistle is commonly found near olive groves and orchards. Testing the acceptability of seedhead fly larvae as a host for *P. cf. concolor* was needed because Daane et al. (2006) reported that another opiine braconid, *Diachasmimorpha kraussii* (Fullaway), reproduced in *C. succinea*. Our parasitoid developed in walnut husk fly in no-choice tests indicating that the pest could serve as an alternative host. However, in choice tests between walnut husk fly and olive fruit fly, the parasitoid showed a higher rate of parasitism of olive fruit fly, suggesting that olive fruit fly may be a more susceptible host.

Distribution of *P. cf. concolor* in California may be limited by climatic conditions, but temperatures and humidities that are suitable for the development of the host (Yokoyama and Miller 2007) are also suitable for the parasitoid. The parasitoid has potential to reduce coastal populations of olive fruit fly that serve as perpetual reservoirs of the pest and where no other method of control is practical or economical. The use of *P. cf. concolor* for augmentative or classical biological control may be limited because of the potential impact on endemic tephritids, lack of mass rearing procedures for olive fruit fly for parasitoid production, and establishment has yet to be achieved.

Acknowledgments

We thank G. T. Miller for help in conducting the research and reviewing the manuscript; G. E. Sergent and P. A. Dwyer, USDA–ARS, San Joaquin Valley Agricultural Sciences Center, Parlier, CA; M. Lopez and A. Aldana, USDA–APHIS–PPQ, MOSCAMED, Guatemala City, Guatemala; M. Johnson and H. Nadel, University of California, Kearney Agricultural Center, Parlier, CA; and R. Wharton, Texas A&M University, College Station, TX, for assistance; and Rancho Bernardo Winery, Rancho Bernardo, CA; G. Turpin, Santa Barbara, CA; and Mirassou Winery, San Jose, CA, for use of their olive orchards for this project. This research was funded in part by the California Olive Committee, Fresno, CA.

References Cited

- Calvitti, M., M. Antonelli, R. Moretti, and R. C. Bautista. 2002. Oviposition response and development of the egg-pupal parasitoid *Fopius arisanus* on *Bactrocera oleae*, a tephritid fruit fly pest of olive in the Mediterranean basin. *Entomol. Exp. Appl.* 102: 65–73.
- Canale, A., and A. Loni. 2006. Host location and acceptance in *Psytalia concolor*: role of host instar. *Bull. Insectol.* 59: 7–10.
- Daane, K. M., M. W. Johnson, K. R. Sime, A. Kirk, R. Wharton, H. Nadel, R. Messing, C. H. Pickett, and F. Zalom. 2006. Host specificity studies on parasitoids of olive fruit fly, pp. 3–5. In D. M. Woods (ed.), *Biological control program annual summary, 2005*. Calif. Depart. Food Agr., Plant Health Pest Prev. Serv., Sacramento, CA.
- GraphPad Software. 2007. GraphPad prism, version 5.00. GraphPad Software, San Diego, CA.
- Johnson, M. W., F. G. Zalom, R. Van Steenwyk, P. Vossen, A. K. Devarenne, K. M. Daane, B. Krueger, J. H. Connell, V. Yokoyama, B. Bisabri, and J. Nelson. 2006. Olive fruit fly management guidelines for 2006. *Univ. Calif. Coop. Ext., UC Plant Protect. Q.* 16: 1–9.

June 2008

YOKOYAMA ET AL.: BIOLOGICAL CONTROL OF OLIVE FRUIT FLY

773

- Kapatos, E., B. S. Fletcher, S. Papas, and Y. Laudeho. 1977. The release of *Opius concolor* and *O. concolor* var. *siculus* [Hym.: Braconidae] against the spring generation of *Dacus oleae* [Dipt.: Trypetidae] on Corfu. *Entomophaga* 22: 265–270.
- Kimani-Njogu, S. W., M. K. Trostle, R. A. Wharton, J. B. Woolley, and A. Raspi. 2001. Biosystematics of the *Psytalia concolor* species complex (Hymenoptera: Braconidae: Opiinae): the identity of populations attacking *Ceratitis capitata* (Diptera: Tephritidae) in coffee in Kenya. *Biol. Control* 20: 167–174.
- Michelakis, S. E. 1990. The olive fly (*Dacus oleae* Gmel.) in Crete, Greece. *Acta Horticult.* 286: 371–374.
- Nadel, H., K. Daane, J. Andrews, and C. Pickett. 2005. Host specificity studies on parasitoids of olive fruit fly, pp. 3–4. In D. M. Woods (ed.), *Biological control program annual summary, 2004*. Calif. Depart. Food Agr., Plant Health Pest Prev. Serv., Sacramento, CA.
- Neuenschwander, P., F. Bigler, V. Delucchi, and S. Michelakis. 1983. Natural enemies of preimaginal stages of *Dacus oleae* Gmel. (Diptera, Tephritidae) in western Crete. I. Bionomics and phenologies. *Boll. Lab. Entomol. Agrar. Portici.* 40: 3–32.
- Rice, R. E. 2000. Bionomics of the olive fruit fly *Bactrocera (Dacus) oleae*. *Univ Calif. Coop. Ext., UC Plant Protect. Q.* 10: 1–5.
- Sime, K. R., K. M. Daane, R. H. Messing, and M. W. Johnson. 2006. Comparison of two laboratory cultures of *Psytalia concolor* (Hymenoptera: Braconidae), as a parasitoid of the olive fruit fly. *Biol. Control* 39: 248–255.
- Wharton, R. A., M. K. Trostle, R. H. Messing, R. S. Copeland, S. W. Kimani-Njogu, S. Lux, W. A. Overholt, S. Mahamed, and J. Sivinski. 2000. Parasitoids of medfly, *Ceratitis capitata*, and related tephritids in Kenyan coffee: a predominantly koinobiont assemblage. *Bull. Entomol. Res.* 90: 517–526.
- Wharton, R. A., K. Dole, R. Stouthammer, and C. H. Pickett. 2006. Developments in the taxonomy of natural enemies of olive fruit fly, *Bactrocera oleae* (Gmelin), pp. 6–9. In D. M. Woods (ed.), *Biological control program annual summary, 2005*. Calif. Depart. Food Agr., Plant Health Pest Prev. Serv., Sacramento, CA.
- Yokoyama, V. Y., and G. T. Miller. 2004. Quarantine strategies for olive fruit fly (Diptera: Tephritidae): low temperature storage, brine, and host relations. *J. Econ. Entomol.* 97: 1249–1253.
- Yokoyama, V. Y., and G. T. Miller. 2007. Olive fruit fly biology and cultural control practices in California. *IOBC/WPRS Bull.* 30: 277–285.
- Yokoyama, V. Y., G. T. Miller, and P. L. Hartsell. 1992. Pest-free period and methyl bromide fumigation for control of walnut husk fly (Diptera: Tephritidae) in stone fruits exported to New Zealand. *J. Econ. Entomol.* 85: 150–156.
- Yokoyama, V. Y., G. T. Miller, and J. Sivinski. 2004. Quarantine control strategies for olive fruit fly in California. *Proceedings of the 6th International Symposium on Fruit Flies Economical Implications, 6–10 May 2002, Stellenbosch, South Africa.*
- Yokoyama, V. Y., G. T. Miller, J. Stewart-Leslie, R. E. Rice, and P. A. Phillips. 2006. Olive fruit fly (Diptera: Tephritidae) populations in relation to region, trap type, season, and availability of fruit. *J. Econ. Entomol.* 99: 2072–2079.
- Yokoyama, V. Y., P. Rendon, and J. Sivinski. 2008. Biological control of olive fruit fly (Diptera: Tephritidae) by releases of *Psytalia* cf. *concolor* (Hymenoptera: Braconidae) in California, parasitoid longevity in presence of the host, and host status of walnut husk fly. *Proceedings of the 7th International Symposium on Fruit Flies Economical Impact. 10–15 September 2006, Salvador, Bahia, Brazil.*

Received 11 April 2007; accepted 7 March 2008.