Commodity Protection and Quality

Stakeholders Conference

2008

Research Summary

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Table of Contents

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

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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 thought 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 culled 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 (5^{th} 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% (LT_{95}) 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% O_2) with one of three protective treatments (10°C storage, 5% O_2, 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.

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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 be 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 l980's and we have obtained nearly 100% upwind flight to a recently isolated volatile complex that will be analyzed for it 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

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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 lead 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.

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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

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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

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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 coddling 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 SF_2O_2, FSO_3^-, 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 gol 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

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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 in 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, Moscamed 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^{o}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

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