where 450 to 500 *T. pallidus* (both sexes) were released into each cage. Parasites were then removed after 24 hours by an aspirator. Trees with the parasitized aphids were held in a cubicle in the greenhouse until mummies began to form. We estimated the number of parasites produced by counting mummies on half of both the tops and bottoms of all leaves of 27 of the 72 trees just before releases and then multiplying by two.

The release sites varied in size, tree varieties present, cultural practices, and pesticide applications made. The Hanford orchard contained Vina and Chico varieties; the Stockton orchard, Payne; the Colusa-North and Colusa-South blocks, Serr, Vina, Chico, and Tehama; and the Gustine block, Payne.

Guthion applications were made before releases at three of the sites: Hanford, at rates of 3 pounds (50WP) per 250 gallons per acre; Stockton, 2.2 pounds (35WP) per 95 gallons per acre; and Gustine, 3.5 pounds (50WP) per 250 gallons per acre. Because the Guthion-resistant strain of *T. pallidus* is cross-resistant to Supracide, we released the parasite into two sites treated with Supracide at 0.75 gallon 4E per 30 gallons per acre. Supracide was applied before parasite releases in the Colusa-North block and after parasite releases in the Colusa-South block. Parasites were released at the various sites on June 23 to 27.

Approximately 9,000 mummies were placed in the Stockton site, 1,800 in Gustine, 30,000 in Hanford, 12,000 in Colusa North, and 24,000 in Colusa South. Releases were made by placing potted trees in the Hanford and Stockton blocks or by stapling foliage to tree trunks at the Colusa North, Colusa South, and Gustine blocks and allowing the parasites to emerge. Releases were timed so that adult parasites should have emerged within 1 to 3 days after releases.

Monitoring

We collected *T. pallidus* from the release sites before releasing the resistant strain to learn whether the wild strain was resistant to Guthion. Parasites were also collected once or twice from each release or nonrelease block after the Guthion-resistant strain was released so that we could monitor establishment and dispersal into nearby nonrelease blocks. Foliage with aphids and/or mummies was taken to the laboratory where mummies were removed and placed in 1-ounce plastic cups with honey and held until parasites emerged. Leaves with aphids were placed on water-soaked cotton in plastic dishes and held at 80°F until mummies formed. After adults emerged, the parasites were counted and sexed, and used to initiate a new colony. Colonies were reared on potted walnut trees infested with walnut aphids. Progeny were tested to determine their tolerance of Guthion.

On each test date, Guthion (35WP) was dissolved in 95% ethanol to form a concentrated stock solution, from which a dilute solution of 50 ppm active ingredient was made. One-ounce plastic cups were filled with the solution, emptied after 5 seconds, and allowed to dry. Control cups were treated with 95% ethanol. Two pieces of black vinyl electrical tape were placed inside each cup and streaked with honey. Adult parasites that had emerged within the past 48 hours were placed in the test cups, which were covered with untreated mesh. Tests were conducted with 10 or 20 parasites (both sexes) per cup, and survival assayed after 24 hours.

Mature foliage in each orchard was sampled every 1 to 2 weeks to monitor the numbers of aphids and mummies. Ten leaves were sampled from each marked sample tree. Ten marked trees were sampled from both the release and adjacent nonrelease sites at Stockton and Gustine, and ten each in the Colusa North and Colusa South blocks. Fourteen marked trees were monitored in the Hanford release block (six release trees and eight sample trees), and eight trees in the nearby (300 feet away) nonrelease block at Hanford.

Survival and dispersal

Survival of wild *T. pallidus* prerelease colonies treated with 50 ppm Guthion in the plastic cup test ranged from 0 to 42% (table 1). The highest survival rate of wild parasites (42% at Gustine) was lower than that of the Selected (Guthion-resistant) laboratory colony (77% to 94%), indicating that clear differences existed between the wild parasites and the Selected colony.

The Guthion-resistant strain of *T. pallidus* became established in four of the five walnut blocks, survived field rates of Guthion or Supracide, persisted through the growing season, and dispersed to nonrelease sites about 300 feet away in two locations (table 1). It also survived applications of Zolone and Omite in the Hanford and Colusa blocks (fig. 1 and data not shown).

Hanford. Samples from the six release trees (fig. 1A) and from eight adjacent sample trees (fig. 1B) in the Hanford block indicated that *T. pallidus* was present through the growing season in the release block. The walnut aphid parasite was also present in the nearby nonrelease block through the growing season (fig. 1C), even though Guthion had been applied 2 weeks before release to all trees in both the release and nonrelease areas. Samples of parasites taken from both areas before release of the Guthion-resistant strain indicated the wild parasites were susceptible to Guthion (16% survival compared with 0 and 94% survival for the Base and Selected colonies, respectively, table 1).

Parasites collected from the release and nonrelease areas at Hanford on July 14 and October 11 were tested with Guthion. Survival was 81% and 80%, respectively, for parasites recovered from the release and nonrelease areas on July 14 and 58% and 69% for the release and nonrelease areas for the October 11 sample (table 1). The high survival rates indicate that the Guthion-resistant strain had become established and

TABLE 1. Survival of *T. pallidus* colonies collected from release (R) and nonrelease (NR) walnut blocks before (prerelease) and after release (postrelease) of Guthion-resistant strain

Sample site	Date collected 1988	No. initiating colony (R/NR)	% survival after 24-hour exposure[*]				
					Controls[◊]		
			R area	NR area	Pre-R	Base	Selected
Hanford							
Prerelease	Jun 24	18	—	—	16.1	0	94.0
Postrelease	Jul 14	3/1	81.1	80.0	22.9	0	92.5
Postrelease	Oct 11	52/47	58.0	68.9	28.0	0	88.7
Gustine							
Prerelease	Jul 23	28	—	—	42.0	0	94.0
Postrelease	Aug 15	6/—	26.4	—	34.0	14.1	90.0
Stockton							
Prerelease	Jun 23	46	—	—	0	0	94.0
Postrelease	Jul 7	16/—	72.5	—	3.0	0	77.5
Postrelease	Sep 12	7/7	46.4	35.0	8.9	7.8	90.5
Colusa-North block[§]							
Prerelease	Jun 27	46	—	—	2.0	0	60.0
Postrelease	Aug 16	107	61.3	—	5.5	14.1	90.0
Postrelease	Oct 12	53	52.0	—	11.1	5.7	85.5
Colusa-South block							
Prerelease	Jun 27	46	—	—	2.0	0	60.0
Postrelease	Aug 16	46	36.5	—	5.5	14.1	90.0
Postrelease	Oct 12	47	24.6	—	11.1	5.7	85.5

[*] Parasites (10 or 20, both sexes) were tested with 50 ppm Guthion by plastic-cup bioassay at 78°F under 16-hour daylength.

[◊] Controls were the Guthion-resistant (Selected) and unselected (Base) colonies, and the wild colony (pre-R) collected from each site before release of Guthion-resistant strain.

[§] Prerelease sample obtained from adjacent Colusa-South block.

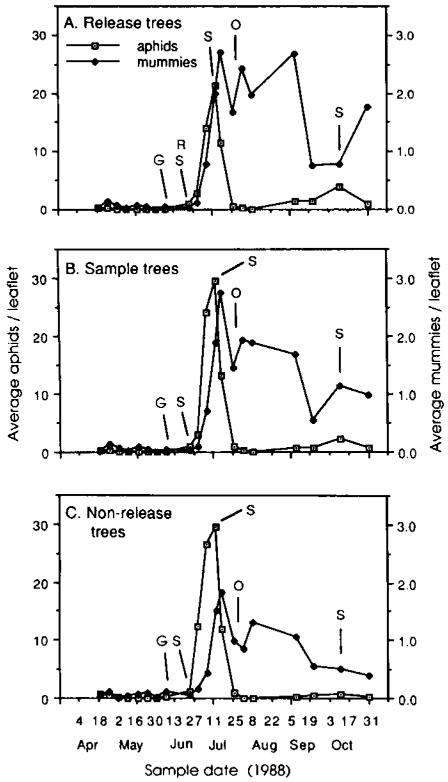

Fig. 1. Numbers of walnut aphids and *T. pallidus* mummies in release and sample trees in the Hanford release site (A, B) and adjacent nonrelease site (C) show the parasite persisted through the season. (G = Guthion 50WP 3 lb/ 250 gal/acre applied June 9; R = 30,000 parasites released June 24; S = parasites sampled for bioassays; O = Omite 30WP 7.5 lb/100 gal/ acre applied July 29.

persisted in the release area and had spread to the nonrelease area by July 14. The decline in survival on the second sample date could be due to intermixture with wild susceptible parasites or to sample errors.

Stockton. None of the wild parasites collected from the Stockton block before release survived 50 ppm Guthion in the prerelease assay (table 1). In contrast, the Selected colony had a 94% survival rate in this test.

Parasites recovered from the release block on July 7 and from both the release and adjacent nonrelease blocks on September 12 had higher survival rates than did wild parasites (table 1). The 72% survival rate of parasites collected on July 7 indicated that the Guthion-resistant strain had successfully survived and reproduced in the block. Parasite survival rates on September 12 (table 1) suggest that the resistant strain had become established, persisted through the growing season, and spread to the nonrelease block despite two Guthion applications (fig. 2).

Colusa North and South. We also recovered the Guthion-resistant strain of *T. pallidus* from the Colusa North and Colusa South blocks, where the parasites were exposed to Supracide rather than Guthion (table 1). They were also exposed to one or two applications of Zolone (phosalone).

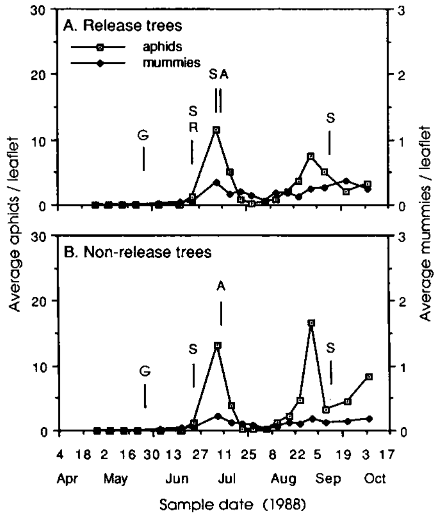

Fig. 2. In the Stockton release (A) and nonrelease (B) blocks, *T. pallidus* also persisted, despite two Guthion applications. (G = Guthion 35WP 2.2 lb/95 gal/acre applied May 24 and 50WP 2 lb/100 gal/acre July 9; S = parasites sampled for bioassays; R = 9,000 resistant parasites released June 23.

Parasites collected from the North and South blocks on October 12 had survival rates of 52% and 24%, respectively, on 50 ppm Guthion (table 1), indicating that they had persisted throughout the growing season. The two blocks provide an interesting contrast, because the resistant parasites were released into the North block after Supracide was applied. This should have selected for the resistant strain, but the fact that few aphids were present (data not shown) would have made establishment more difficult. By contrast, the resistant parasite strain was released into the South block before the Supracide application. In this case, wild *T. pallidus* were abundant. The released parasites could have interbred with the susceptible wild strain, and they would have had to compete with the wild strain for aphid hosts.

Gustine. Based on counts of aphids and mummies (data not shown) and the bioassay of parasites recovered, there is no evidence that the Guthion-resistant strain became established in the Gustine block (table 1). This failure could have been due to low aphid densities in the block throughout the growing season and the relatively low numbers (1,800) of parasites released.

Conclusions

The Guthion-resistant strain of the walnut aphid parasite *T. pallidus* survived on field rates of Guthion or Supracide, parasitized aphids, and persisted through the growing season in four of the five walnut blocks where it was released. It also dispersed to nearby walnut blocks in two sites. The resistant strain performed well in geographically distant commercial walnut blocks that differed in aphid densities, cultural practices, and pesticide applications. The laboratory-selected strain met the goals set for it during the 1988 growing season.

Genetic improvement of parasites and predators involves a series of steps. It is necessary to identify the trait needing improvement and to find variability for that trait so that selection can occur. It is highly desirable that the mode of inheritance and the fitness of the improved strain be known before field releases. In the case of *T. pallidus*, we did not know the mode of inheritance of the Guthion resistance, and fitness tests of the Selected and Base colonies have not yet been completed. However, no matter how fit the strain appears under laboratory conditions, the ultimate evaluation must occur under field conditions. To date, the Guthion-resistant strain has met the criteria that are essential if it is to be implemented in walnut IPM programs in California. The overwintering success of the Guthion-resistant strain is being monitored.

Implementing the Guthion-resistant strain will probably require its long-term establishment in commercial walnut orchards. At present, no commercial companies produce and sell *T. pallidus*. If mass-rearing is necessary, rearing techniques for this parasite will need to be improved for production to be cost-effective and reliable.

Should the Guthion-resistant strain overwinter successfully, its dispersal, impact on aphid populations, maintenance of Guthion resistance levels, and persistence over several seasons remain to be evaluated. Implementation will require additional information, but the first steps have been taken to integrate a genetically manipulated, pesticide-resistant parasite of the walnut aphid into walnut IPM.

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