Efficacy of *Beauveria bassiana* Against *Lygus hesperus* (Hemiptera: Miridae) at Low Temperatures[1]

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Abstract The western tarnished plant bug, *Lygus hesperus* Knight, is susceptible to the naturally-occurring pathogen, *Beauveria bassiana* (Balsamo) Vuillemin, in the San Joaquin Valley of California. Recent research efforts have focused on selection of *Beauveria* strains that were effective against *Lygus* under the high-temperature conditions typical of the cotton (*Gossypium* spp.) production season. However, the most appropriate use of this pathogen may not be as a rescue treatment. Alternatively, *B. bassiana* may be useful to efforts to target overwintering populations of *Lygus* if isolates are available that are highly virulent under the low temperature conditions typical of winter and early-spring in the San Joaquin Valley. One commercially-available isolate and 4 native isolates of *B. bassiana* were assayed against *L. hesperus* adults under constant temperatures of 12.8, 18.3, and 23.9°C. Although decreasing temperatures were associated with diminished *Beauveria*-induced mortality of *Lygus* and slower development of disease symptoms, no differences in efficacy were detected among the tested isolates. Differences in the patterns of occurrence of *Beauveria* disease symptoms were observed among isolates at some temperatures, but those differences were not substantial. Furthermore, results at some temperatures suggested potential influences of *Lygus* adult age or gender on susceptibility to *B. bassiana*. Those effects should be further investigated. Overall, the results did not indicate that any of the tested isolates of *B. bassiana* were superior to the commercially-available isolate under low temperature conditions.

Key Words western tarnished plant bug, *Lygus hesperus*, *Beauveria bassiana*, entomopathogen

The western tarnished plant bug, *Lygus hesperus* Knight, is a key pest of cotton (*Gossypium hirsutum* L., *G. barbadense* L.) in western production regions of the U.S. Despite the availability of cultural methods of reducing the impacts of *Lygus* on cotton (Stern et al. 1967), most efforts to manage *Lygus* in California cotton involve conventional pesticides applied according to nominal thresholds. Development of ecologically benign management tactics for *Lygus* represents a major focus of the mission of the Western Integrated Cropping Systems Research Unit (WICSRU). *Beauveria bassiana* (Balsamo) Vuillemin is a fungal pathogen endemic to the San Joaquin Valley of California that infects *L. hesperus* (McGuire 2002). Previous research efforts of the WICSRU focused on selecting isolates of *B. bassiana* that were effective under the high temperature environment typical of the cotton production season (Leland et al. 2005a, McGuire et al. 2005). However, field tests of these isolates indicated less impact on population levels of *Lygus* spp. than was anticipated given the high levels of observed disease symptoms (Leland et al. 2005b, McGuire et al. 2006).

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Beauveria bassiana has several disadvantages as a *Lygus* control tactic in cotton. First, the pathogen is sensitive to high temperatures and solar radiation (Leland et al. 2005a). In addition, mortality produced by *B. bassiana* occurs relatively slowly, and feeding behavior of infected *Lygus* may be modified to increase the extent of plant injury (Noma and Strickler 2000). Therefore, use of *B. bassiana* as a rescue treatment against *Lygus* in cotton may not represent the most appropriate role for this pathogen.

McGuire et al. (2006) suggested that a possible approach to managing *Lygus* in cotton was to target overwintering populations before they grew to a large size. This approach would negate the disadvantages of slow production of *Lygus* mortality, and persistence of *B. bassiana* conidia may be enhanced by the heavy fogs typical of the winter and early-spring in the San Joaquin Valley. However, additional information is required to assess the potential for success of this tactic. First, the overwintering ecology of *L. hesperus* in the San Joaquin Valley must be better understood so treatments of *B. bassiana* can be directed to the appropriate habitats at the optimal times. Secondly, it would be advantageous if *B. bassiana* attacked other insects, such as pests of alfalfa (*Medicago sativa* L.) including larvae of the alfalfa weevil, *Hypera postica* (Gyllenhal), and Egyptian alfalfa weevil, *H. brunnipennis* (Boheman). Such impacts on non-*Lygus* pests could provide incentive for use of *B. bassiana* by producers who do not grow cotton. This scenario seems possible based on reports of efficacy of *B. bassiana* against the clover root weevil, *Sitona lepidus* Gyllenhal (Willoughby et al. 1998). Finally, results of preliminary studies (Spurgeon and Hudson 2008) suggest variations among *B. bassiana* isolates in their efficacy against *Lygus* at low temperatures should be examined to determine the likelihood of major differences in effectiveness. The objective of the studies reported herein was to examine the relative effectiveness of selected *B. bassiana* isolates against *L. hesperus* adults under temperature conditions typical of daytime highs during the winter and early-spring in the San Joaquin Valley of California.

Materials and Methods

The climatic conditions under which an entomopathogen will be used are thought to be an important consideration in selecting an isolate (Fargues et al. 1997). Therefore, 3 isolates of *B. bassiana* previously collected from infected *L. hesperus* during the winter in the San Joaquin Valley (designated 1 - 25, 3 - 3, and 4 - 11) were selected for study. Additional treatments included a previously studied isolate collected during the summer in the San Joaquin Valley (s44, referred to as WTPB2 by Leland et al. 2005a, b), a commercially available isolate (strain GHA, Laverlam International, Butte, MT), and a control without *Beauveria*. *Beauveria bassiana* conidia were either obtained from previously prepared stocks, dried or stored in 15% glycerol at –80°C, or were freshly harvested from colonies grown on Sabouraud dextrose agar (Becton-Dickson, Cockeyesville, MD) supplemented with 0.2% (w/v) yeast extract (Sigma Chemical, St. Louis, MO). Before each repetition of the assays, the viability of conidia was assessed by culturing an aliquot of each isolate overnight in potato dextrose broth (Sigma) and examining the conidia for germination at 400×. Conidia were considered viable if the germ tube was longer than the diameter of the conidia. Each preparation used in the study exhibited ≥80% germination. Preparations of conidia were diluted to a final concentration of 1×10^6 viable conidia per ml in 0.01% Silwet–L77 (GE Silicones, Friendly, WV). This concentration was intended to provide a concentration of ~6.8×10^3 conidia per square cm of sprayed surface. This concentration was slightly higher than the LC_{50} previously reported for GHA after 7 days at 28°C (Leland et al. 2005a).

The insects used were obtained from a long-standing laboratory colony maintained on pods of green bean (*Phaseolus vulgaris* L.) and raw sunflower seeds (*Helianthus annuus* L.) at the WICSRU. Field-collected *L. hesperus* were periodically introduced into the colony to maintain vigor. Two days before each assay, all adults were aspirated from the rearing containers. The following day, newly-emerged adults were collected for the assays. Collected adults were held overnight with green bean pods for food in an environmental chamber maintained at 23.9°C. This holding period was intended to allow hardening of the cuticle. Therefore, *L. hesperus* adults were 1 - 2 days old at the time the assays were initiated.

Temperatures examined were 12.8, 18.3, and 23.9 ± 1°C, which were maintained within environmental chambers with a photoperiod of 14:10 (L:D) h. Each assay included 20 adult *L. hesperus* of mixed sexes for each isolate and temperature combination. The assays were repeated 4 times.

Experimental procedure. Immediately before each assay adult *L. hesperus* were aspirated into 15-dram vials (Thornton Plastics, Salt Lake City, UT) each containing a foam plug in the bottom. A separate vial contained the insects for each respective combination of isolate and temperature. It was not practical to randomly assign individual *L. hesperus* to the experimental treatments. Therefore, thorough mixing of the bugs among treatments was accomplished by aspirating 10 insects into each vial, followed by an additional 10 insects per vial, followed by 5 additional insects per vial for a total of 25 adult *L. hesperus* per vial. Vials of *L. hesperus* were then randomly assigned to combinations of *B. bassiana* isolate and temperature.

Immediately before each treatment was applied, bugs within the designated vial were lightly anesthetized with CO_2 (12 - 15 sec at 1.5 L/min of CO_2). Anesthetized bugs were decanted into a 100 × 15-mm Petri plate lined with filter paper. The Petri plate was gently rocked to distribute the bugs over the bottom of the plate, and then it was placed in the center of the rotating floor of a spray chamber described by McGuire et al. (2005). A 5-ml aliquot of the designated isolate, or the control treatment of 0.01% Silwet–L77, was delivered at 138 kPa through a TG 0.4 full cone nozzle (Spraying Systems, Wheaton, IL). Immediately following spray application, 20 of the treated bugs were distributed individually into 5-dram plastic vials. Each vial contained a short (~2 - 3 cm) section of green bean pod. Vials were then closed with a foam plug. Before application of the next treatment, each vial was inspected to ensure the treated bugs were alive. *Lygus hesperus* adults that did not revive were replaced by the extra treated bugs. Vials of treated bugs and the Petri plates containing extra treated bugs were transferred to the assigned temperature condition. Vials were inspected a second time 1 - 2 h after treatment, and bugs dead from handling were replaced.

Vials were inspected daily for 21 days after treatment to detect mortality of the insects. This study duration was selected based on preliminary studies (Spurgeon and Hudson 2008). When a dead *L. hesperus* adult was identified, its death and its gender was recorded, and the foam plug closing the vial was replaced by a solid snap-cap lid marked with an identifying number. Green bean sections were retained within the vials with the dead bugs to maintain high humidity levels. Vials containing dead bugs were then placed into sealable plastic bags, each labeled with the respective *B. bassiana* isolate and temperature treatment. Bags of vials were held in the chamber maintained at 23.9°C for detection of mycelial growth or sporulation. *Lygus hesperus* adults that remained alive at each inspection were provided a fresh section of green bean pod 3 times weekly until the end of the assay.

Cadavers were inspected daily under a dissecting microscope to detect *B. bassiana* mycelia or conidia. Cadavers were discarded after sporulation occurred, or if mycelia had not appeared on the exterior of the cadaver by the 5th day after death. In rare instances, mycelia were detected by the 4th or 5th day after death, but sporulation did not occur by the 5th day. In those cases, the vial was retained for a 6th day to verify that the *Beauveria* infection resulted in sporulation.

Statistical analyses. Survival functions of *L. hesperus* adults were compared among *B. bassiana* treatments within temperatures using the LIFETEST procedure of SAS (SAS Institute 2008). The analyses controlled for repetition of the experiment using the STRATA statement, and bug gender was included as a covariate in the TEST statement (Allison 1995). Statistical differences in bug survival among the treatments were declared based on the log-rank statistic. Pairwise comparisons of the survival functions within each temperature were made. The experiment-wise error rate was controlled using the ADJUST = SIMULATE option in the STRATA statement.

Because it is unknown whether both genders of *L. hesperus* are equally susceptible to *B. bassiana*, it seemed useful to examine whether the proportions of bugs that were female were similar among the temperature treatments. For this comparison, proportions of bugs that were female for each combination of experimental repetition, temperature, and *B. bassiana* isolate were categorized as <35%, 35 - 44%, 45 - 55%, 56 - 65%, or >65% female. Because both temperature and the classes of female proportions were ordinal, the distributions of female proportions were compared among temperatures using the Cochran-Mantel-Haenszel (CMH) nonzero correlation statistic (Stokes et al. 2000).

Beauveria bassiana isolates were compared to determine whether there were differences in the extent to which cadavers exhibited symptoms of disease (presence of mycelia or conidia) by day 5 after death using the CMH row mean scores statistic (Stokes et al. 2000). This comparison among isolates controlled for repetition of the experiment and temperature. Although all dead *L. hesperus* were held at the same temperature, the purpose of this examination was to determine whether there were residual effects on pathogen growth and development from previous exposure to the different temperatures. Because none of the bugs in the controls ever exhibited symptoms of infection, they were excluded from all analyses of symptom occurrence.

Patterns of occurrence of mycelia or conidia were compared among temperatures controlling for repetition of the experiment, *B. bassiana* isolate, and day after death. Because both the response (no symptoms, presence of mycelia, presence of conidia) and temperature were ordinal, these comparisons were made using the CMH nonzero correlation statistic.

Finally, the patterns of appearance of fungal mycelia and conidia were examined among *B. bassiana* isolates within temperatures using the CMH row mean score statistic. These analyses controlled for repetition of the experiment and day after death. When statistically significant differences within a temperature were indicated in these patterns, the data were analyzed by temperature and day after death to identify the times when differences occurred.

Results

LIFETEST results indicated differences among the *Lygus* survival functions corresponding to *B. bassiana* treatments at each temperature (12.8°C, $\chi^2 = 20.54$, df = 5, $P < 0.01$; 18.3°C, $\chi^2 = 37.11$, df = 5, $P < 0.01$; 23.9°C, $\chi^2 = 81.40$, df = 5, $P < 0.01$; Fig. 1). Survival was too high at 12.8°C for estimation of the median survival times. At

18.3°C, median survival times of *B. bassiana* treated bugs ranged from 11 d (isolates 3 - 3, 4 - 11; 95% C.L., 9 - 14 d) to 16 d (GHA; lower 95% C.L., 10 d, upper 95% C.L. could not be estimated). At 23.9°C, median survival times of treated bugs ranged from 7 d (isolates 3 - 3, 4 - 11; 95% C.L., 6 - 10 d) to 9 d (GHA, 95% C.L., 6 - 11 d). At each temperature, survival of *Beauveria*-treated *Lygus* was lower than for the corresponding controls, except for the isolate s44 at 12.8°C. Survival of bugs treated with s44 at 12.8°C was not different from that of the controls ($\chi^2 = 7.91$, adjusted $P = 0.056$). No differences in *Lygus* survival were detected among *B. bassiana* isolates ($P > 0.05$). *Lygus* gender was not a significant covariate at 12.8 ($\chi^2 = 0.23$, $P = 0.63$) or 18.3°C ($\chi^2 = 0.06$, $P = 0.80$), but survival of males was higher than that of females at 23.9°C ($\chi^2 = 8.65$, $P < 0.01$). Between days 7 and 21, male survival was about 10% higher than female survival.

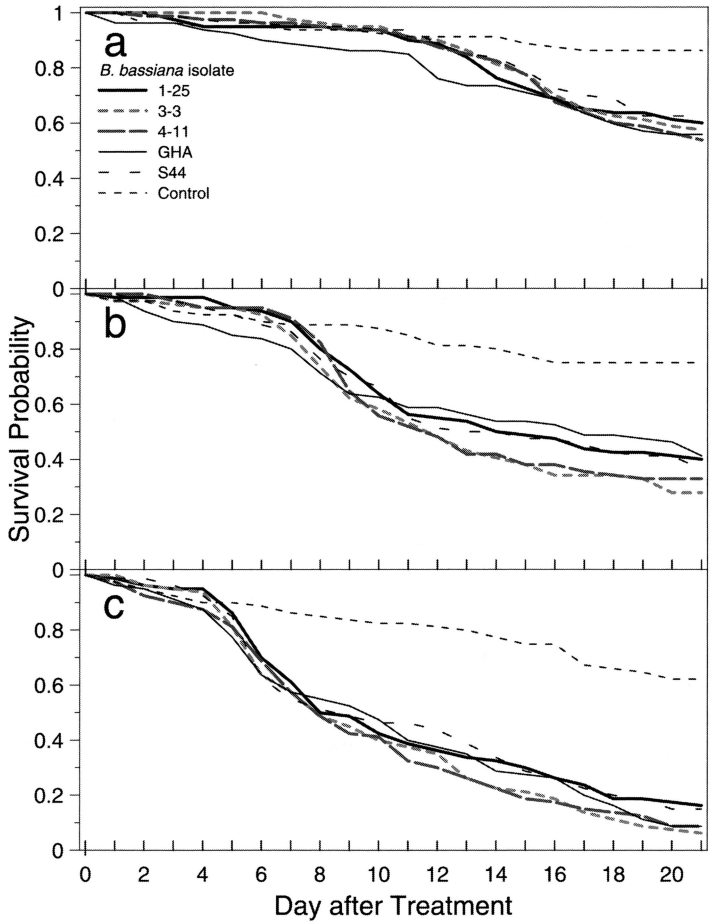

Fig. 1. Predicted survival functions for *Lygus hesperus* adults treated with *Beauveria bassiana* isolates at (a) 12.8°C, (b) 18.3°C, and (c) 23.9°C.

Only 19% of individual cohorts contained equal proportions of female and male bugs, but 50% of cohorts were between 45 and 55% female. Only 14% of cohorts contained less than 35% or more than 65% female bugs. The CMH nonzero correlation statistic (0.81, df = 1, $P = 0.37$) indicated no differences in the distributions of sex ratios assigned to the various temperatures.

Not all of the mortality of *B. bassiana*-treated *Lygus* could be attributed to the pathogen. The percentage of cadavers exhibiting symptoms of *B. bassiana* was about 80% regardless of temperature (12.8 = 82.2%, 18.3 = 81.6%, 23.9°C = 80.1%), and no differences were demonstrated among isolates in the proportions of dead *Lygus* that exhibited either mycelia or conidia by day 5 after death (CMH row mean score = 5.25, df = 4, $P = 0.26$). Of the *Lygus* exhibiting mycelia, only 2 bugs (one treated with GHA at 12.8°C, one treated with 1 - 25 at 23.9°C) did not sporulate by day 5 after death. Both of these bugs sporulated on the 6th day.

Mycelia were observed soon after the death of most bugs. However, considering only those bugs eventually exhibiting symptoms of disease, the CMH nonzero correlation statistic (47.01, df = 1, $P < 0.01$) indicated differences among the temperature treatments in the patterns of appearance of mycelia and conidia (Fig. 2). Both symptoms of *B. bassiana* disease tended to appear more slowly at 12.8°C than at the 2 higher temperatures, but >90% of cadavers sporulated by day 3 after death regardless of temperature.

Comparisons of the patterns of appearance of mycelia and conidia among isolates within temperatures did not indicate differences at 12.8°C (CMH row mean score = 2.39, df = 4, $P = 0.67$). However, differences among isolates were indicated at 18.3 (CMH row mean score = 14.44, df = 4, $P < 0.01$) and 23.9°C (CMH row mean score = 9.51, df = 4, $P = 0.0496$). Further examination of the data for 18.3° indicated a difference in the respective patterns of occurrence of mycelia and conidia only on day 2 after death (CMH row mean score = 11.23, df = 4, $P = 0.02$; Fig. 3a). On that day, a lower proportion of *Lygus* infected with isolates 1 - 25 and s44 sporulated compared with other isolates. Although similar analyses of data for 23.9°C did not indicate a statistical difference among isolates on day 2 (CMH row mean score = 8.37, df = 4, $P = 0.08$), observed trends were similar to, but less distinct than, those observed at 18.3° (Fig. 3b).

Discussion

Previous preliminary studies suggested the potential to identify *B. bassiana* isolates with improved efficacy at low temperatures (Spurgeon and Hudson 2008). However, comparisons among 3 isolates collected during the winter, an isolate collected during the summer, and a commercial isolate did not indicate significant differences in *Lygus* mortality. Although differences were observed among the isolates in the time required for sporulation, those differences were not great enough to serve as a basis for isolate selection.

Decreases in temperature were accompanied by decreased *Lygus* mortality. However, the proportions of cadavers exhibiting symptoms of disease in the *Beauveria* treatments were similar among temperature treatments. Therefore, estimates of survival functions corresponding to the various isolates were not confounded by differences in background mortality. The similarity among temperatures in the proportions of dead *Lygus* exhibiting symptoms of disease have other implications regarding estimates of mortality. If only 80% of the dead treated *Lygus* were killed by *Beauveria*, final estimates of mortality would be reduced by 8 - 9%, 12 - 14%, and 17 - 19%, at 12.8,

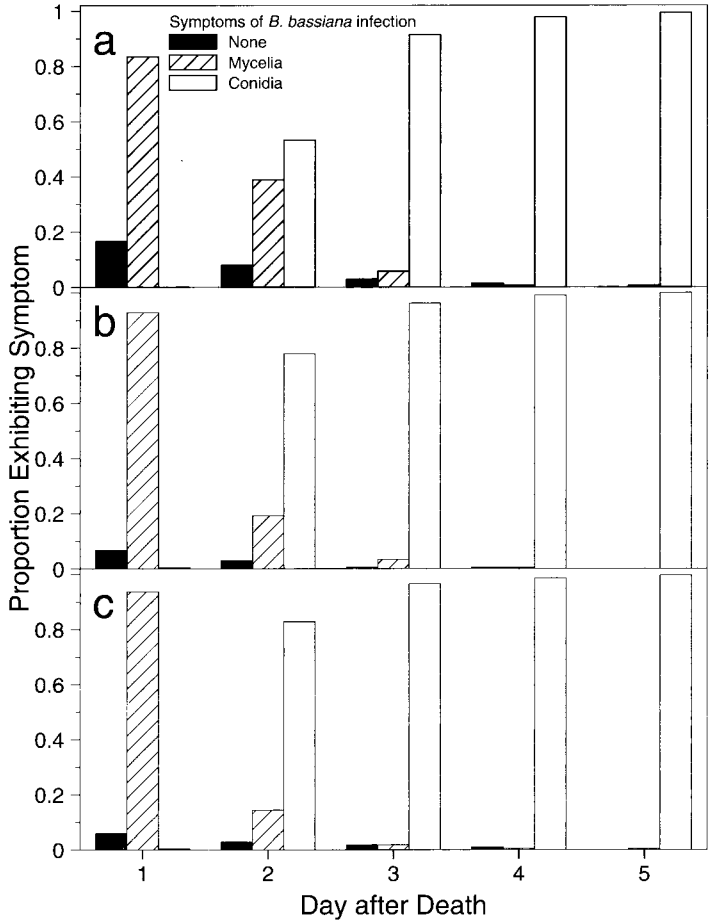

Fig. 2. Patterns of occurrence of *Beauveria bassiana* infection symptoms in adult *Lygus hesperus* treated at (a) 12.8°C, (b) 18.3°C, and (c) 23.9°C. All *Lygus* were held at 23.9°C for 5 days after death for development of symptoms.

18.3, and 23.9°C, respectively. Also, the continued infection of *Lygus* until the end of the 21-d test period, especially for bugs held at 23.9°C, suggests that either some of the mortality caused by *B. bassiana* was replaceable, or the susceptibility of *Lygus* adults increased with age.

The significant effect of the *Lygus* gender covariate at 23.9°C is difficult to interpret in the absence of similar effects at the lower temperatures. It seems likely that the observed difference in survival of respective genders at 23.9°C was either a type I error, or the lower overall levels of mortality observed at 12.8 and 18.3°C masked the influence of gender. Differences in the susceptibility of *Lygus* genders to *B. bassiana* would be of practical and ecological importance if they occur, and this observation should be confirmed or refuted through additional experiments.

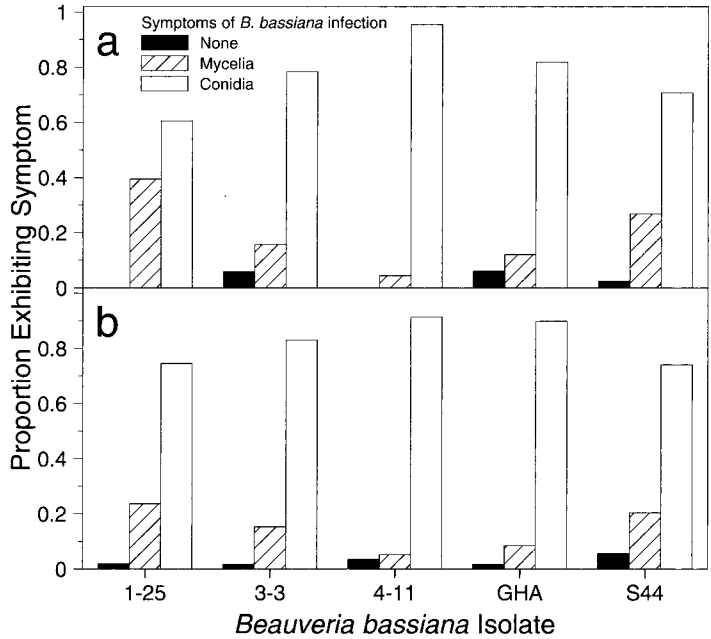

Fig. 3. Distributions of infection symptoms exhibited on day 2 after death by adult *Lygus hesperus* treated with five isolates of *Beauveria bassiana* at (a) 18.3°C and (b) 23.9°C. All *Lygus* were held at 23.9°C after death for development of symptoms.

In summary, the results do not indicate that any of the tested native isolates of *B. bassiana* are superior to the commercially-available isolate for use under low temperature conditions. In addition, the decreased *Lygus* mortality observed with decreasing temperature may not preclude the effective use of *B. bassiana* during the winter and early-spring if low temperature conditions also prolong the viability of applied conidia. While these results have implications regarding the development of *B. bassiana* as a control tactic for overwintering *Lygus*, they may also be useful in the development of *Lygus* management plans for high-value crops grown under low temperature conditions on the Pacific Coast of California.

Acknowledgment

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture.

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