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A genetically-manipulated strain of the walnut aphid parasite, *Trioxys pallidus* (Haliday), was evaluated to determine whether its azinphosmethyl resistance was stable, the resistant strain could enter and terminate diapause, and the strain could persist in three walnut orchards where releases were made during 1988-1991. The laboratory-selected strain was held in population cages with and without selection with azinphosmethyl for 13 generations without losing resistance. However, the level of azinphosmethyl resistance in mixed populations of resistant and susceptible parasitoids declined over 13 generations without selection. During laboratory selection, the resistant strain was reared for more than 27 generations without entering diapause. However, when this colony, and a freshly-collected wild colony, were reared at 15-16°C or 13-14°C under 8L:16D conditions, diapause induction rates in the two colonies were similar, suggesting that the laboratory-selected strain should be able to overwinter in walnut orchards. Rates of diapause termination were also similar for the two colonies. Bioassays on colonies of *T. pallidus* collected during 1989-1992 from three commercial walnut orchards where releases had been made in 1988, 1989 and 1991 indicated these populations are tolerant of azinphosmethyl and support the conclusion that the resistant strain has established.

**KEY WORDS:** *Trioxys pallidus*; walnut aphid; biological control; genetic improvement; Aphididae; pesticide resistance; azinphosmethyl; *Chromaphis juglandicola*; diapause; fitness
INTRODUCTION

Genetic improvement of arthropod natural enemies involves purposeful genetic changes designed to enhance their efficacy in biological control programs (Hoy, 1990a). Laboratory selection of parasitoids for resistance to pesticides offers a potentially useful method for integrating biological control agents into integrated pest management (IPM) systems. However, relatively few parasitic Hymenoptera have been selected in the laboratory for resistance to pesticides (Adams and Cross, 1967; Croft, 1990; Havron et al., 1991a,b; Hoy, 1990b; Javier et al., 1991; Pielou and Glasser, 1952; Robertson, 1957; Rosenheim and Hoy, 1988; Spollen, 1991; Spollen and Hoy, in press a, b), and even fewer have been released into the field for evaluation.

*Trixys pallidus* Haliday, a parasitoid of the walnut aphid, *Chromaphis juglandicola* Katlenbach, was selected in the laboratory for resistance to azinphosmethyl (Hoy and Cave, 1989; 1991). This resistant strain was released in 1988 into several walnut orchards in California, where it survived field rates of azinphosmethyl, had an impact on aphid populations, dispersed to adjacent walnut blocks and overwintered (Hoy et al., 1990). This strain is cross resistant to methidathion, phosalone, chlorpyrifos and endosulfan, materials used in IPM programs in California walnut orchards (Hoy and Cave, 1989). An analysis of the mode of inheritance of the azinphosmethyl resistance indicated the resistance is determined by more than one gene, although resistance in the reciprocal F1 females is partially dominant (Brown et al., in press).

There is no consensus about what attributes or methods should be used to evaluate the quality and efficacy of genetically-manipulated arthropod natural enemies because relatively few case histories have been studied. Nor are all potentially-interesting evaluations equally easy to perform. It is difficult to evaluate certain traits, such as longevity or ability to locate hosts or prey, under field conditions but relatively easy to measure life table attributes under laboratory conditions for most species. The specific use for which the strain is intended should dictate the types of evaluations made. For example, if the genetically-manipulated strain is to be mass reared for augmentative releases, then attributes such as the ability to diapause and overwinter would not be of interest, but fecundity, sex ratio, and developmental rate would (Hoy, 1976).

Genetically-manipulated strains released for long term establishment in a region should be able to overwinter and persist through several field seasons and disperse. Thus, each genetically-manipulated strain will have to be evaluated in terms of its intended use and its specific biological, ecological and behavioral characteristics.
In the case of azinphosmethyl-resistant *T. pallidus*, it is clearly relevant to determine whether the resistance is stable and of a sufficiently high level that the strain can survive relevant pesticide applications in the field. Because *T. pallidus* is difficult to mass produce and annual augmentative releases are therefore unlikely, this genetically-manipulated strain will have to become permanently established in California walnut orchards if it is to be useful in walnut IPM programs (Caprio *et al.*, 1991; Hoy *et al.*, 1991). Thus, this strain should be able to overwinter and persist in walnut orchards. Diapause attributes may be modified inadvertently during long-term laboratory rearing of many arthropod species (Hoy, 1978), and alteration of diapause characteristics could preclude successful establishment of *T. pallidus*.

This paper describes laboratory experiments designed to compare diapause attributes of the azinphosmethyl-resistant strain and a wild strain of *T. pallidus*. The persistence of resistance in population cages of homogeneous and mixed populations of *T. pallidus* held with and without selection with azinphosmethyl was evaluated in order to assess whether selection has resulted in a strain with a stable resistance. Ultimately, of course, the performance of the laboratory-selected strain must be assessed in the field, and evidence is provided that the azinphosmethyl-resistant strain has overwintered and persisted in three California walnut orchards for one, two and three years, respectively.

**MATERIALS AND METHODS**

*Colony sources and culture methods.* The Selected colony of *T. pallidus* was derived by conducting additional selections with azinphosmethyl on a colony obtained by pooling three colonies that had been selected with increasing concentrations of azinphosmethyl (Hoy and Cave, 1988, 1991). Adults of the Selected colony were selected with azinphosmethyl every 1-12 generations, averaging every other generation. When these tests began, the Selected colony had been selected 27 times (Selected-27). The Base colony was derived by pooling the three corresponding unselected base colonies. *T. pallidus* colonies were maintained on walnut aphids reared on potted Persian walnut seedlings (*Juglans regia* L.) grown in a University of California greenhouse at Berkeley. Synchronous colonies were reared on the potted trees containing walnut aphids in cloth-covered sleeve cages (45 by 45 by 86 cm) at 27 ± 1.5 °C under continuous light.

Field colonies of *T. pallidus* were initiated by collecting foliage with aphids and mummies from walnut orchards. The foliage was placed in a chilled ice chest and returned to the laboratory, where mummies were removed from the foliage. Parasitized aphids were held on walnut leaves resting on water-soaked cotton in plastic dishes until mummies were formed. Mummies were
placed in a 29-ml plastic cup with honey, held until adults emerged, and adults were counted and sexed: any hyperparasitoids present were eliminated. The adult parasitoids from each collection site were used to initiate a colony on potted walnut trees infested with walnut aphids, as described above, and their progeny were tested with azinphosmethyl in a standard bioassay.

**Standard bioassay.** Adults were tested in treated 29-ml plastic cups (Serco-100, Anchor Hocking Plastics, St. Paul, Minnesota) covered with an untreated mesh lid. Azinphosmethyl (Guthion 35WP or 50WP, Mobay Chemical Corporation, Kansas City, Missouri) was dissolved in 95% ethanol to form a concentrated stock solution each test day. Test cups were filled with a 0 or 50 ppm solution of azinphosmethyl, held for 5 seconds, emptied, and inverted on paper toweling until dry. Two pieces of black vinyl electrical tape approximately 1.5 mm wide and 12 mm long were placed on the inside of each cup and streaked with two or three lines of undiluted honey. Adult parasitoids that had emerged within 0-48 h were aspirated into the test cups. An untreated mesh cover for each cup was held on with a plastic cap from which all but the rim had been removed. Tests were conducted using 5 to 20 parasitoids (both sexes) per cup with 10 cups per colony per test. Cups were placed into a growth chamber at 25°C and 16L:8D, and survival was assessed after 24 h.

**Stability of azinphosmethyl resistance.** Population cages were set up to monitor resistance to azinphosmethyl in Selected-27 and Base populations, and in mixed populations initiated with equal numbers of Selected and Base parasitoids held without selection for 13 generations. In addition, the Selected-27 colony was selected with azinphosmethyl every other generation (Selected-28,-29,-30,-31) as a control. Two populations of the Selected-27 colony held without selection were each initiated with 100 mated adult females and were called Relaxed-Selection-A,B. Two populations of the Base colony were maintained as controls; one was initiated with 130 adult females, the other with 140. Three populations (Persistence-A, -B, -C) each were obtained by combining 70 mated Selected-27 females and 70 mated Base females and holding them without selection. Because these populations were initiated with mated females, the progeny of these females were not hybrids. However, the progeny of the parental females had the opportunity to mate with progeny from the other colony and thus the following generation produced the F1 generation. After six generations, selections with azinphosmethyl were performed with 100 ppm ai azinphosmethyl on a subset of the Persistence populations to determine whether, and how rapidly, azinphosmethyl resistance would increase. This subset colony (Persistence-Selected) was subjected to two selections on alternate generations.
The number of populations was reduced following the ninth generation by combining the Base A and B populations into a single Base colony, continuing the Persistence-B population, but discarding the -A and -C populations, continuing the Relaxed-Selection-A population, but discarding Relaxed-Selection-B, continuing the Selected-31 population, and discarding Persistence-Selected-2. Populations were screened in the initial, F1 and every other subsequent generation using the standard bioassay. Mean percentage survival (± SE) was calculated for each treatment. A regression analysis was performed to determine the slope of the responses of each population over time using arcsin-transformed percentage survival rates.

Comparison of diapause attributes.

Diapause induction. Diapause induction rates were compared in two experiments using the Selected-32, the Base, and a newly-collected colony (Yuba) obtained from Sutter County, California. The first experiment compared responses of all three colonies held at 15-16°C, 8L:16D. The second experiment compared only the Selected-32 and Yuba colonies held at 13-14°C, 8L:16D. For each experiment, approximately 150 individuals (males and females) from each colony were introduced into cages where the females could parasitize aphids on each of two walnut trees for 24 h.

Both aphids on walnut trees and parasitoids were acclimatized at 15-16°C or 13-14°C for seven to eight days before the parasitoids were allowed to sting their aphid hosts. However, because parasite activity was minimal under these cool conditions, parasitoids and aphid-infested trees were held at 26-27°C 16L:8D for 24 h to allow parasitoids to sting the aphids. The trees containing the parasitized aphids then were placed into a large growth chamber for four to five weeks at either 15-16°C or 13-14°C. The mummies that formed were removed and separated into three classes based on color and thickness; mummies that were light tan and thin enough to allow the enclosed parasite to be seen through a dissecting microscope were classified as non-diapausing (van den Bosch et al., 1962). Mummies that were so thick that the parasite could not be detected by examination with a dissecting microscope were classified as diapausing. The thicker mummies were of two colors (dark and light tan), but the significance of the different colors is unknown. The three different classes of mummies were placed into 29-ml plastic cups, with a maximum of 50 per cup. The cups were then held at 15-16°C or 13-14°C, 75% RH and 8L:16D and the number of adult parasitoids that emerged each day under these conditions were counted. Approximately 12 days after the last emergence was recorded, ten mummies from which parasitoids did not emerge were dissected from each replicate to determine whether the
parasitoids were dead or alive; live prepupae were assumed to be in diapause. Chi Square analyses were conducted to compare diapause induction rates.

**Diapause termination.** Diapause termination rates for each colony were estimated by transferring mummies containing diapausing parasitoids from the first experiment into new cups with 25 mummies per cup and holding these in a growth chamber set at 4-5°C, 75% RH and continuous darkness. Subsamples of the Yuba (100 individuals), Selected (50) and Base (25) colonies were removed approximately every four weeks for five months and placed in a growth chamber at 25°C, 75% RH and 16L:8D. The number of adult parasitoids that emerged was recorded twice a week. Two weeks after the last emergence was recorded, ten or 20 mummies from each colony from which no adults emerged were dissected to determine whether the parasitoids were dead or remained in diapause.

**Persistence of the azinphosmethyl-resistant strain in three California walnut orchards.**

**Hanford.** Approximately 30,000 azinphosmethyl-resistant *T. pallidus* were released into the Hanford, California orchard during 1988 and the survival of azinphosmethyl-resistant parasitoids over the 1988-89 winter was reported by Hoy et al. (1990). Longer-term persistance was evaluated in 1989, 1990 and 1991 for this paper. In 1989, colonies of *T. pallidus* were collected in April, July, and August from the orchard and their survival rates were compared to those of the Yolo (Y), Base (B) and Selected (S) colonies, which served as controls. The Yolo colony is a susceptible laboratory colony (Hoy and Cave, 1991). In addition, we collected *T. pallidus* colonies during 1989 from four other walnut orchards (Paramount, Pilibus, Westside, and Barker) in the Central Valley of California where no releases of the Selected strain had been made. Survival rates of these colonies were compared to survival rates for the Yolo, Base and Selected colonies using the standard bioassay. In 1990, *T. pallidus* were collected from this orchard after each of three azinphosmethyl applications (H1, H2, H3), and their resistance to azinphosmethyl assessed using the standard bioassay with the Yolo, Base and Selected colonies serving as controls. During 1991, we collected two colonies of *T. pallidus* (H1, H2) and tested them using the standard bioassay procedure. Survival rates were compared with a freshly-collected field colony from Red Bluff (RO) and the Yolo and Selected colonies.

**Gridley.** Approximately 20,000 azinphosmethyl-resistant *T. pallidus* were released into the Gridley, California orchard during 1989 (Hoy et al., 1990). During 1990, parasitoids were
collected from the Gridley block twice (G1, G2) and one colony was collected in 1991. These colonies were tested in the laboratory using the standard bioassay and survival rates compared to the Yolo, Base and Selected colonies during 1990 and to the Yolo, Red Bluff pre-release and Selected colonies during 1991.

Red Bluff. We reared and released a total of approximately 40,000 of the azinphosmethyl-resistant *T. pallidus* into a 100-acre block near Red Bluff, California in May and June 1991. Before the two releases were made, colonies were collected from the release site (R0) and two nearby orchards (C and L). Survival of these three colonies was evaluated using the standard bioassay and compared to the Selected and Yolo colonies. Parasitoids were collected three times during the 1991 field season after the releases (R1, R2, R3) and once in April 1992. Survival rates of these and the RO, Yolo, and Selected colonies were compared using the standard bioassay.

**RESULTS AND DISCUSSION**

*Stability of azinphosmethyl resistance in population cages.* Survival of the Selected-28, -29, -30, and -31 colonies averaged 98.4% in the standard bioassay (Fig. 1, line 1). Regression analysis indicated the slope of line 1 was not significantly different from zero (F=0.008, P = 0.931). The two Relaxed-Selected colonies also maintained a high level of resistance over 13 generations without selection, averaging 96.4% survival (Fig. 1, line 2). There was no significant departure from a slope of zero (F= 0.646, P = 0.437), suggesting that azinphosmethyl resistance in the Selected-27 colony is stable over an interval equal to nearly two field seasons. The reason for the decline in survival rates in generation seven in line 2 (and lines 4 and 5) is unknown, but may be due to inadvertent changes in experimental conditions. The Base colonies remained susceptible to azinphosmethyl, with survival rates averaging 4.7% over the 13 generations and a slope not significantly different from zero (F= 0.136, P= 0.136, Fig. 1, line 5).

The mean survival of the Persistence colonies declined over 13 generations from 43.5 to 13.5 % (Fig. 1, line 4) and the slope departed significantly from zero (F= 7.215, P=0.0156), indicating that azinphosmethyl resistance may be selected against in mixed populations. However, after a subset colony (Fig. 1, line 3) was selected twice with azinphosmethyl the survival rate rebounded to 72.5%, indicating that the heterogeneous population could respond to selection with azinphosmethyl.
Possible differences in development time, mortality rates of immatures, sex ratio, or reproductive rate, as well as differential mate selection, may have altered the ratio of the resistance alleles in the initial and subsequent generations in the Persistence populations. This experiment may provide an assessment of what occurs when the azinphosmethyl-resistant strain is released in areas where susceptible *T. pallidus* populations are present and no azinphosmethyl applications are made. Results of tests conducted to determine the mode of inheritance of azinphosmethyl resistance in the Selected strain indicated we should expect the initial resistance level in F₁ females to be intermediate between the Selected and Base strains (Brown *et al.*, in press). The decline in azinphosmethyl resistance over time in these population cages suggests that selection against allele(s) conferring resistance to azinphosmethyl may have occurred, or that other life history attributes of the Selected strain were impaired, either by the selection process itself or by inadvertent selection, inbreeding, or genetic bottlenecks that occurred during 1986-1990.

*Comparison of diapause attributes.*

*Induction.* Both males and females of *T. pallidus* entered diapause at the prepupal stage within mummies. Incidence of diapause in the Selected-32 colony reared and held at 15-16°C and 8L:16D was 41.6% (SE=15.4) compared to 51.3 (5.4) % in the Yuba, and 36.3 (11.5) % in the Base colonies (Fig. 2). Parasitoids reared at 13-14°C had a slightly higher rate of diapause induction, with 46.6 (3.8) % of the Selected and 66.2 (0.6)% of the Yuba colony parasitoids entering diapause (Fig. 2). Thus, the Selected strain retained the ability to enter diapause despite more than four years of laboratory rearing when it was not exposed to diapause-inducing conditions. While the Yuba colony demonstrated a significantly greater rate of diapause induction than did the Selected colony in the second experiment at 13-14°C (Chi Square = 67.9, df = 1, P< 0.01), we do not know whether this difference reflects the fact that this colony had been reared for only a brief interval in the laboratory before being tested. The moderate rates of diapause induction recorded in these experiments (36 to 66%) suggests either that we did not use optimal induction conditions or that diapause incidence is less than 100% in these *T. pallidus* populations.

*Termination.* Diapause termination rates for the Selected colony after 30, 60, 86, 114, and 142 days of chill at 4-5°C were 44, 60, 60, 58, and 56%, respectively (Fig. 3). The percentage of Base parasitoids terminating diapause declined over time, with 52, 44, 24, 24, and 26% terminating diapause after 30, 60, 86, 114, and 142 days, respectively. Emergence rates of the Yuba colony were 38, 48, 39, 41, and 37% over the same intervals. Diapause termination
rates for the Selected colony (44 to 60%) thus appeared to be somewhat higher than those for the freshly-collected Yuba colony (37 to 48%). Dissections of the mummies from which no parasitoids emerged indicated that the parasitoids usually remained in diapause, although 0 to 50% of these mummies held dead parasitoids.

Persistence of the azinphosmethyl-resistant strain in California walnut orchards.

Hanford. Survival of the T. pallidus colony collected in April 1989 was 11.5%, compared to 0, 0, and 86% for the Yolo, Base, and Selected colonies, respectively. Survival of the Hanford colony collected in July 1989 was 7.5%, compared to 90.3% for the Selected colony and 0% each for the Yolo and Base colonies. Survival of the Hanford colony collected in August 1989 was 57%, compared to 5.1, 1.5, and 99.5% for the Yolo, Base and Selected colonies, respectively, suggesting that the Selected strain had overwintered during 1988-89 and increased in frequency in the orchard between April and August 1989.

Survival of the colonies collected in 1989 from the Paramount, Westside, Pilibus and Barker orchards, where no Selected parasitoids had been released, was 51, 52, 22, and 3.5%, respectively. We do not know whether the high survival rates of parasitoids from the Paramount (51%) and Westside (52%) orchards are due to dispersal and establishment of the Selected colony after its release in five walnut orchards during 1988, or whether wild parasitoids in these orchards had responded to selection with azinphosmethyl in the field.

The mean percentage survival of the H1 colony (70.0%) collected in May 1990, after an azinphosmethyl application in April, was significantly higher than that of the Yolo (7.5%) and Base (2.5%) susceptible colonies, and close to that of the Selected (100%) colony (Fig. 4). These results were repeated with the two colonies collected from this orchard after the second (H2) and third (H3) applications of azinphosmethyl during 1990 (Fig. 4).

Because these colonies were collected in 1990 from the entire 65-acre block, and because the azinphosmethyl-resistant strain was only released into 2.5 acres of the orchard in 1988, the data indicate the resistant strain has successfully persisted during two years and has dispersed throughout the entire 65-acre block. It is likely that the strong selection exerted during the 1990 field season, when the Hanford orchard received three applications of azinphosmethyl, enabled the resistant strain to increase in numbers. Unfortunately, we did not monitor aphid and parasite interactions from this
orchard biweekly during the 1990 field season. According to R. Beede, University of California Cooperative Extension (personal communication), aphid densities in the block did exceed the current treatment levels in the orchard in June. However, most of these aphids were parasitized and the population collapsed without the grower applying a pesticide application for aphids. Despite high densities of aphids early in the season, subsequent walnut yield and quality were not affected (R. Beede, personal communication), suggesting that the azinphosmethyl-resistant parasitoids had an impact on the aphid population.

Parasitoids were collected from the Hanford orchard twice during 1991 to determine whether the resistant strain had overwintered a second winter (Fig. 5). Percentage survival of the colony collected in July (70.7%) was significantly higher than that of the Yolo (3.5%) and Red Bluff colonies (20.5%), and nearly as high as that of the Selected colony (98.0%). A second colony collected in September (H2) again had a significantly greater survival rate (74.2%) than the Yolo and Red Bluff prerelease colonies (2.5% and 29.0%) and a survival rate nearly as high as that of the Selected colony (98.0%). These data indicate resistant parasitoids have overwintered and persisted through three growing seasons in this orchard, when this orchard received a total of five applications of azinphosmethyl and one of chlorpyrifos.

Gridley. The survival rates of the colonies collected from the Gridley block during 1990 indicates the resistant strain has overwintered and persisted in this orchard, as well (Fig. 6). Survival of the colonies collected after the first chlorpyrifos application in 1990 was significantly higher (84%) than that of the Yolo (4.5%) and Base (7%) colonies, and nearly as high as that of the Selected colony (99%). Similar results were obtained with the second colony collected from this orchard during 1990. Survival of the parasitoids collected after the application of chlorpyrifos and an application of azinphosmethyl plus propargite averaged 76% compared to 5, 4, and 98% for the Yolo, Base and Selected colonies, respectively.

The results obtained from the Gridley block during 1991 confirm the ability of azinphosmethyl-resistant parasite to overwinter (Fig. 7). Survival of the colony collected in August 1991 was significantly higher (70.4%) than that of the susceptible Yolo (2.5%) and Red Bluff pre-release (26.0%) colonies, and nearly as high as that of the Selected colony (95.0%). These data suggest the resistant parasitoids have successfully survived both the 1989-90 and 1990-91 winters and survived
applications of chlorpyrifos, azinphosmethyl, and propargite during the 1990 and 1991 field seasons.

**Red Bluff.** Survival rates of a pre-release colony (RO) from the release site and two colonies collected from nearby orchards (C, L) during 1991 indicated field populations from these sites were moderately susceptible to azinphosmethyl. Survival rates were 24.3 to 27.8% for the RO, C, and L colonies, compared to 1.5% for the Yolo and 98.5% for the Selected colonies.

Three parasite colonies were collected during 1991 after releases were made and their survival rates were compared with the pre-release RO colony, the Yolo, and Selected laboratory colonies (Fig. 8). Survival of the first post-release colony collected in June was 56.9% compared to 20.5, 3.5 and 98.0% for the pre-release, Yolo, and Selected colonies, respectively. Similarly, survival for the second post-release colony (collected in September) was 60.0%, compared to 29.0, 2.5 and 98.0% for the same control colonies. Survival of the third post-release colony (collected in October) was 64.7%, compared to 30.5, 2.0, and 99.5% survival in the control colonies. Two or three applications of azinphosmethyl were made in the 100-acre block during the 1991 growing season; one before release of the resistant strain and one or two following release. Survival rates of parasitoids recovered following azinphosmethyl applications indicate that resistant *T. pallidus* have established in this Red Bluff orchard and persisted through the 1991 field season.

Survival of a colony collected in April 1992 from the Red Bluff release site was 35.6%, compared to 5.5, 3.0 and 96.5% for the Red Bluff pre-release, Yolo, and Selected-35 colonies, respectively. This survival rate suggests, but is not conclusive, that the Selected strain has overwintered in the Red Bluff site.

**GENERAL DISCUSSION**

There is no consensus on how to evaluate genetically-manipulated natural enemies in order to determine their potential to provide effective biological control in agricultural crops or glasshouses. Certainly, the ability to successfully overwinter and persist is a minimal requirement if azinphosmethyl-resistant *T. pallidus* are to establish in California walnut orchards. The data presented here for three different walnut orchards indicate that azinphosmethyl-resistant parasitoids can persist for one to three years (Figs. 4,5,6,7,8). It remains to be determined
whether the azinphosmethyl-resistant strain of *T. pallidus* will establish throughout the walnut-growing region of California. As pointed out by Caprio, *et al.* (1991), establishment is influenced by which pesticides are used in walnut IPM, the number of pesticide applications and their rates, the number of untreated refugia, and dispersal rates and relative fitness of the resistant and susceptible parasitoids. Caprio, *et al.* (1991) suggested that up to ten years may be required for the azinphosmethyl-resistant strain to become established in a large region of the Central Valley of California.

The population cage study suggests that if the resistant strain were released in a site where susceptible *T. pallidus* were absent, this strain should maintain high levels of resistance to azinphosmethyl for at least two years without selection (Fig. 1, line 2). Periodic selection with azinphosmethyl, or the other pesticides to which it is resistant, should enable the resistance to persist for longer intervals. The decline in resistance levels of the Persistence colonies (Fig. 1, line 4) indicates that periodic selection with azinphosmethyl, chlorpyrifos, endosulfan, or diazinon will be important in maintaining the resistant strain in California walnut orchards (Hoy and Cave, 1989).

The results of the diapause experiments are difficult to interpret in one regard. It is common to assume that all individuals of a population have the ability to overwinter in diapause and that there is strong natural selection to maintain high levels of diapause in a population. No previous studies of diapause in *T. pallidus* have been conducted, so we do not know what the optimal induction conditions are. Diapause in a related species, *Trioxys complanatus* Quillis, a parasite of the spotted alfalfa aphid, *Theroaphis trifolii* (Monell), has been studied (Flint, 1980). *T. complanatus* colonies from California, Iran and Italy were evaluated for both aestival and hibernal diapause. The Italian strain failed to enter aestival diapause, although both the California and Iranian strains did so. All three colonies of *T. complanatus* exhibited hibernal diapause (Flint, 1980). Diapause induction rates of the three *T. complanatus* colonies held at 13.3°C ranged from approximately 55 to 68%, which is about the range of hibernal diapause induction observed for the *T. pallidus* colonies tested. Thus, it appears that both *Trioxys* species have a significant degree of phenotypic or genetic variability for diapause induction and that, under the conditions tested, a significant proportion of the population fails to enter a hibernal diapause. Our laboratory results provide no evidence that diapause induction or termination rates of the Selected colony are unusual compared to diapause induction and termination rates of the newly-colonized Yuba colony. Thus, we have no evidence that the Selected strain's diapause attributes have been influenced by long-term rearing and selection under laboratory conditions.
It is frustrating that our only evidence for establishment and persistence of the azinphosmethyl-resistant strain of *T. pallidus* in walnut orchards is based on laboratory bioassays for tolerance to azinphosmethyl. Pre-release colonies of *T. pallidus* were collected from orchards where releases were made and their tolerance to azinphosmethyl was assessed. If post-release colonies collected from these sites were more tolerant to azinphosmethyl, we used this as evidence that the azinphosmethyl-resistant strain had established. However, in 1989, we found that tolerance to azinphosmethyl was surprisingly high in colonies collected from two walnut orchards (Paramount and Westside), and we have found moderate tolerances in other colonies collected subsequently (Hoy and Cave, unpublished). We do not know whether this is due to dispersal of the Selected strain from our release sites or to selection of these wild populations in the field, or to some other factor.

It is unclear how much interbreeding occurs between the released Selected and endemic susceptible *T. pallidus* populations. Releases of the Selected strain were made after an application of azinphosmethyl, which should have reduced the susceptible endemic populations. Azinphosmethyl has a long residual activity for *T. pallidus* (Hoy and Cave, 1989), which also should have favored establishment of the Selected strain. Subsequent applications of azinphosmethyl, or other pesticides to which the Selected strain is more tolerant than the endemic population, should have favored the establishment of the allele(s) for azinphosmethyl resistance. However, the question is: Does the Selected strain persist as a more-or-less reproductively isolated biotype in the release site and in sites to which it disperses, or does the Selected strain interbreed extensively with the endemic (susceptible) population, yielding hybrid progeny that are subsequently selected by field applications of azinphosmethyl and other pesticides for increased resistance levels? Without a more sensitive method for detecting establishment and dispersal of specific biotypes of arthropod natural enemies, this question cannot be resolved.

The laboratory data indicate that the laboratory-selected strain of *T. pallidus* has an apparently normal capacity to diapause and that azinphosmethyl resistance can persist in laboratory populations for over 13 generations without selection, although resistance levels do decline in mixed populations of resistant and susceptible parasitoids held without selection. The field data indicate that azinphosmethyl-resistant *T. pallidus* can establish and persist in California walnut orchards. Long term studies, and other techniques, are required to determine whether, and how extensively, the azinphosmethyl-resistant strain will disperse and colonize additional walnut orchards in the Central Valley of California.
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FIG. 1. Stability of azinphosmethyl resistance in *T. pallidus* populations held with and without selection for 13 generations. Populations were tested with the standard bioassay every other generation. Line 1 shows survival of the Selected strain periodically selected with azinphosmethyl; line 2 shows mean survival ± SE of two Relaxed-Selected populations held without selection for 13 generations; line 4 mean survival of three populations obtained by mixing equal numbers of Selected-27 and Base (susceptible) parasitoids and holding them without selection; line 5 mean survival of two populations of the Base strain. Line 3 shows the rapid response of a subsample of the mixed (line 4) populations to selection with azinphosmethyl in generations 6 and 8.

FIG. 2. Mean percentage diapause (± SE) in the azinphosmethyl-resistant (Selected) strain of *T. pallidus* compared to the Base and Yuba colonies. Diapause was induced by rearing parasitized walnut aphids at 15-16°C or 13-14°C and 8L:16D.

FIG. 3. Percentage of diapausing *T. pallidus* from experiment 1 (held at 15-16°C, 8L:16D) that terminated diapause following return to conditions of 25°C, 75% RH and 16L:8D after being held at 4°C, 75% RH for 30, 60, 86, 114 or 142 days.

FIG. 4. Resistance levels in three colonies of *T. pallidus* (H1, H2, H3) collected from the Hanford walnut orchard after three applications of azinphosmethyl during 1990 compared to the Yolo (Y), Base (B) and Selected (S) laboratory colonies.
FIG. 5. Resistance levels in colonies of *T. pallidus* collected from the Hanford walnut orchard during 1991 compared to the Yolo (Y), Base (B) and Selected laboratory colonies, and the Red Bluff colony (RO) using the standard bioassay.

FIG. 6. Resistance levels in colonies of *T. pallidus* collected from the Gridley walnut orchard during 1990 compared to the Selected (S), Base (B) and Yolo (Y) laboratory colonies.

FIG. 7. Azinphosmethyl resistance levels in the *T. pallidus* colony collected from the Gridley walnut orchard (G) during 1991 compared to a colony collected from a Red Bluff walnut orchard (RO) before releases were made, and the Yolo (Y) and Selected (S) laboratory colonies.

FIG. 8. Establishment of azinphosmethyl-resistant *T. pallidus* indicated by survival of parasitoids collected in July, September and October from the Red Bluff release site (R1, R2 and R3, respectively) following the May 1991 releases of the azinphosmethyl-resistant strain, compared to the Red Bluff pre-release (R0), Yolo (Y) and Selected (S) colonies tested with the standard bioassay.
Figure 2: Mean percent entering diapause (±SE) at different temperatures for Select, Yuba, and Base strains.

- **Select**: Solid bar
- **Yuba**: Crossed bar
- **Base**: Cross-hatched bar

Temperature (°C):
- **15-16 °C**
- **13-14 °C**
Fig. 3

Percent terminating diapause vs. Chill period (days)

- Selected
- Yuba
- Base
Hanford

Percent survival

H1 S
H2 S

Collection date (1991)

July

R0 Y

September

R0 Y

Fig. 5
Gridley

Percent survival

G1

S

G2

S

June

October

Collection date (1990)
Figure 8: Percent survival of Red Bluff fish collected at different collection dates (1991).