

Generic Vapor Heat Treatments to Control *Maconellicoccus hirsutus* (Homoptera: Pseudococcidae)

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ABSTRACT Vapor heat treatments were developed against life stages of the mealybug *Maconellicoccus hirsutus* (Green) (Homoptera: Pseudococcidae). Treatments tested were 47°C for 5–50 min in 5-min increments and 49°C for 3, 5, 8, 10, and 12 min. All tests were conducted with mixed age *M. hirsutus* on Chinese pea, *Pisum sativum* L. Treatment at 47°C required 45 min to kill all *M. hirsutus*, whereas treatment at 49°C required 10 min. The adult female and nymphal stages were the most heat tolerant at 47°C, but the egg stage was the most heat tolerant at 49°C. Use of the vapor heat treatments on other commodities will require achieving or exceeding the proper temperature and duration at all locations on the host where *M. hirsutus* may reside.

KEY WORDS quarantine pest, phytosanitary treatment, quarantine heat treatment

THE MEALYBUG *Maconellicoccus hirsutus* (Green) (Homoptera: Pseudococcidae) is a recent introduction to the continental United States that poses a serious threat to agriculture, forestry, and the nursery industry (Zettler et al. 2002). *M. hirsutus* was discovered in Calexico and El Centro, Imperial County, California, in 1999 (CDFA 1999). Its appearance caused Mexico and several Central American countries to impose embargoes or quarantine restrictions on host commodities originating from the infested areas of Imperial County (Zettler et al. 2002). Current *M. hirsutus* populations in Imperial County are restricted to urban areas only, and it has not been found on agricultural crops. Nevertheless, phytosanitary measures are needed to cope with quarantine restrictions. *M. hirsutus* was discovered in Hawaii in 1983 (Beardsley 1985) and is currently of interest in Hawaii because it is a federal quarantine pest of several tropical exotic fruits for export, including atemoya, durian, longan, rambutan, and sapodilla (Follett 1999). *M. hirsutus* is also presently found in Mexico, Central America, and throughout the Caribbean and is expected to invade the southern United States. The economic impact of this polyphagous pest on U.S. agriculture should it become widely established is estimated at \$750 million in the absence of control measures (Sagarra and Peterkin 1999).

A number of postharvest treatment methods could be adapted to treat commodities attacked by *M. hirsutus*, including methyl bromide fumigation, irradiation, vapor heat, and hot water immersion. A methyl bromide treatment developed for mealybugs on various commodities (USDA 1998) was shown to be effective against *M. hirsutus* (Zettler et al. 2002). Methyl bromide is an ozone-depleting substance scheduled for phase out under the Montreal Protocol (UNEP

1992). Quarantine and preshipment uses of methyl bromide were exempted from the phase out to avoid nontariff trade barriers because alternative treatments or technologies were not widespread and available. However, this exemption could be rescinded, and the cost of methyl bromide has increased significantly in recent years with the drop in demand. Alternative quarantine treatments are needed. Irradiation treatment with a dose of 250 Gy may be effective against *M. hirsutus* (Jacobsen and Hara 2003), but large-scale testing is needed to confirm the efficacy of this dose. Heat treatment is a potential alternative to control *M. hirsutus* on many agricultural commodities. The objective of this study was to develop generic vapor heat treatments against life stages of *M. hirsutus*.

Materials and Methods

A colony of *M. hirsutus* was started with nymphs and adults collected from hibiscus plants in Hilo, HI, in 2000. The colony was maintained in the laboratory first on Japanese pumpkin, *Curcubita moschata* (Duchesne) variety *chirimen*, by using the method described by Meyerdirk et al. (1998), and later on common bean, *Phaseolus vulgaris* L., in ventilated 3.8-liter plastic tubs (Rubbermaid, Wooster, OH) containing moistened paper towels. Fresh beans were provided every 2 d. Rearing conditions were 25°C, 85% RH, and a photoperiod of 0:24 (L:D) h (darkness inhibits crawlers from leaving host material). Five life stages were treated: eggs, crawlers, nymphs, male pupae, and adult females (with and without egg masses).

Tests were conducted at the USDA-ARS laboratory in Hilo by using a computer-controlled vapor heat treatment chamber specifically designed for research on postharvest treatments of fresh tropical commod-

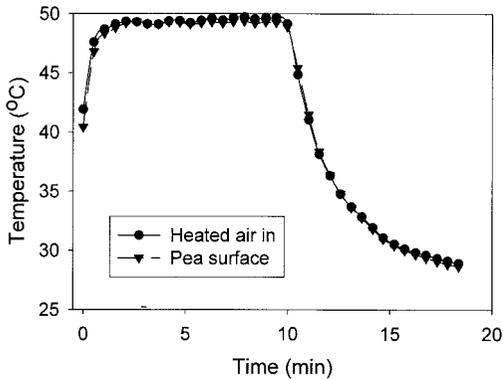


Fig. 1. Temperature profile for Chinese pea subjected to vapor heat treatment (49°C, 10 min) and subsequent passive cooling.

ities (Gaffney and Armstrong 1990). Vapor heat treatments tested were 47°C for 5–50 min in 5-min increments, and 49°C for 3, 5, 8, 10, and 12 min. Thermocouples were used to monitor the temperature of air entering and exiting the vapor heat chamber, and the temperature at the surface of four fruiting structures infested with mealybugs during each test.

All tests were conducted with mixed age *M. hirsutus* on Chinese pea, *Pisum sativum* L. Treatment of mealybugs on Chinese pea, which is a relatively two-dimensional (thin and flat) host, rather than a larger fruit or vegetable host, facilitated counting of various mealybug life stages and measurement of heat experienced by the mealybugs (i.e., at any time the surface temperature of the pea is nearly equal to the air temperature in the chamber). To minimize warm up time during treatment, the chamber was preheated to the target temperature, and infested peas from the colony were introduced through a small door cut into the main door of the chamber. The peas were suspended freely by paper clips from a ring stand in the center of the chamber during treatment. A typical vapor heat treatment temperature profile is shown in Fig. 1. Relative humidity in the chamber was 95–100% during all treatments. The main door to the chamber was opened at the end of each treatment to allow passive cooling of the peas with mealybugs.

After vapor heat treatment and cooling to ambient temperature, peas with *M. hirsutus* life stages were transferred to petri dishes rimmed with Teflon and sealed with parafilm until scoring for survivorship. Life stages of *M. hirsutus* are described in Meyerdirk et al. (1998). Crawlers and nymphs were distinguished by size and mobility, and adult females were identified by their size or the presence of a cottony egg mass or ovisac attached to the dorsum. Male puparia are distinctive. Crawlers, nymphs, and adults were scored for mortality after 24 h by touching the thorax and abdomen with a camel-hair brush to elicit any leg movement. Eggs were separated from egg masses, transferred onto double-sided sticky tape (Scotch brand, 3M) attached to the bottom of a petri dish, and held for emergence (typically <7 d). Crawlers emerging

from eggs immediately stick to the tape and are easily counted. Puparia were held for 1 wk for emergence in covered petri dishes.

To make comparisons of heat tolerance between life stages, dose–response data were subjected to logistic regression and analysis of covariance (ANCOVA) by using the standard least squares model (SAS Institute 2002). The logistic transformation was calculated by $\log_e [(\% \text{ mortality}/100) / (1 - (\% \text{ mortality}/100))]$ and was used to help normalize the mortality data and linearize the response function. Data used in the logistic regression model included any heating duration causing mortality between 0 and 100%, and the lowest duration causing 100% mortality. For each replicate, mortality values <100% were adjusted for control mortality by using Abbott's formula (Abbott 1925). Residual plots were evaluated to ensure regression model assumptions were met for each treatment combination. Covariance analysis requires the slopes of the regression lines fitted to each group to be parallel, so the assumption of parallelism (nonsignificant stage \times treatment duration interaction effect) was tested before evaluating intercepts (stage effects) (Sokal and Rohlf 1981).

Results

Survivorship decreased with increasing dose for all life stages at both temperatures (Tables 1 and 2). The lowest treatment duration showing no survival varied by stage. At 47°C, no male pupae survived ≥ 25 min, and no eggs or crawlers survived ≥ 35 min (Table 1). A few nymphs and adult females survived in the 40-min treatment but none survived in the 45- and 50-min treatments. At 49°C, no individuals of any life stage survived in the 10- or 12-min treatments (Table 2).

ANCOVA was used to determine the most tolerant life stage. Life stage by treatment duration interaction effects were significantly different only for the comparison between adult females and nymphs at 49°C ($F = 10.4$, $df = 1$, $P = 0.003$), indicating slopes of the lines differed and therefore the effect of stage could not be evaluated. Life stage \times treatment time interaction effects were not significantly different for any other pair of life stages at either temperature, indicating that slopes of the regression lines did not violate the assumption of parallelism. At 47°C, adult females were significantly more tolerant of heat than eggs ($F = 7.2$, $df = 1$, $P = 0.009$) and crawlers ($F = 10.4$, $df = 1$, $P = 0.002$) but not significantly different than nymphs ($F = 0.2$, $df = 1$, $P = 0.65$). Nymphs also were significantly more tolerant of heat than eggs ($F = 7.0$, $df = 1$, $P = 0.0002$) and crawlers ($F = 13.7$, $df = 1$, $P = 0.0004$). Crawlers and eggs did not differ in their tolerance of heat ($F = 0.0001$, $df = 1$, $P = 0.99$). At 49°C, eggs were significantly more tolerant of heat than adult females ($F = 9.7$, $df = 1$, $P = 0.005$), nymphs ($F = 4.2$, $df = 1$, $P = 0.05$), and crawlers ($F = 9.9$, $df = 1$, $P = 0.004$). Nymphs were significantly more tolerant of heat than crawlers ($F = 7.1$, $df = 1$, $P = 0.01$). Adults and crawlers were not significantly different ($F = 0.9$, $df = 1$, $P = 0.35$). The predicted doses to achieve 100%

Table 1. Effect of vapor heat treatment at 47°C for various times on *M. hirsutus* mortality

Time (min)	Stage	Replicates	Treated		Untreated	
			Total	% survivors (\pm SE)	Total	% survivors (\pm SE)
5	Eggs	2	363	52.4 (\pm 19.1)	141	72.3 (\pm 0.0)
	Crawlers	2	360	73.7 (\pm 16.6)	127	92.8 (\pm 0.2)
	Nymphs	2	486	90.6 (\pm 3.8)	98	90.8 (\pm 2.3)
	Pupae ♂	0	0	-	0	-
	Adult ♀	2	37	100.0	11	100.0
10	with eggs	2	7	41.7 (\pm 41.7)	0	-
	Eggs	3	217	22.7 (\pm 16.8)	199	76.8 (\pm 8.2)
	Crawlers	5	356	48.1 (\pm 6.5)	86	81.6 (\pm 6.5)
	Nymphs	5	1210	63.0 (\pm 7.6)	458	91.2 (\pm 4.2)
	Pupae ♂	1	2	100.0 (\pm 0.0)	0	-
15	Adult ♀	5	302	73.7 (\pm 7.7)	80	97.0 (\pm 2.2)
	with eggs	3	5	100.0	4	100.0
	Egg	5	875	6.2 (\pm 2.7)	110	71.9 (\pm 11.9)
	Crawlers	6	345	6.9 (\pm 4.5)	105	76.7 (\pm 16.0)
	Nymphs	6	1643	13.1 (\pm 4.1)	283	94.9 (\pm 1.4)
20	Pupae ♂	5	28	14.3 (\pm 9.4)	2	100.0 (\pm 0.0)
	Adult ♀	6	364	24.5 (\pm 6.9)	85	99.2 (\pm 0.8)
	with eggs	5	14	3.3 (\pm 3.3)	3	66.7 (\pm 33.3)
	Eggs	3	836	3.4 (\pm 2.5)	169	47.5 (\pm 17.7)
	Nymphs	5	1210	4.4 (\pm 3.3)	809	95.8 (\pm 1.7)
25	Crawlers	5	530	4.1 (\pm 3.1)	364	87.2 (\pm 4.6)
	Pupae ♂	1	1	0.0	0	-
	Adult ♀	5	332	5.6 (\pm 3.1)	73	97.5 (\pm 1.5)
	with eggs	2	7	16.7 (\pm 16.7)	2	100.0 (\pm 0.0)
	Eggs	3	543	0.0	172	87.0 (\pm 8.4)
30	Crawlers	5	584	0.0	365	85.5 (\pm 7.2)
	Nymphs	5	2529	0.9 (\pm 0.4)	587	94.8 (\pm 1.6)
	Pupae ♂	1	13	0.0	1	100.0
	Adult ♀	5	162	21.5 (\pm 19.7)	80	98.2 (\pm 1.8)
	with eggs	2	5	0.0	2	50.0 (\pm 50.0)
35	Eggs	5	4059	0.02 (\pm 0.02)	811	87.4 (\pm 2.1)
	Crawlers	5	1266	1.9 (\pm 1.9)	217	88.7 (\pm 7.7)
	Nymphs	5	3624	0.9 (\pm 0.9)	573	98.8 (\pm 0.6)
	Pupae ♂	5	23	0.0	3	100.0
	Adult ♀	5	175	4.0 (\pm 4.0)	26	75.0 (\pm 15.8)
40	with eggs	5	39	0.0	10	60.0 (\pm 18.7)
	Eggs	5	3732	0.0 (\pm 0.0)	952	76.6 (\pm 7.2)
	Crawlers	5	2167	0.0 (\pm 0.0)	446	93.0 (\pm 2.0)
	Nymphs	5	4639	0.2 (\pm 0.2)	529	98.3 (\pm 0.5)
	Pupae ♂	5	30	0.0	1	100.0
45	Adult ♀	5	245	0.0	102	98.6 (\pm 1.4)
	with eggs	5	42	0.0	9	93.3 (\pm 6.7)
	Eggs	4	1711	0.0	516	89.4 (\pm 2.4)
	Crawlers	5	2026	0.0	963	81.8 (\pm 10.0)
	Nymphs	5	4227	0.2 (\pm 0.2)	748	98.6 (\pm 0.7)
50	Pupae ♂	2	2	0.0	2	100.0
	Adult ♀	5	259	0.5 (\pm 0.5)	44	100.0
	with eggs	4	21	0.0	6	75.0 (\pm 14.4)
	Eggs	4	2475	0.0	859	60.9 (\pm 18.0)
	Crawlers	7	1032	0.0	388	68.4 (\pm 12.5)
55	Nymphs	7	2789	0.0	883	97.3 (\pm 0.8)
	Pupae ♂	4	31	0.0	5	75.0 (\pm 25.0)
	Adult ♀	7	307	0.0	112	99.0 (\pm 1.0)
	with eggs	5	55	0.0	12	100.0
	Eggs	3	4389	0.0	836	86.8 (\pm 5.9)
60	Crawlers	4	1915	0.0	404	91.5 (\pm 4.2)
	Nymphs	4	2342	0.0	583	95.7 (\pm 2.9)
	Pupae ♂	2	10	0.0	16	33.3 (\pm 33.3)
	Adult ♀	4	95	0.0	26	96.7 (\pm 3.3)
	with eggs	3	48	0.0	23	63.2 (\pm 18.4)

mortality at 47°C were 33.0, 31.3, 41.4, and 40.8 min for eggs, crawlers, nymphs and adult females, respectively (Table 3). The predicted doses to achieve 100% mortality at 49°C were 10.7, 8.0, 9.7, and 8.1 min for eggs, crawlers, nymphs, and adult females, respectively (Table 3). Adults and nymphs were the most heat-tolerant stages at 47°C, whereas the egg was the most heat-tolerant stage at 49°C. Male pupae and adult females

with eggs were not included in the analyses because of low numbers in all treatments.

Discussion

In our study, treatment at 47°C required 45 min to kill all *M. hirsutus*, whereas treatment at 49°C required 10 min (Tables 1 and 2). Surface heating data for

Table 2. Effect of vapor heat treatment at 49°C for various times on *M. hirsutus* mortality

Time (min)	Stage	Replicates	Treated		Untreated	
			Total	% survivors (\pm SE)	Total	% survivors (\pm SE)
3	Eggs	3	367	41.6 (\pm 11.9)	122	42.5 (\pm 7.0)
	Crawlers	4	849	57.0 (\pm 18.3)	144	72.8 (\pm 16.5)
	Nymphs	4	1091	69.4 (\pm 15.9)	196	94.6 (\pm 1.6)
	Pupae ♂	1	1	0.0	1	100.0
	Adult ♀	3	27	67.3 (\pm 19.7)	9	100.0
5	with eggs	2	3	75.0 (\pm 25.0)	5	100.0
	Eggs	6	1607	24.2 (\pm 10.8)	476	66.0 (\pm 3.8)
	Crawlers	6	543	11.4 (\pm 6.7)	124	95.2 (\pm 2.9)
	Nymphs	6	1897	8.5 (\pm 2.8)	813	82.0 (\pm 16.4)
	Pupae ♂	3	9	16.7 (\pm 16.7)	0	-
8	Adult ♀	5	275	16.2 (\pm 5.7)	42	77.1 (\pm 19.5)
	with eggs	5	15	23.3 (\pm 14.5)	3	25.0 (\pm 25.0)
	Eggs	4	671	2.4 (\pm 2.2)	228	86.6 (\pm 3.7)
	Crawlers	5	562	0.0	99	61.5 (\pm 18.3)
	Nymphs	5	1713	0.1 (\pm 0.1)	338	94.7 (\pm 1.9)
10	Pupae ♂	1	2	0.0	0	-
	Adult ♀	5	159	0.0	34	96.7 (\pm 3.3)
	with eggs	4	4	0.0	1	100.0
	Eggs	8	4385	0.0	1404	79.6 (\pm 4.7)
	Crawlers	10	1940	0.0	539	83.8 (\pm 6.5)
12	Nymphs	10	2298	0.0	1034	97.4 (\pm 1.0)
	Pupae ♂	6	36	0.0	12	72.2 (\pm 24.2)
	Adult ♀	10	299	0.0	88	97.1 (\pm 2.5)
	with eggs	7	55	0.0	16	100.0
	Eggs	5	2901	0.0	988	77.3 (\pm 10.4)
49	Crawlers	5	394	0.0	516	93.7 (\pm 3.4)
	Nymphs	5	2571	0.0	632	98.7 (\pm 0.9)
	Pupae ♂	2	4	0.0	2	100.0
	Adult ♀	5	370	0.0	44	97.7 (\pm 2.3)
	with eggs	5	17	0.0	9	73.3 (\pm 19.4)

specific commodities are needed before recommending specific time-temperature treatments. Disinfesting *M. hirsutus* on commodities with a smooth surface will probably require treatment times similar to those reported for Chinese pea. Infested commodities with rough, invaginated, or hirsute skin or other protective anatomical features (e.g., the calyx) may require longer treatment times to apply heat for a sufficient duration to all locations. However, if 47°C can be applied for 45 min, or 49°C for 10 min, to all parts of the commodity infested with *M. hirsutus*, quarantine security can be achieved. Large-scale confirmatory tests should be performed to validate efficacy on specific commodities. Lower temperature treatments may be required to maintain commodity quality for

heat-sensitive commodities, which would require new time-temperature efficacy data. For example, during preliminary tests in this study control of *M. hirsutus* with vapor heat at 45°C required >2.5 h (P.A.F., unpublished data).

M. hirsutus is a federal quarantine pest of atemoya, durian, longan, rambutan, and sapodilla in Hawaii. Hawaii has an approved vapor heat quarantine treatment to control tephritid fruit flies (internal pests) in rambutan before export that involves heating the fruit to a seed surface (fruit center) temperature of 47.2°C in 1 h and then holding the seed surface temperature at 47.2°C for 20 min (Follett and Sanxter 2000). This protocol should provide complete control of *M. hirsutus* in addition to fruit flies. Vapor heat treatments

Table 3. Logistic regression of percentage mortality against heating duration (minutes) for life stages of *M. hirsutus*

Temp (°C)	Stage ^a	N ^b	Intercept \pm SE	Slope \pm SE	R ²	Predicted time (95% CL) for 100% mortality (min) ^c
47	Egg	10,625	-0.9 \pm 2.1	0.38 \pm 0.08	0.55	33.0 (28.2-42.3)
	Crawler	5,608	-2.6 \pm 1.8	0.45 \pm 0.07	0.56	31.3 (27.6-37.4)
	Nymph	22,357	-3.1 \pm 1.0	0.35 \pm 0.03	0.72	41.4 (38.2-45.6)
	Adult ♀	2,183	-4.7 \pm 1.8	0.39 \pm 0.06	0.54	40.8 (36.4-47.6)
49	Egg	1,954	-11.4 \pm 2.5	2.3 \pm 0.31	0.76	10.1 (9.1-11.6)
	Crawler	7,030	-8.9 \pm 2.3	2.6 \pm 0.40	0.79	8.0 (7.1-9.6)
	Nymph	461	-6.9 \pm 1.1	1.9 \pm 0.14	0.89	9.7 (9.2-10.3)
	Adult ♀	8,020	-11.2 \pm 0.9	2.8 \pm 0.15	0.98	8.1 (7.8-8.4)

^a Male pupae and adult females with eggs were excluded from analysis due to low numbers of test insects.

^b Total no. of treated insects included in the analysis.

^c Inverse prediction times from logistic regression for 100% mortality are shown for comparative purposes only and are not intended to suggest treatments to control *M. hirsutus*.

have not been developed for the other fruits. A hot water immersion treatment at 49°C for 20 min to control fruit flies is approved for longan (Follett and Sanxter 2002), but its effect on *M. hirsutus* is untested. *M. hirsutus* and other mealybugs also infest tropical cut flowers and foliage and must be removed or controlled before plants are exported from Hawaii. Vapor heat treatment for 1 h at 46.6°C or 2 h at 45.2°C was shown to control a complex of mealybugs on several Hawaiian cut flowers (Hansen et al. 1992). Hot water immersion at 49°C for 12–15 min did not kill all obscure mealybugs, *Pseudococcus affinis* (Maskell), and long-tailed mealybugs, *Pseudococcus longispinus* (Targioni-Tozzetti), on red ginger flowers, *Alpinia purpurata* (Vieill.), probably because the mealybugs were insulated deep inside leaf sheaths and flower bracts (Hara et al. 1996, 1997).

The egg stage was the most heat-sensitive stage at 47°C and the most heat-tolerant stage at 49°C. This may be explained by the presence of the ovisac. The fibrous waxy ovisac may effectively insulate the egg cluster during the early stages of heating, and eggs on the inside of the cluster within the ovisac may be doubly protected from direct exposure to heated air. If this is true, the egg stage may seem more heat tolerant during short duration heat treatments, such as those tested at 49°C (3–8 min), whereas the other exposed life stages suffer high mortality.

The term “generic” as it applies to quarantine treatments has several definitions. It can mean a treatment that provides quarantine security for a broad group of pests at dose levels that do not affect the quality of commodities (Follett and Armstrong 2004), a treatment for all pests infesting a single commodity, or a treatment for a single pest on many different commodities, such as we propose for *M. hirsutus*. Traditionally, quarantine treatments were developed for a single pest on a single commodity, and involved finding a balance between killing the pest and minimizing the adverse effects of the treatment process on commodity quality (Paull and Armstrong 1994). Most quarantine treatments have been developed against internal feeding pests because commodities infested by the pests are difficult to identify by visual inspection. Surface feeders such as *M. hirsutus* are usually visible with the naked eye or with the aid of a hand lens. Therefore, they do not always require a penetrating quarantine treatment per se, and physical treatments such as high pressure water or brushes may be sufficient to dislodge the pest from its host (Walker et al. 1999). However, a postharvest quarantine treatment may be required for a surface pest if infestations can go undetected, such as when individuals reside underneath the calyx (e.g., limes, Gould and McGuire 2000) or other plant parts (e.g., flowers, Hara et al. 1996, 1997), in deep fissures (e.g., Japanese pumpkin, Meyerdirk et al. 1998), or inside clusters of fruit (e.g., grape or banana bunches, Armstrong 2001, Zettler et al. 2002), which is the case with *M. hirsutus*.

M. hirsutus has a wide host range, including forest trees, fruit trees, ornamentals, root crops, and vegetables (Mani 1989). *M. hirsutus* hosts with export po-

tential may have different tolerances to high temperatures and different heating properties. Therefore, rather than conduct heat sensitivity tests on *M. hirsutus* on many hosts, we conducted tests using a model host (Chinese pea) with simple heating properties. *M. hirsutus* readily settles and develops on Chinese pea. When Chinese pea is placed inside a vapor heat chamber, the surface of the pea rapidly attains the same temperature as the surrounding air, making it a convenient substrate for conducting heat treatment tests.

M. hirsutus is a minor pest in Hawaii and seems to be under fortuitous biological control. A classical biological control program against *M. hirsutus* in the Caribbean and Central America has been highly successful (Meyerdirk 2001). Release of biological control agents in California has significantly reduced *M. hirsutus* populations by 96% in infested areas in Imperial County and may be partly responsible for the absence of *M. hirsutus* from rigorous inspections of agricultural crops in the area (Zettler et al. 2002). Nevertheless, *M. hirsutus* populations are established and persist in Hawaii, the Caribbean, Mexico, and Central America and may migrate to the southern U.S. Quarantine treatment options including heat, irradiation, or fumigants are needed to prevent disruption of agricultural trade. Effective biological control will reduce *M. hirsutus* numbers on export crops and improve any quarantine system that includes postharvest treatments.

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