Genetic improvement of a parasitoid: response of *Trioxys pallidus* to laboratory selection with azinphosmethyl

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Summary
Colonies of *Trioxys pallidus* Haliday (Hymenoptera: Aphidiidae) were collected from California walnut orchards during 1985-86 and screened for variability of responses to azinphosmethyl. Variability was found and laboratory selection was initiated with four colonies. All colonies responded to selection; after 7 to 12 selections, three colonies were combined (Select colony) and selection was conducted an additional 27 times. The corresponding base colonies were also combined and maintained for comparisons (Base). Concentration/mortality lines obtained for the Select and Base colonies after 5 and 27 selections, indicated 7- and 9.2-fold differences in LC50 values, respectively. This selection response appeared inadequate to allow the resistant strain to survive field rates of azinphosmethyl, but when the resistant strain's survival was evaluated with several different bioassay methods, different conclusions were reached regarding the potential ability of the selected strain to survive field rates of azinphosmethyl.

Introduction
Genetic improvement of arthropod natural enemies is remains a controversial tactic in biological control because the number of cases in which laboratory-selected strains have been shown to be effective in the field remains small (Hoy, in press). Several phytoseiid species (Acari: Phytoseiidae) and an insect predator (*Chrysoperla carnea* (Stephens), Chrysopidae) have been selected in the laboratory for high levels of resistance to pesticides (Grafton-Cardwell & Hoy, 1986; Hoy, in press). Recently, a parasitoid, *Aphytis melinus* DeBach (Hymenoptera, Aphelinidae) was selected successfully for resistance to carbaryl (Rosenheim & Hoy, 1988). However, pesticide-resistant strains of parasitic Hymenoptera remain rare and no laboratory-selected strains have yet been shown to be effective under field conditions (Croft & Strickler, 1983; Havron, 1983; Delorme et al., 1984; Horn & Wadleigh, 1988; Hoy, 1990; Thelting & Croft, 1989).

In commercial walnut orchards in California, *Trioxys pallidus* Haliday can be an effective parasite of the walnut aphid, *Chromaphis juglandicola* Kaltenbach (Schlinger et al., 1960; van den Bosch et al., 1982; van den Bosch et al., 1970; Frazer & van den Bosch, 1973; Riedl et al., 1979; van den Bosch et al., 1979). Unfortunately, codling moth, *Cydia pomonella* (L.) and navel orangeworm, *Amyelois transitella* Walker, remain key pests that are commonly controlled with pesticides and these often disrupt biological control of walnut aphid by *T. pallidus*. Azinphosmethyl applications have been associated with secondary outbreaks of walnut aphid (Riedl et al., 1979; Sibbett et al., 1981), yet growers prefer to use azinphosmethyl because it provides more effective control of codling moth and navel orangeworm than alternative pesticides. Thus, development and implementation of an azinphosmethyl-resistant strain could improve the usefulness of *T. pallidus* in integrated pest management programs in California walnut orchards.

We report the results of screening colonies of *T. pallidus* collected from commercial walnut orchards to determine whether variability in responses to azinphosmethyl exist. Responses to laboratory selection for azinphosmethyl resistance are reported for four colonies and for a pooled colony derived from three of the four colonies. Finally, we compared the responses of the resistant and susceptible colonies using several bioassay methods to
determine whether the azinphosmethyl resistance level obtained might be sufficient to justify field evaluation of this genetically-selected strain and discuss the significance of choosing appropriate bioassay techniques.

Methods

Colony sources and culture methods. The Valley colony (number initiating the colony \( n = 12 \)) was collected during June 1985 from four walnut orchards in Tulare County, California with unknown pesticide use histories. The Christensen colony \( (n=314) \) was collected during September 1985 from an orchard in Kings County, California; it had been treated once with azinphosmethyl during 1985 but had no previous history of azinphosmethyl treatments because the grower traditionally used phosalone or chlorpyrifos. The Los Banos colony \( (n=155) \) was collected in July 1986 from an orchard in Merced County, California that had been treated "every year" with azinphosmethyl. The Yolo colony \( (n=32) \) was collected during July 1986 from an orchard in Yolo County, California that had been treated twice a year for the preceding seven years with azinphosmethyl. The Aggregate colony \( (n=153) \) was collected from two orchards in Kings County, three orchards in Merced County, and one orchard in Tulare County during September 1986. Several of these orchards had been treated with azinphosmethyl but details were not available.

\( T. \) pallidus is a bisexual, solitary, endoparasitoid. It attacks all stages of the aphid, but "prefers to oviposit" in the younger stages (van den Bosch et al., 1960). In the laboratory, approximately 11-14 days are required to complete a generation at 26-28°C and up to 350 progeny may be produced by each female during her lifetime. \( T. \) pallidus colonies were maintained as discrete generations on walnut aphid-infested walnut seedlings; approximately 200 parasites were introduced into a cage with an aphid-infested walnut seedling and removed after 24 hours. The seedling tree was held in the cage until adult parasites emerged approximately 11-14 days later and these adults were used to initiate the next generation after they were selected (or not).

Selection and bioassay methods. The Valley and Christensen colonies were combined in January 1986 (VC colony); a VC base colony was maintained until March 1987 without selection while the VC select colony was selected 12 times with azinphosmethyl (Figure 1). Likewise, the Los Banos colonies were maintained as an unselected base (LB base) and a select (LB select) colony that was selected nine times by February 1987. The Aggregate colonies were maintained as a base (Agg base) and a select (Agg select) colony that was selected seven times by February 1987. The Yolo select colony was selected three times and discarded while its base colony was maintained as a susceptible colony for comparisons (Figure 1). The VC, LB, and Agg colonies that had been selected were then pooled into a single colony (Select) by placing 75 mated adults of each colony into each of three cages (total = ca. 675). The Select colony was selected an additional 27 times, beginning with the F1 generation, by October 1989. The VC, LB, and Agg base colonies were also pooled into a combined Base colony in March 1987 and maintained through October 1989.

Selections were conducted using 700 to 4975 (mean = 1813) adult male and female parasites one to two days old, using concentrations of azinphosmethyl that permitted 10-50% survival. Selections were not conducted each generation because of time constraints, insufficient parasite emergences, insufficient aphid hosts, or other logistical problems.

Initially, selections, concentration/mortality tests, and bioassays were conducted using one ounce (29.6 ml) clear plastic cups with solid plastic lids (Serco-L-100, Anchor Hocking Plastics, St. Paul, Minnesota). Azinphosmethyl (Guthion 50 WP or 35 WP, Mobay Chemical Corporation, Kansas City, Missouri) was dissolved in 95% (190 proof) ethanol to form a concentrated stock solution each test day. Additional dilutions were made from the
stock with 95% ethanol. The plastic cups were treated by placing one ml of dilute solution in the cup and swirling and rolling the cup and lid to obtain complete coverage. Control cups were treated with 95% ethanol. Cups and lids were inverted on paper toweling to dry. Two pieces of black vinyl electrical tape ca. 1.5 X 12 mm were placed on the inside of each treated cup and 2 or 3 lines of undiluted honey were streaked on each. Adult parasites that had emerged within one or two days were aspirated into the test cups. Suction for the aspirator was provided by a vacuum pump that could be adjusted to eliminate injury to the parasites. To minimize contamination the rubber stopper of the aspirator was covered with organdy cloth and parasites were added to the vials starting with the water control and then the selection concentration. The cloth was discarded and rubber stopper cleaned after each test. Selections were conducted with 50 parasites (both sexes) per cup. Cups were held lid side down in trays at 25°C (16L:8D) in an environmental chamber and scored after 48 h by counting the number of parasites dead or alive. These test methods were used until May 1987 for bioassays, concentration/mortality tests, and selections of the VC, LB, Agg, and Yolo colonies (Figure 1).

Because we suspected the treated plastic cap and cup method overestimated parasite mortality, we compared mortality data obtained with cups closed with the solid plastic caps or caps made of treated or untreated cloth mesh. When treated mesh was substituted for the solid plastic cap, cloth pieces were soaked in the pesticide solution, removed, and allowed to air dry. For both treated and untreated mesh caps, cups were treated by filling them with pesticide solution for five seconds, pouring the solution out, and allowing the cups to dry on paper toweling. Mesh was held on each cup with a plastic cap which had all but the rim removed. Honey was placed on two strips of black tape in each cup. Cups with mesh lids were placed on their sizes in the trays so that air exchange was enhanced and so that parasites were exposed to treated surfaces. Preliminary observations had indicated that parasitoids spent most of their time on the upper most surface of the container, so by placing the cups with mesh on their sides the parasitoids spent little time on the untreated mesh surface. Survival of the Select-2 and Base colony at 15 ppm azinphosmethyl were compared with the three cup treatments after 24, 48, and 68 hours using three replicates of 25 parasites/cup. Survival was compared by a Mantel-Haenszel Chi Square test (Lee, 1980). Complete concentration/mortality lines were obtained for the Yolo, Base, and Select-5 colonies using the capped cups and cups with untreated mesh lids using 20 parasites/cup for 6 or 8 replicates of each of 5 to 7 concentrations plus water controls. All concentration/mortality data were analyzed using the POLO program with the probit option (Russell et al., 1977).

Beginning with the Select-3 colony (September 1987), selection methods were altered by substituting untreated mesh cloth for the solid plastic lids and holding cups on their sides in trays in the growth chamber. Concentration/mortality lines were conducted with Yolo, Base and Selected colonies after 5 and 27 selections with the untreated mesh lids.

Other bioassays to evaluate resistance

After selection had been conducted for more than a year, we were concerned whether we could obtain a strain with a level of resistance suitable for survival in azinphosmethyl-treated walnut orchards. The resistance levels obtained in the selected lines, as estimated by the plastic cup assays, did not appear to be sufficient to allow the parasitoids to survive field rates of azinphosmethyl; 15 ppm, the standard selection rate, is approximately 2.5% of 600 ppm (which is at the low end of the field rate which ranges from 450 to 1125 ppm). This apparent discrepancy between selection response and apparent need for a much higher level of resistance led us to consider discontinuing the project. However, before discarding the colony, we decided to conduct bioassays that would compare the survival of the resistant and susceptible strains under conditions that came closer to mimicking field conditions. Specifically, we wanted to know whether the selected parasites might survive higher rates of azinphosmethyl on treated foliage than in the plastic cups. Initially, we evaluated survival of
parasites contained in clip cages on seedling walnut trees sprayed with azinphosmethyl by hand. Then we compared the ability of the selected and susceptible parasites to reproduce on aphids on treated seedling trees in the greenhouse. Finally, we evaluated survival rates of the selected and susceptible parasites contained in clip cages on field-treated foliage.

Three seedling walnut trees each were sprayed to drip with 1.2 g 50WP azinphosmethyl/liter, 0.6 g 50WP azinphosmethyl/liter, or water using a Hudson sprayer. Twenty parasites were placed in each clip cage on the treated foliage 4 hours, 14 days, 28 days, or 42 days after treatment and a total of 160 parasites of the Select-3, Base, and Yolo colonies each were evaluated on each residue age. Parasites were anesthetized with CO2 when placed in the clip cages and again when cages were attached to the leaflets. Clip cages were made from 31.5 mm (inner diameter) clear acrylic tubing cut into 8 mm rings. One side of the ring was padded with 12 mm thick foam to cushion and provide a good seal against the leaf surface. The other side of the ring was covered with cloth mesh to allow air circulation. The cage was attached to the underside of the walnut leaf with a hair clip and supported with a stiff wire so the under surface of the leaf was the top of the cage. Previous observations with clip cages indicated the upper surface of the cage is where the parasites spend most of their time. Survival was evaluated after 4, 8, 18, 24, 48, and 72 hours of exposure to the residues by Mantel-Haenszel Chi Square test (Lee, 1980).

The ability of the Select-3 and the susceptible Yolo colonies to reproduce on azinphosmethyl residues on sprayed seedling walnut trees held in cages was compared. The parasites had to search for hosts on treated foliage and oviposit, and the progeny had to develop and emerge from the host aphids on treated residues. Seedling walnut trees (5-7 leaves) were sprayed to drip with 1 lb 50WP azinphosmethyl/100 gallon water (1.2 g 50WP/liter) or water using a 4-liter Hudson sprayer. Trees were then placed in coarse-meshed cages outdoors for 22 to 24 days until use. (Tests of parasite mortality on aged residues had indicated that azinphosmethyl residues were still highly toxic after this time interval (Hoy & Cave, 1988, 1989).) Aphid populations on the trees were augmented, if necessary, a few days before tests began so that approximately equal numbers of aphids were present. Tests were started by placing 40 females in a sleeve cage with a single tree for a 2-h period. Parasite mortality was assessed when parasites were removed. Trials were conducted over three consecutive days, with one water and azinphosmethyl treatment tested in the morning and afternoon of each day, for a total of six replicates, although one replicate of the susceptible Yolo colony (untreated control) had to be discarded because of predation by cecidomyid larvae. After the parasites were removed, the trees were placed in large cages in a greenhouse where daily temperatures ranged from 17-370 C. Mummies formed after six days and after nine days leaves were clipped off each tree. Leaves from each tree were placed in individual cages and the number of adult parasites from each tree were counted after all had emerged.

The survival of the susceptible Yolo and Selected-3 colonies were compared using foliage containing azinphosmethyl residues from a commercial walnut orchard. Eight trees in the Blossom Farms orchard, near Stockton, California, were sprayed with a rate of 1 lb 50WP azinphosmethyl/100 gallons (1.2 g 50WP/liter) on August 1, 1987. Leaf samples were taken on August 4 and 18 (3 and 17 days after treatment) from 8 treated and untreated trees. Six leaves each were sampled from approximately 2 and 4.5 m heights on each tree and taken to the laboratory in ice chests. The two penultimate leaflets were removed, one for each of the parasite strains evaluated. The day before the leaves were sampled, clip cages were set up with ten parasites per cage. Parasites were anesthetized with CO2 when placed in the clip cages and again when cages were attached to the leaflets. The leaflet was then oriented so the under side formed the top of the cage. Parasites were exposed to the residues on the same day the foliage was collected and mortality was scored after 4, 8, 18, 24, 48, and 72 h. Tests
were conducted at 25°C under 16L:8D. Survival through time was compared for each residue age using the Mantel-Haenszel Chi Square test.

Results and Discussion

Variability in responses to azinphosmethyl

Concentration/mortality lines obtained for the Valley, Christensen, Yolo, Aggregate, and Los Banos colonies of T. palidus indicate that variability in responses to azinphosmethyl are present (Figure 2). LC₅₀ values for the different colonies were obtained on three different dates, so it may not be appropriate to compare them directly, but the values ranged from 3 to 8 ppm for the Valley and Los Banos colonies, respectively, a 2.7-fold difference (Table 1).

Selection responses

The VC, LB, and Agg colonies responded slowly to selection with azinphosmethyl. Interestingly, although the Yolo colony was collected from a site which was reported to be treated twice a year with azinphosmethyl for at least seven years, it yielded a minimal response to selection. Concentration/mortality lines were obtained for the Yolo and VC colonies after 3 and 11 selections, respectively (Table 2). The LC₅₀ values for the Yolo base and Yolo-3 colonies were 3.5 and 4.5 ppm azinphosmethyl, respectively, a 1.3-fold difference. The LC₅₀ values for the VC base and VC-11 colonies were 10.5 and 17.4 ppm, respectively, a 1.7-fold difference. Complete concentration/mortality lines were not obtained for the LB and Agg selected colonies before they were combined with the VC-select colony to form the Select colony. However, survival of the selected lines of both the LB and Agg colonies increased slowly during their nine and seven selections, respectively. Initial survival of the LB colony treated with 9 ppm azinphosmethyl was 92%; subsequent selections with 15 ppm yielded 27.5, 38.0, 32.4, 34.8, 47.9, 14.6, 74.0, and 49.0% survival rates during selections 2-9. The Agg colony was treated with 15 ppm azinphosmethyl and yielded survival rates of 27.8, 16.3, 26.0, 44.8, 14.0, 51.3, and 55.6%, respectively, during the seven selections.

After the VC, LB, and Agg colonies were pooled and selected five times, two concentration/mortality tests indicated that response to selection continued (Table 2). LC₅₀ values (obtained with the cups capped with solid plastic lids) were 2.5 ppm for the Yolo (unselected) colony, 5.0 ppm for the Base (unselected) colony, and 18.8 ppm for the Select-5 colony, a 7.5-fold difference between the Yolo and the Select-5 colony. When the same colonies were tested in cups with untreated mesh lids, LC₅₀ values were 5.6, 8.7 and 39.0 ppm azinphosmethyl, respectively, for the Yolo, Base, and Select-5 colony, a 7-fold difference between the Yolo and Select-5 colony (Table 2, Figure 3).

At the end of 1989, concentration/mortality lines were obtained for the Yolo, Base, and Select-27 colonies to determine if additional selection response had occurred (Table 2, Figure 4). The LC₅₀ values (obtained using cups capped with untreated mesh) for the Yolo, Base, and Select-27 colonies were 10.9, 11.3, and 100.6 ppm azinphosmethyl, respectively, a 9.2-fold difference between the Yolo and Select-27 colonies. Thus, only a modest increase in resistance was obtained between May 1987 and December 1989, after an additional 22 selections.

Results of different bioassays

Different test methods resulted in different survival rates at the same concentration of azinphosmethyl, although they did not alter the conclusions about the relationship between the selected and susceptible colonies (Table 2, Figures 3, 5).

Significant differences in survival of the Select-2 and Base colonies were found during a timed response test using three different test methods (Figure 5): treated cups (15 ppm) with solid treated lids, treated cups with treated mesh lids, and treated cups with untreated

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mesh lids. Significant differences in survival rate (Mantel-Haenszel Chi Square > 6.2, P < 0.05, d.f. = 1) of the Base colony were found between the three cup treatments. Significant differences were also found in survival of Select-2 parasites held in cups with solid lids and either mesh-capped treatment (Chi Square = 59, P < 0.05, d.f. = 1). There was no significant difference in survival of the Select-2 parasites tested in cups with treated or untreated mesh, however (Chi Square = 0.1, P > 0.05, d.f. = 1). The fact that the solid treated lids resulted in higher mortality for both colonies suggests that there could be a fumigant effect from the azinphosmethyl, or that the parasites were exposed to more azinphosmethyl when confined in cups with treated caps.

Concentration/mortality lines obtained for the Yolo, Base, and Select-5 colonies with the two test methods (capped cups and cups with untreated mesh lids) confirmed the results with the timed response test using a single concentration of azinphosmethyl (Table 2, Figure 3). The untreated mesh caps resulted in lower mortality rates in all three colonies. For example, the LC50 value for the Yolo colony was 2.5 ppm when a solid cap was used but was 5.6 ppm when an untreated mesh lid was used, a 2.2-fold difference. The LC50 for the Select-5 colony held in cups with a solid cap was 18.8 ppm while it was 39.0 for the untreated mesh lid, a 2.1-fold difference.

The Select-3 colony survived better than the Base and Yolo colonies when parasitoids were contained in clip cages on azinphosmethyl-treated walnut seedlings (not all data presented). For example, 39.6 and 68.8% of the Select-3 colony, 8.2 and 14.4% of the Base colony, and 0 and 1.2% of the Yolo colony, respectively, were alive after 4 hours on the two different rates of one-day-old azinphosmethyl residues. Survival of the Select-3 colony after 4 hours on 42-day-old residues was 8.0 and 34.4%, respectively, for the two rates of azinphosmethyl, while survival of the Base colony was 0.6 and 5.0% for the two azinphosmethyl rates, and survival of the Yolo colony was 1.9 and 15.5%.

Reproductive success of Select-3 females on azinphosmethyl-treated caged seedlings was greater than that of the Yolo females (t-test, t= 4.69, P < 0.001, d.f. = 9). Yolo females produced an average of 6.4 (S.D. ± 2.5) progeny while the Select-3 females produced a mean of 42.7 (± 6.9) progeny/cage. However, azinphosmethyl residues decreased reproductive success of both Select-3 and Yolo females; Yolo females produced 279 progeny (± 25.8) while Select-3 females produced 298.6 (± 24.1) progeny/cage on the water-treated trees. Progeny production was not significantly different for the two colonies on the control trees, however (P= 0.60, d.f. = 8).

When Sel-3 and Yolo females were contained on azinphosmethyl-treated foliage collected from the walnut orchard after 3 and 17 days, survival was again significantly different (Χ² = 398.5 and 443.7, P < 0.05, d.f. = 1, respectively, for the 3- and 17-day-old residues). Survival rates on 3-day-old residues for the Select-3 colony were 89.7, 81.8, 62.4, 53.7, 36.5, and 25.8% after 4, 8, 18, 24, 48, and 72 hours. Survival rates for the Yolo colony were 53.6, 38.1, 21.3, 17.6, 10.1 and 5.1%, respectively, for the same intervals. The 17-day-old residues remained toxic, indicating that azinphosmethyl has a long residual impact on T. pallidus populations in the field. Survival rates of the Select-3 colony were 87, 68.7, 54.9, 47.3, 34.1, and 25.6% after 4, 8, 18, 24, 48, and 72 hours on the 17-day-old residues. Survival rates of the Yolo colony were 45.3, 29.2, 18.4, 12.8, 6.1, and 2.9%, respectively, for the same intervals. These data suggested that the Select colony survived significantly better on azinphosmethyl residues on foliage than the Yolo colony. Because the treated foliage was collected from a walnut orchard where it was exposed to sunlight, and coverage might be comparable to that in commercial orchards, plans were made to release the Select colony into several walnut orchards during the 1988 field season after the Select colony had undergone additional selections (Hoy et al., 1990).
General Discussion

Variability in responses to azinphosmethyl was found in the populations of *T. pallidus* collected from commercial walnut orchards in California. This suggests that local populations may be responding to selection with azinphosmethyl. Because the azinphosmethyl-resistant strain is cross resistant to chlorpyrifos, endosulfan, methidathion, and phosalone (Hoy & Cave, 1989), they may also be responding to selection with these pesticides as well. Extensive intraspecific variability in responses to pesticides have been found in colonies of the California red scale parasite *Aphytis melinus* DeBach collected from California citrus (Rosenheim & Hoy, 1986), in the common green lacewing, *Chrysoperla carnea*, from alfalfa in California (Grafton-Cardwell & Hoy, 1985), and the phytoseiid predator, *Metaseiulus occidentalis* (Nesbitt), collected from pears, almonds, and grapes in California (Hoy, 1985).

The variability found in *T. pallidus* was not expected for several reasons. Parasitic Hymenoptera are considered to have low levels of genetic variability (Grauer, 1985). Furthermore, because *T. pallidus* is an exotic species that was introduced into California in a classical biological control program, it may have been subject to genetic bottlenecks during quarantine and colonization so that genetic variability could have been reduced. Whether the genetic variability which provided the basis for the variable responses to azinphosmethyl was present in the initial colony(ies) of *T. pallidus* released in California is unknown. Because *T. pallidus* has been established in California for approximately 20 years (van den Bosch et al., 1970) and population densities have been very high, it is possible that variability in responses could have been derived by selection on new mutations.

All colonies of *T. pallidus* responded to selection with azinphosmethyl (Table 2). Because three colonies were combined for logistical reasons after 7 to 12 selections, we don't know whether they would have yielded different levels of resistance had the selection continued on each colony. The combination of colonies was necessary because *T. pallidus* is costly and time consuming to rear in the laboratory because three trophic levels must be maintained (parasitoid, herbivore and plant).

Artificial selection for resistance should be most efficient if all parasites are uniformly exposed to a specific concentration of pesticide. The plastic cup with solid plastic lid provided such an effective and efficient selection method. As a bioassay technique this technique also provided reliable information on comparative survival rates between the selected and base colonies. However, because the selected colony was only able to survive such low rates of azinphosmethyl in the plastic cup relative to the field rate, it was difficult to predict whether selection had yielded a field-usable level of resistance. When the lid was modified by using a mesh lid, both the Select and Base colonies survived higher concentrations of azinphosmethyl. When parasites were tested in the clip cages on walnut foliage treated with higher rates of azinphosmethyl, survival of the Selected strain was increased sufficiently that field trials appeared warranted. Thus, the choice of a specific bioassay technique for this parasitoid could lead to different conclusions regarding resistance levels and field rates, although all methods indicated the Selected colony was more tolerant of azinphosmethyl than the Yolo or Base colonies.

Ultimately, the survival, persistence, and efficacy of the resistant strain in azinphosmethyl-treated commercial walnut orchards will determine whether the selection project has yielded a genetically improved strain. Field trials conducted during the 1988 field season in commercial walnut orchards demonstrated that the Select-19 strain survived field rates of azinphosmethyl, persisted in the orchard, had an impact on aphid populations, and dispersed to nearby blocks (Hoy et al., 1990). Additional information on this laboratory-selected strain is needed, including the mode of inheritance of the resistance, the stability of the resistance, the strain's overwintering ability and general fitness. Implementation of this
laboratory-selected strain will probably require that it be released inoculatively and replace or displace the susceptible wild population because augmentative releases are limited by our current inability to mass produce this strain easily or inexpensively. However, if this resistant strain can persist and control walnut aphids, it will be an example of genetic improvement serving as a practical tactic for enhancing the role of biological control in integrated pest management programs.

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References


Figure 1. Outline of selection scheme with *T. pallidus*. The solid rectangles indicate colonies were selected, while the dashed rectangles indicate unselected base colonies. Arrows indicate which colonies were combined and their subsequent names.
Figure 2. Variability in concentration/mortality lines for five colonies of *T. pallidus* collected from walnut orchards in California during 1985 and 1986. Tests were conducted in plastic cups with treated solid lids.
Figure 3. Survivorship curves for the Base and Select-2 colonies of *T. pallidus* compared using different techniques; plastic cups with solid plastic lids, plastic cups with treated mesh lids, and plastic cups with untreated mesh.
Figure 4. Concentration/mortality lines for the Yolo (triangles), Base (dots), and Select-5 (squares) colonies of *T. pallidus* using two different techniques. Solid lines and symbols indicate the plastic cups were closed with untreated mesh lids.
Figure 5. Concentration/mortality lines for the Yolo (triangles), Base (dots), and Select-27 (squares) colonies of *T. pallidus* using plastic cups closed with untreated mesh lids.

![Graph showing concentration/mortality lines for different colonies]
Table 1. Concentration/mortality tests conducted to determine if variability in responses to azinphosmethyl are present in different field-collected colonies of \( T. \) pallidus.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Test date*</th>
<th>LD(_{10}) (95% CI)</th>
<th>LD(_{50}) (95% CI)</th>
<th>LD(_{90}) (95% CI)</th>
<th>Slope (SD)</th>
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<td>Christensen</td>
<td>Jan 1986</td>
<td>1.6 (0.7-2.1)</td>
<td>3.4 (2.7-4.1)</td>
<td>7.3 (5.5-15.1)</td>
<td>3.81 (0.54)</td>
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<td>Valley</td>
<td>Jan 1986</td>
<td>1.2 (0.9-1.4)</td>
<td>3.0 (2.6-3.4)</td>
<td>8.3 (6.8-10.9)</td>
<td>2.92 (0.21)</td>
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<td>Yolo</td>
<td>Nov 1986</td>
<td>1.8 (1.3-2.2)</td>
<td>3.5 (3.0-3.9)</td>
<td>6.8 (5.9-8.2)</td>
<td>4.45 (0.26)</td>
</tr>
<tr>
<td>Aggregate</td>
<td>Mar 1987</td>
<td>3.9 (2.7-4.9)</td>
<td>7.1 (6.0-8.0)</td>
<td>12.9 (11.5-15.3)</td>
<td>4.97 (0.45)</td>
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<tr>
<td>Los Banos</td>
<td>Mar 1987</td>
<td>3.9 (3.1-4.5)</td>
<td>8.0 (7.3-8.8)</td>
<td>16.8 (15.1-19.2)</td>
<td>4.01 (0.23)</td>
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* The test container consisted of one ounce (29.6 ml) treated plastic cups with a treated solid lid. Results are reported as ppm azinphosmethyl.
Table 2. Concentration/mortality tests conducted with different colonies of *T. pallidus* to evaluate responses to selection with azinphosmethyl.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Test date</th>
<th>Test method*</th>
<th>LD₁₀ (95% CI)</th>
<th>LD₅₀ (95% CI)</th>
<th>LD₉₀ (95% CI)</th>
<th>Slope (SD)</th>
</tr>
</thead>
<tbody>
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<td>Yolo</td>
<td>Nov 1986</td>
<td>cap</td>
<td>1.8 (1.3-2.2)</td>
<td>3.5 (3.0-3.9)</td>
<td>6.8 (5.9-8.2)</td>
<td>4.45 (0.26)</td>
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<tr>
<td>Yolo-3</td>
<td>Nov 1986</td>
<td>cap</td>
<td>2.5 (1.8-3.0)</td>
<td>4.5 (3.9-5.0)</td>
<td>8.3 (7.4-9.9)</td>
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<td>cap</td>
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<td>10.5 (9.3-11.8)</td>
<td>20.2 (16.8-28.3)</td>
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<td>cap</td>
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<td>17.4 (16.0-18.6)</td>
<td>27.9 (25.0-33.9)</td>
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<td>cap</td>
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<td>2.5 (2.2-2.8)</td>
<td>4.9 (4.3-4.9)</td>
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<td>11.2 (10.0-13.2)</td>
<td>3.64 (0.26)</td>
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<td>cap</td>
<td>9.2 (5.6-11.6)</td>
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<td>13.0 (11.3-15.6)</td>
<td>3.51 (0.20)</td>
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<td>75.7 (66.0-92.5)</td>
<td>4.46 (0.28)</td>
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The "cap" test method consisted of 29.6 ml treated plastic cups with a treated intact lid; the "mesh" method consisted of the treated cup with an untreated mesh lid. Results are reported as ppm azinphosmethyl.

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<th>6.4 (5.1-7.3)</th>
<th>10.9 (10.0-12.0)</th>
<th>18.6 (15.9-24.3)</th>
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<td>100.6 (93.0-108.2)</td>
<td>160.1 (143.9-188.1)</td>
<td>6.34 (0.54)</td>
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