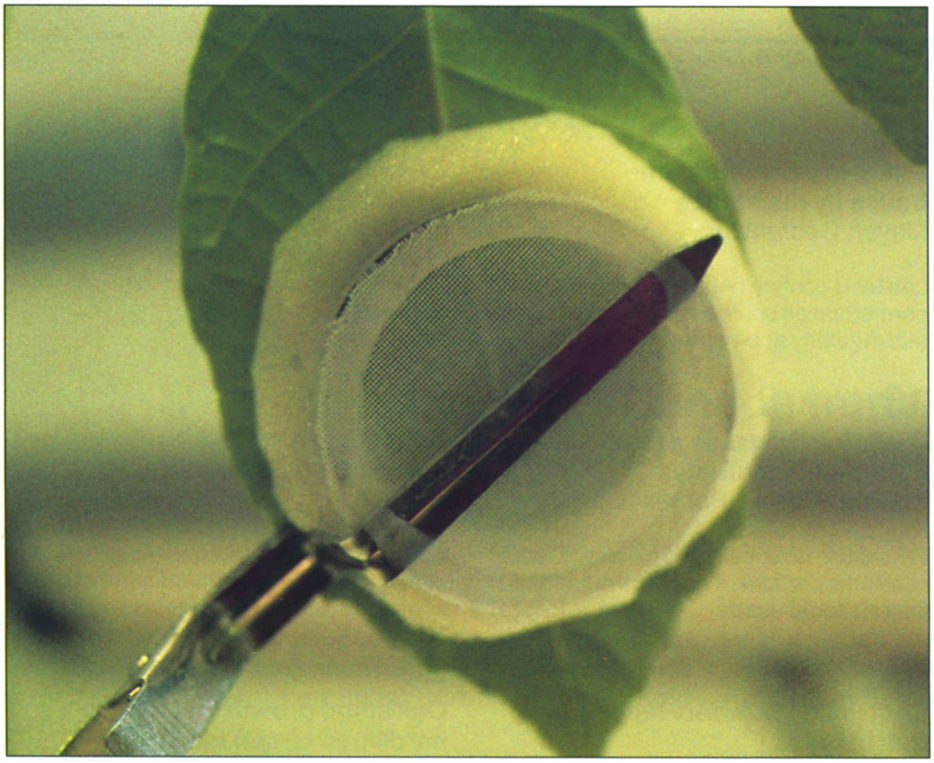

The aphid parasite's ability to survive on Guthion-treated foliage was tested in cages clipped to the undersides of walnut leaves.

Guthion-resistant strain of walnut aphid parasite

Marjorie A. Hoy ❑ Frances E. Cave

A laboratory-selected strain of the parasitic wasp Trioxys pallidus *is ready for field trials*

One of the challenges in integrated pest management (IPM) programs is integration of biological and chemical control. The incompatibility of the two types of control often results in pest resurgences, secondary pest outbreaks, and the development of pesticide resistance. Some of the problems can be overcome by using ecologically or physiologically selective insecticides to preserve natural enemies. The use of naturally or artificially selected pesticide-resistant strains of natural enemies can also enhance the compatibility of chemical and biological controls.

This approach may be effective in the control of walnut pests. The development of resistance to Guthion (azinphosmethyl) in the walnut aphid parasite, *Trioxys pallidus* Haliday, could allow growers to use this insecticide to control codling moth, *Cydia pomonella* (L.), or navel orangeworm, *Amyelois transitella* Walker, without disrupting the effective biological control of the walnut aphid, *Chromaphis juglandicola* Kalt., by *T. pallidus*. Use of Guthion has frequently been associated with secondary outbreaks of the walnut aphid.

During 1985 and 1986, we collected colonies of the aphid parasite from walnut orchards in California to determine whether variability in responses to Guthion existed in wild populations. Our goal was to develop a Guthion-resistant population of the parasite for use in walnut orchards.

Selection for resistance

The parasite colonies were reared on walnut aphids, which were maintained on potted walnut trees in the greenhouse at the University of California, Berkeley. We then tested the parasites with Guthion in the laboratory to obtain concentration/mortality lines for each colony. One-ounce clear plastic cups with plastic lids were treated with five to seven concentrations of Guthion in a 95 percent ethanol mixture and allowed to dry. Twenty *T. pallidus* adults were placed in each of five to ten cups per concentration. Survival was assessed after 48 hours, and data were statistically analyzed (probit analysis).

The five colonies varied in response to Guthion (fig. 1). Valley, a susceptible colony, was initiated from samples collected from Fresno, Tulare, and Kings counties. Los Banos, the most tolerant colony, was from Merced County. However, the colonies appeared to have only a slight tolerance to Guthion, as measured by this bioassay technique, when the concentrations used in the test were compared with those used in the field (shaded portion of fig. 1).

We selected *T. pallidus* colonies for Guthion resistance by placing adults in treated plastic cups, using an average of 1,900 parasites per selection (range, 700-4,975). Survivors after 48 hours were used to begin a new colony. After the first selection, a base colony was maintained for each selected colony, and 100 to 200 parasites were tested at each selection as a control. The Guthion concentration used for selection was increased whenever more than about 50 percent of the parasites survived.

All colonies responded to selection. After seven selections for the Aggregate colony, nine for the Los Banos, twelve for the Valley-Christensen, and three for the Yolo, all selected colonies but the Yolo colony were pooled. The base colonies were also pooled, and the pooled Base colony was tested as a control at each subsequent selection. The Yolo base colony was maintained as a susceptible comparison colony.

After the pooled Selected colony had been selected five times, the difference in LC_{50} values (concentrations lethal to 50 percent of the test population) between the Yolo strain and the Selected colony was approximately 7.5-fold (fig. 2). Using the plastic cup bioassay technique, however, the response to selection appeared to be modest and the resistance level inadequate for use in walnut orchards, because the concentration/mortality line for the Selected strain was still lower than the field rate.

Cage tests

Because the test we used with the plastic-capped cup could overestimate mortality of

the Selected strain of *T. pallidus*, we evaluated the Selected colony in three other tests designed to more closely mimic walnut orchard conditions. For two of these we used clip cages containing the parasite, which were attached to Guthion-treated walnut leaves. Each cage was clipped to walnut foliage so that the undersurface of the leaf formed the top of the cage. Parasites commonly spend time searching the undersurfaces of walnut leaves for aphids to parasitize.

Using this clip cage, we tested the ability of the Selected and susceptible (Yolo) colonies to survive on Guthion-treated walnut foliage on potted walnut trees (data not shown) and tested the ability of adult parasites to survive on treated and untreated foliage collected from a walnut orchard near Stockton, California. We also compared the ability of the susceptible and Selected strains to parasitize aphids on Guthion-treated potted trees placed in sleeve cages.

The walnut orchard used for the foliage test was treated with 1 pound 50WP Guthion per 100 gallons water on August 1, 1987. Foliage had been taken from the orchard before treatment to test for the presence of previously applied pesticides, as well as 3 and 17 days after treatment. Twelve leaves were removed from each of eight treated trees; four leaves were sampled from each of eight unsprayed trees on each sample date. Treated and untreated foliage was taken to the laboratory, where clip cages containing ten parasites each were attached. For each sample date, 960 Selected and 960 Yolo parasites were tested on the treated foliage and 320 of each strain on the untreated foliage.

Survival of the Selected and Yolo parasites was compared after 4, 8, 18, 24, 48, and 72 hours (fig. 3). The clip cage assay indicates that 26 percent of the Guthion-resistant strain of *T. pallidus* were able to survive for at least 72 hours on Guthion-treated foliage collected from a walnut orchard 3 and 17 days after treatment, compared with less than 6 percent survival of the susceptible Yolo strain. Mortality of both Selected and Yolo parasites was less than 8 percent on the untreated walnut foliage.

In a separate experiment to evaluate parasitization rates, we released 40 *T. pallidus* females into each cage (18 x 18 x 34 inches) containing a Guthion-treated (five replicates) or water-treated (six replicates) potted walnut tree. Guthion-treated trees had been sprayed to drip with 1 pound 50WP per 100 gallons of water before parasite release. Female parasites were removed after two hours and the trees held in the greenhouse until progeny emerged and were counted. The Selected females produced 6.7-fold more progeny than susceptible females did on Guthion-treated walnut trees.

This parasitization rate may be a conservative estimate of the ability of the Selected strain to parasitize aphids on Guthion-treated foliage because of the complete coverage obtained under these laboratory conditions and the limited time allowed to parasitize aphids (two hours). Coverage is likely to be less complete in the field, and the Selected strain's ability to parasitize aphids may be relatively greater the longer the parasites are on the Guthion residues.

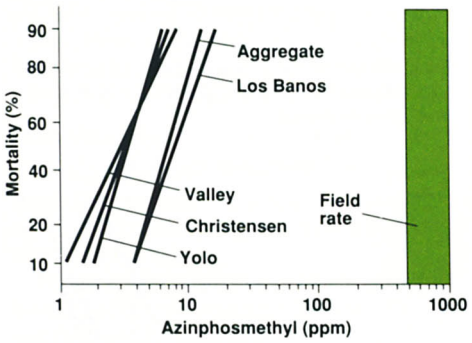

Fig. 1. Five *Trioxys pallidus* colonies from California walnut orchards varied in response to Guthion (azinphosmethyl). In the plastic-cup test, all appeared susceptible to rates well below field rates of 1.5 to 3.3 lb a.i./400 gal water.

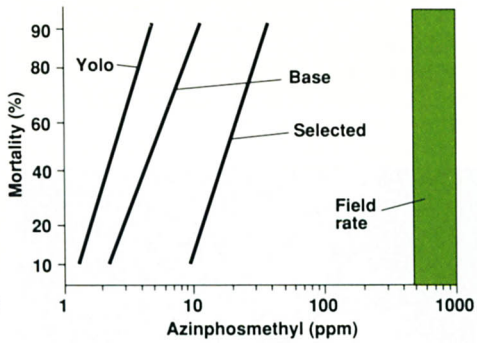

Fig. 2. The selected *T. pallidus* strain was less susceptible to Guthion than the susceptible Yolo and unselected Base strains, but resistance appeared inadequate by the plastic-cup bioassay technique used.

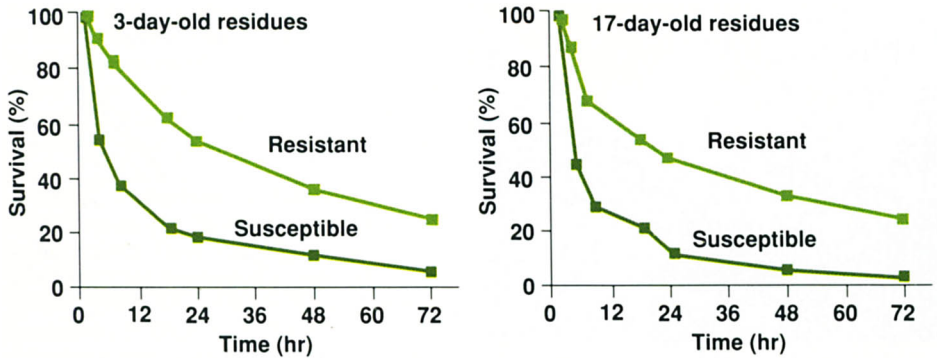

Fig. 3. In clip-cage tests on treated walnut foliage, which more closely resembled orchard conditions, 26 percent of the resistant strain survived field rates for at least 72 hours.

Conclusions

The different bioassay results lead to different conclusions regarding the ability of the Selected strain of *T. pallidus* to survive field rates of Guthion. The bioassays using the plastic cups suggest that the Selected strain is not sufficiently resistant to survive field rates of Guthion. However, this strain's survival on treated foliage from a walnut orchard and the ability of Selected females to produce 6.7-fold more progeny parasites than the susceptible strain on treated potted walnut trees indicate that field trials are justified.

Field trials this season will investigate whether the Selected strain can become established in a walnut orchard, persist, and survive Guthion applications applied to control codling moth or navel orangeworm. We will also monitor to determine if the Selected strain can overwinter in the walnut orchard and spread to surrounding walnut orchards. If the laboratory-selected strain meets these standards, it may be possible to increase the efficacy of *T. pallidus* in the walnut IPM program. Long-term establishment of a Guthion-resistant parasite strain could lead to more effective biological control of the walnut aphid, decreasing the pesticide applications needed to control walnut aphid when Guthion is applied to control codling moth.

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