PRUNE APHIDS: OVERWINTERING BIOLOGY AND BIOLOGICAL CONTROL

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INTRODUCTION

Aphids have become an important problem in prunes in California with the development of the Bt (*Bacillus thuringiensis*) spray program to replace in-season use of organophosphate sprays for peach twig borer (*Anarsia lineatella*). Two different aphid species cause damage to prune trees in the Central Valley: the mealy plum aphid (MPA), *Hyalopterus pruni;* and the leaf curl plum aphid (LCPA), *Brachycaudus helichrysi*. The mealy plum aphid develops large populations on the undersides of prune leaves in the spring and migrates from older orchards to cattails by mid June, but remains in young orchards all through the year. These populations sap tree vigor, slow the growth of young trees, cause splitting of fruit, and appear to lower fruit sugar content. The leaf curl plum aphid rapidly builds populations on new foliage in spring causing affected spurs to develop tightly curled leaves. The aphids migrate from the orchards to their summer host plants (Asteraceae) in May, but are also implicated in reducing tree growth and fruit sugar content and causing the splitting of fruit.

Traditional dormant oil sprays in prune orchards applied in December to February contribute to rain induced runoff of insecticides into the surrounding watersheds, and are considered by the State Water Resources Control Board as a significant source of nonpoint source pollution with regard to water quality. In addition, use of within-season organophosphate insecticide treatments often disrupts the natural control of the San Jose scale and mites, adding further to management costs. The need to develop alternatives to dormant oil sprays for the control of aphids in prunes has generated interest in the late season and winter phases of the life cycles of both MPA and LCPA. One alternative is to be able to treat aphid populations either in the fall before the dormant period, or in early spring after the dormant period but before the aphids hatch from their overwintering eggs. For fall treatment of aphids we have determined that the start of the return migration of male aphids is mid October, but it remains unclear how late in the season egg laying occurs in the orchards, information that would facilitate decisions regarding the timing of treatments for aphid control. For early spring treatment of aphids it is important to know the timing of egg hatch for both aphid species, and this could be predicted through experimental evaluation of the thermal requirements of post-diapause overwintering eggs.

In addition, as both of the aphid species are exotic insects, originating in Eurasia, they have not been kept under control in California by specialized insect parasitoids. The recent establishment of *Aphidius colemani* in the Central Valley has led to substantial levels of parasitism of LCPA and a general reduction in damage caused by this aphid species. In recent years, we have also imported and field released different biotypes of *Aphidius transcaspicus*, a parasitoid of mealy aphids from the Mediterranean for control of MPA. The success of this approach is dependent upon finding a biotype

that is compatible with MPA in California, adapted to climatic conditions in California, and effective in using cues presented by MPA for host location.

OBJECTIVES

1. To monitor the development of egg-laying oviparae of MPA and LCPA in orchards in the fall.

2. To estimate the timing of termination of diapause and subsequent thermal requirements for egg hatch in early spring for both MPA and LCPA.

3. To test the impact of a range of potential insecticides, for use in fall or early spring treatments of aphids, on mummies of *Aphidius transcaspicus*.

4. To continue to field release and monitor biotypes of *Aphidius transcaspicus* for control of MPA.

PROCEDURES AND RESULTS

Objective 1. To monitor the development of egg-laying oviparae of MPA and LCPA in orchards in the fall.

Background

One management option for the control of aphids in prunes is to break the life cycle of the aphids by preventing returning migrants from laying overwintering eggs on the twigs of the trees in the orchards. The life cycle of MPA and LCPA are typical of a holocyclic heteroecious aphid (Fig. 1). Overwintering eggs hatch in spring and a series of parthenogenetic generations take place on the prune trees before the aphids migrate to their summer host plants (early May for LCPA and mid June for MPA). The aphids then complete a series of parthenogenetic generations on the summer host plant before switching to the sexual phase of the life cycle in the fall. The sexual phase of the life cycle begins with the production of gynoparae, winged aphids that migrate back to prune orchards to give live birth to young nymphs that develop on the tree foliage into adult egg-laying oviparae. A couple of weeks after the gynoparae migrate back to the orchards, winged males are produced on the summer host plant and these males migrate back to prune orchards to mate with the adult egg-laying oviparae. Two management options for breaking the life cycle in the fall are (1) insecticide treatment, or (2) early defoliation of the prune trees using zinc sulfate to prevent the successful development of the egg-laying oviparae. In both cases, the timing for the management options is critical and dependent on a sound knowledge of the overwintering ecology of the aphids.

Methods

We monitored development of MPA through November in both 2004 and 2005 in a prune orchard in the Winters area to determine the age structure of the aphid populations and the likely timing of egg laying in the orchards. At weekly intervals 10 leaves with colonies of developing oviparae were placed into ziplock bags and returned to the laboratory for assessment of the numbers of individuals present in the successive instars. The numbers of winged male aphids

present on each leaf were counted, and the size of all nymphs was determined by measurement of the length of the hind tibia. Nymphs were then assigned to each of the five successive instars (four nymphal and one adult) assuming a linear increase in tibia length between instars. Thus the extreme range of observed hind tibia lengths was separated into five equal size classes to assign individuals to instars.

Results

In 2004, nymphal instars of oviparous MPA and winged males were observed on the foliage of the prune trees from Nov 10 through to Nov 24 (Fig. 2). These results suggest that although the return migration of male aphids begins in mid October, the majority of the oviparae are not maturing in the orchard until mid November, indicating that there is an extended interval in the fall when insecticide applications are likely to be successful in the disruption of egg laying in prune orchards.

On Nov 10, 2005, in the same orchard, there were far fewer MPA, but greater numbers of a less well known aphid the rusty plum aphid (*Hysteroneura setariae*). As temperatures were unusually warm this year all the oviparae had left the foliage by Nov 21, and eggs were evident on the twigs. This again confirms that for MPA, nymphal development of egg laying MPA takes place in early November, with oviposition peaking around mid November.

We were unable to find orchards with LCPA on the trees again this fall, despite visiting orchards that have a history of LCPA activity in spring. It remains unclear whether this is because LCPA is generally far less abundant than MPA, or whether the development of egg laying oviparae of LCPA occurs earlier in the year. Water trap catches of gynoparae of males of LCPA in previous years suggest a similar timing of the return flight for both MPA and LCPA, but the lack of direct observation of oviparae in prune orchards is puzzling. Even more so, as we found trees with oviparae of both rusty plum aphid and waterlily aphid (*Rhopalosiphum nymphaeae*) in November this year; two aphids that are also infrequent in orchards in spring.

2. To estimate the timing of termination of diapause and subsequent thermal requirements for egg hatch in early spring for both MPA and LCPA.

Methods

To determine the thermal requirement for egg hatch of MPA and LCPA, a large set of eggs were collected on cut twigs from an orchard in the Winters area on December 3, 2004. Small sections of spurs with eggs at bud bases were cut from the twigs collected and placed into Petri-dishes and held in an incubator at 1.6°C to allow chilling to bring about the termination of egg diapause. The eggs were removed from chilling on February 14, 2005 and for each aphid species five replicate batches of 25 eggs were placed at each of four constant temperatures (5.6, 9.7, 15.1 and 19.9°C) to await egg hatch. Eggs were monitored daily to note the number of eggs hatching on each day. The nymphs hatching from the eggs were also checked to ensure that they were MPA and LCPA respectively, rather than nymphs of any other aphid that may have laid eggs on the same twigs collected from the field. Linear regression was used to examine the relationship

between the rate of egg development and temperature, using the intercept on the x axis to estimate the lower threshold temperature for development and the reciprocal of the slope to estimate the thermal requirement for development.

The date when diapause of MPA eggs was terminated in 2005 was also estimated, as this defines the date after which temperatures above the threshold for egg development begin to accumulate. Termination of diapause was estimated for MPA by collecting spurs with overwintering eggs at regular two-week intervals from December 6, 2004 through to February 15, 2005. The spurs were trimmed and held in Petri-dishes as described above. For each sampling date, 8 sets of Petri-dishes containing 10 eggs each were held at 18°C to determine the median time from field collection to egg hatch for each Petri-dish, and the mean of the median times for each sample date. If eggs have not completed their diapause chilling requirement, they take far longer to hatch than would be expected from the thermal requirement for egg hatch. Thus the date of termination of diapause can be estimated from the collection date at which the actual degree day accumulation for egg hatch matches the estimated thermal requirement (see above).

Results

The thermal requirements for egg hatch for MPA and LCPA are presented in Fig. 3. Fitting a linear regression to the data on rate of development in relation to temperature, we estimate the threshold temperature for development of MPA to be 1.3°C, and that for LCPA to be 3.9°C. The reciprocal of the slope of each regression provides an estimate of the thermal requirement for egg hatch of 244°D(C) for MPA, and of 79°D(C) for LCPA. By collecting the eggs from the field in early December, 2004 and storing them at a temperature that is close to or below the threshold for development for 2 months, we were able to complete the diapause requirement for the eggs and to accurately assess the thermal requirement for egg hatch of both MPA and LCPA.

The degree day requirement for egg hatch of MPA eggs when collected at regular two week intervals over the winter of 2004-05 are shown in Fig. 4. Eggs collected on Dec 6 required 763 °D(C) before hatching, which is clearly well above the estimated thermal requirement for egg hatch of 244 once diapause is terminated. The time taken for eggs to hatch decreased linearly until the Feb 1 sample date, when the time taken to hatch in the lab once collected from the field was rather less than the estimated thermal requirement for egg hatch. This indicates that egg diapause was completed in the Winters area in 2004-05 between the sample dates of Jan 18 and Feb 1. Thus for the last two sample dates, diapause had already been completed in the field and the eggs had already begun to accumulate temperature toward egg hatch before being collected. To better estimate overall average date of termination of diapause in Winters we can then use the final sample date of Feb 15 to work backwards from the thermal accumulation in the lab once collected to match the estimated total thermal requirement for egg hatch (Fig. 3) based on temperatures that the eggs experienced in the field before collection. Thus for the eggs collected on Feb 15, the thermal accumulation to egg hatch in the lab was only 61°D(C), indicating that the difference (244 - 61 = 183) was accumulated already in the field before being collected. Working back from the sample date of Feb 15, the difference of 183°D(C) above a threshold of 1.3°C would have accumulated from a start date of Jan 25, the estimated date of termination of egg diapause in the field. Working forward from Jan 25 with field temperatures above 1.3°C we

can then estimate that 244°D(C) would have been reached on Feb 18 in the Winters area, suggesting that the peak of egg hatch for MPA in this region would have occurred at that time in 2005.

3. To test the impact of a range of potential insecticides, for use in fall or early spring treatments of aphids, on mummies and emerging adults of *Aphidius transcaspicus*.

Methods

Although diapausing mummies of *A. transcaspicus* would be the most appropriate experimental subject for testing the impact of dormant or delayed dormant insecticide treatments, the long duration of diapause development adds to the difficulty of effectively monitoring the impacts of the insecticides. In view of this constraint, non-diapause mummies which have only a short interval before adult emergence were used. In view of the experiences of earlier studies with aphid parasitoids, the insecticides were applied through dipping rather than contact spraying to provide more consistent results. From the surprising results of these tests in 2004, we repeated the test of these products again in 2005, paying particular attention to maintaining a pH below 4 for Imidan through use of Trifol buffer. The same four insecticides were tested, with 100% rates of 4.8 oz/ 100 gallons for Asana XL, 15 gallons/ 100 gallons for Omni oil, 4.25 lb/ 100 gallons for Imidan 70W, and 2 pints/ 100 gallons for Diazinon 4E. In each case the full rate of 100% was tested together with a 50%, 10% and 0% (water control) rate.

Ten replicate sets of leaves with 10 parasitoid mummies each, standardized in age to 2-3 days old, were dipped for 3 secs in each of the treatment materials, allowing the material to run off and the leaves and mummies to air dry. Once dry, individual mummies were cut out from the leaves, leaving only a very small piece of leaf attached to minimize the surface of insecticide residue, and each set of 10 mummies was placed into a glass vial and kept at 20°C and a 16h photoperiod to allow emergence. Emergence and the sex of the emerging adults were monitored daily, and mummies removed from the vials as the adults emerged.

Results

The percentages of wasps emerging form the treated mummies were corrected for mortality rates occurring in the control treatments using Abbots formula. The resulting percentage emergence for the four insecticides is presented in Fig. 5. Omni oil had the greatest lethal effect on mummies of *A. transcaspicus*, resulting in very low emergence at all rates tested. Diazinon had a greater lethal impact than Asana, with only the 10% rate allowing the same levels of wasp emergence as Asana, and all rates of Asana having a similar impact. As in our earlier tests, Imidan had no lethal impact on *A. transcaspicus* at any of the rates tested, even with the pH more rigorously controlled. We will follow up on these simple tests with others focusing on application of the same products to adult wasps rather than mummified aphids, as it is possible that direct application to adults would show a different pattern of mortality. However, as the mummy stage of aphid parasitoids represents almost one half of the generation time, insecticides that are compatible with this stage of the life cycle are of particular significance in developing effective IPM strategies for aphid management.

4. To continue to field release and monitor biotypes of *Aphidius transcaspicus* for control of MPA.

Methods

In May 2005, a survey was made of the occurrence of mealy aphids on *Prunus* trees in Israel, Crete and Cyprus. These regions were selected for their higher summer temperatures and low winter rainfall, more similar to those found in the Central Valley of California, in comparison to previous collections from eastern Spain. Both aphids and mummies were collected from a total of 24 locations in these regions and were sent to the quarantine facility at UC Berkeley for parasitoid rearing and analysis of host tree specific and geographic variation among mealy aphid populations in the Mediterranean.

Field releases of parasitoids of the almond biotype of *Aphidius transcaspicus* collected from mainland Greece in May 2003, and of new almond biotypes collected from Israel and Crete in 2005 were carried out in prune orchards in the Sacramento Valley in June and July 2005. All parasitoids that were field released this year were reared on MPA on potted *Phragmites* rather than from black bean aphids on potted beans. Although it is more difficult to produce parasitoids continuously on MPA it avoids the problem encountered in 2004, when parasitoids reared on black bean aphids began to reject MPA as suitable hosts. Some of the parasitoids were released into sleeve cages on aphid infested branches where parasitism could be more closely monitored, while other were released openly into the orchards to allow the parasitoids greater freedom of movement in selecting trees and resting sites.

Results

In contrast to our experiences in 2004, we were able to get live parasitoids back to our quarantine facility at UC Berkeley from all three locations surveyed this year. Despite increased security measures applied to the importation of natural enemies over the intervening year we were able to obtain a special permit from USDA to hand carry aphid parasitoids back to California. Thus we now have three new colonies of *Aphidius transcaspicus* collected from Israel, Crete and Cyprus. We have also just received new information from USDA that biological control importations into the U.S. will be facilitated in the future, and thus we should be able to avoid the disasters of 2004 in which all parasitoids died in transit.

Fewer parasitoids were field released this year in comparison to previous years, due to the lower production of parasitoids from MPA on *Phragmites* and due to the earlier migration of aphids from prune orchards in 2005. A total of 1,550 parasitoids were field released during the 2005 field season (Table 1). The majority of the parasitoid releases this year were of two of the new almond biotypes collected from Israel and Crete, but releases were also made of the almond biotype from mainland Greece collected in 2003. Unfortunately, this latter biotype did not performed well in sleeve cages and so we concentrated on the new biotypes for both sleeve cage trials and open field releases. Of the new biotypes, the parasitoids from Crete appeared a little

more aggressive than those from Israel, but both performed well in the sleeve cage trials producing good numbers of mummies.

CONCLUSIONS

The need to develop alternatives to dormant oil sprays for the control of aphids in prunes has generated interest in the late season and winter phases of the life cycles of both MPA and LCPA. While male aphids begin their return migration into prune orchards in mid October, observations this season have again confirmed that egg-laying oviparae that are produced by the return migration of gynoparae develop through to the adult stage in mid November. This indicates that fall treatments for the control of aphids on foliage could be as late as early November and still have a substantial impact on overwintering aphid populations. From chilled field collected eggs the thermal requirement for egg hatch, after completion of diapause, was estimated to be 244°D(C) above 1.3°C for MPA, and 79°D(C) above 3.9°C for LCPA. In addition, regular field collections of MPA eggs from the Winters area indicated that the termination of egg diapause occurs around Jan 25 and we estimated that the peak of egg hatch in this region in 2005 was around Feb 18. We are currently monitoring the corresponding termination of egg diapause for LCPA this winter. These data will be very helpful for understanding the timing of sprays for aphids in spring

In addition to possibilities to develop acceptable treatments for aphids in the fall or early spring, the most cost-effective way to manage aphids in prunes, as invasive pests, is to establish effective biological control. An analysis of the impact of four insecticides that could be used for aphid control in fall and spring applications on survivorship of parasitoids within aphid mummies, and their subsequent adult longevity produced some interesting results. Surprisingly, Omni oil had the greatest detrimental effect, whereas there was no measurable impact from Imidan, with Asana and Diazinon falling in between. Thus Imidan would clearly be the product of choice with regard to compatibility with aphid parasitoids. Previous tests of the temperature and humidity tolerances of two biotypes of *A. transcaspicus* clearly indicated a better climatic match with the Central Valley for parasitoids from Greece versus Spain, indicating that future collections from the eastern and southern Mediterranean would provide a better climatic match. Our collections of parasitoids this year consequently focused on the eastern Mediterranean, and the biotypes collected not only show better climatic matching, but also appear to be more aggressive in the attack of MPA than those collected previously from the western Mediterranean. Finally, the rearing of parasitoids on MPA on *Phragmites* has proved necessary to ensure their fidelity to MPA, but has at the same time proved to be more difficult than our previous rearing on black bean aphid on beans, with a consequent reduction in the number of parasitoids that we can produce for filed release. We are currently exploring other alternate aphid hosts, with a focus on grass aphids that are more closely related to MPA, in the hope that we can increase parasitoid production for field release in the future without compromising host preference.

Parasitoid Strain	Open	Sleeve	Mummies
Almond, Israel 2005	700	100	73
Almond, Crete 2005	500	100	120
Almond, Greece 2003		150	0
Total	1,200	350	0

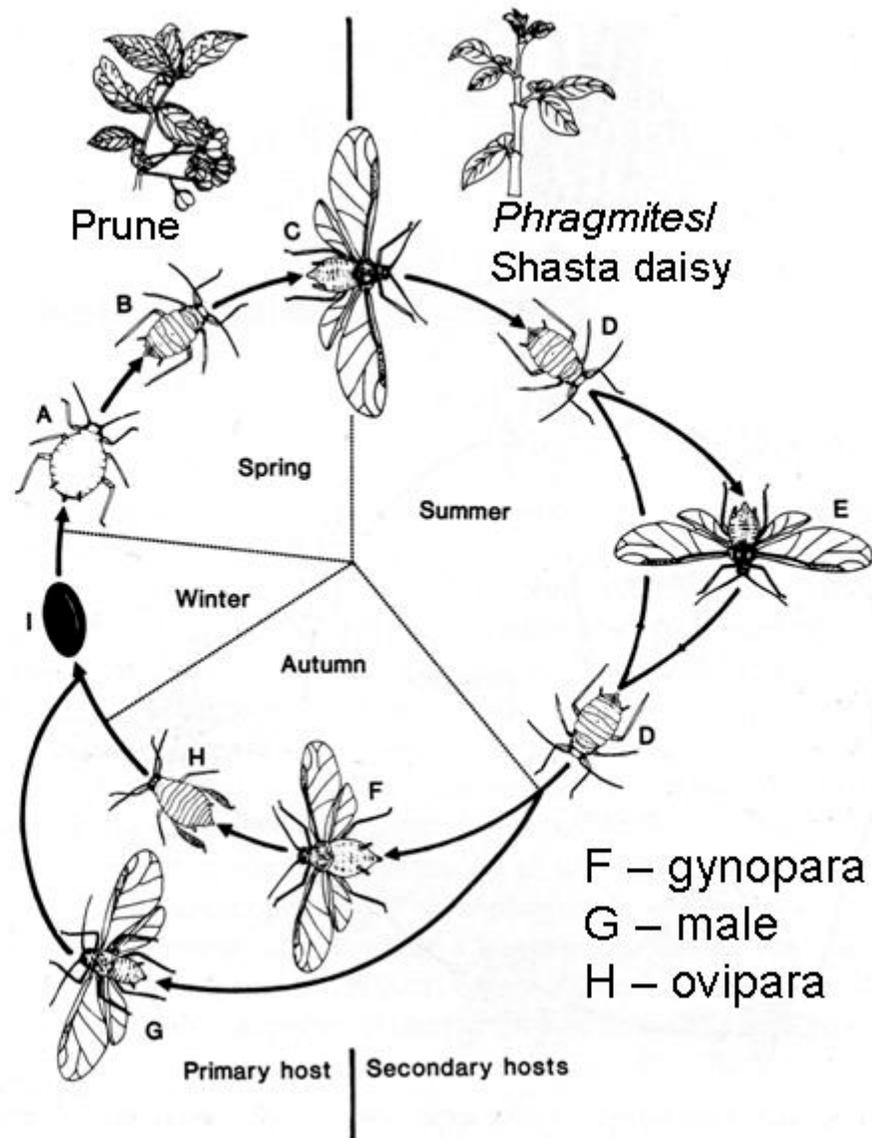Table 1. A summary of the releases and recoveries of parasitoids in prune orchards in 2005.

Fig. 1. A schematic diagram of the life cycles of the mealy plum aphid, that migrates between prunes and reeds or cattails, and of the leaf curl plum aphid, that migrates between prunes and composite plants such as Shasta daisy. The asexual stages of the life cycles are labeled A-D, while the sexual stages are labeled F-H.
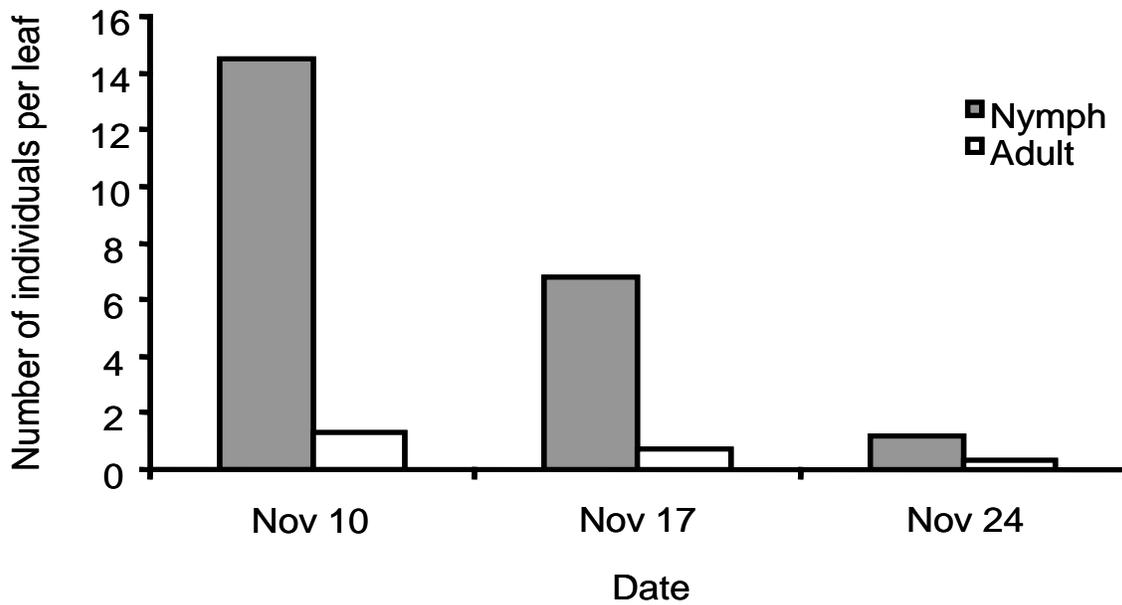
Fig. 2. The decline in the number of nymphs of the egg-laying oviparae as they develop through to the adult stage and adults move from the leaves to the twigs in an orchard near Winters in November, 2004.
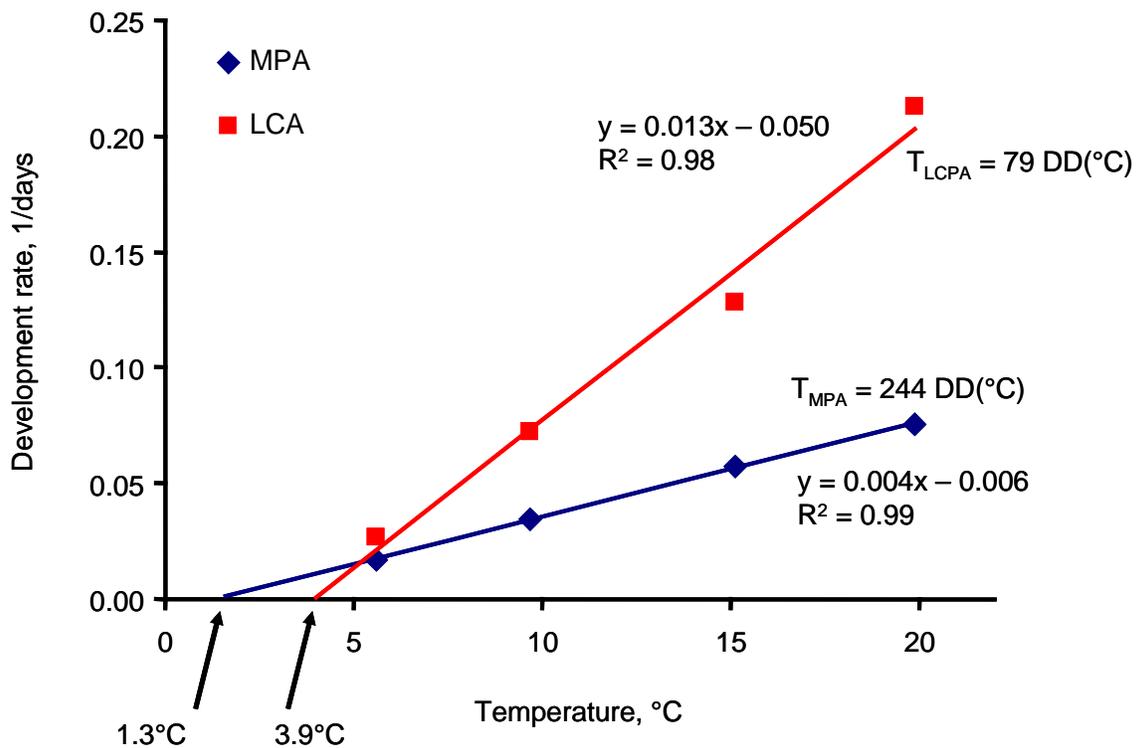

Fig. 3. Estimation of the threshold temperatures and the thermal requirements for development of MPA and LCPA eggs collected from Winters on December 6, 2004 and chilled at 1.6°C until February 14, 2005.
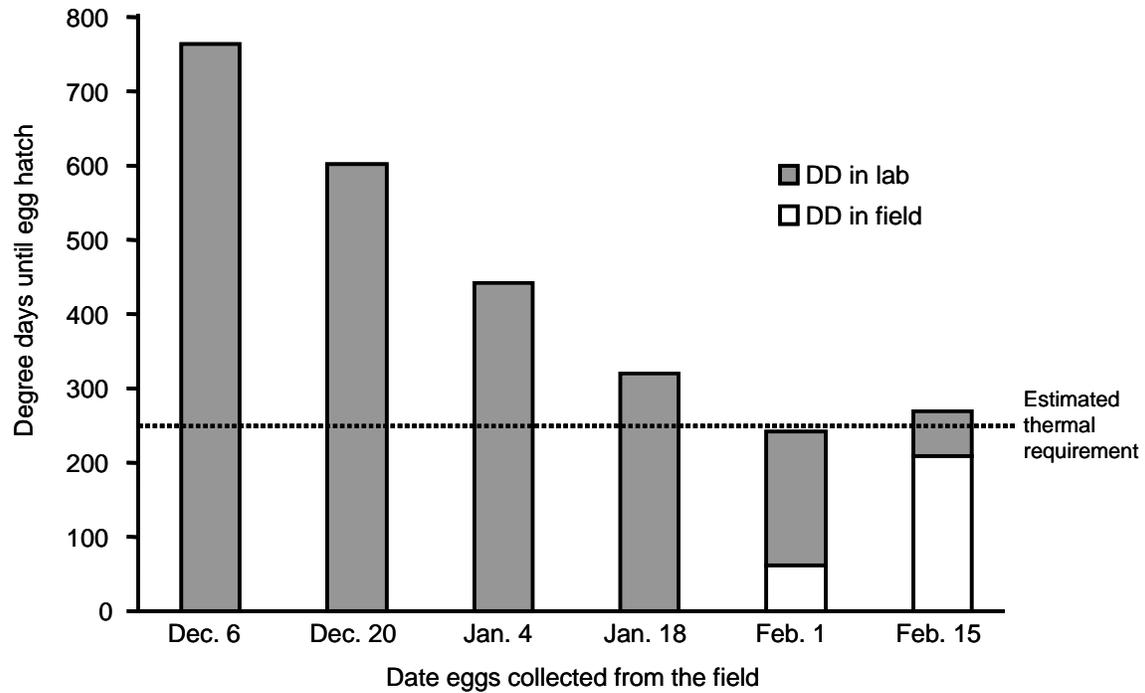
Fig. 4. Estimation of the date of termination of egg diapause for MPA eggs collected from Winters at regular intervals through the winter of 2004-05. The estimated thermal requirement for egg hatch (from Fig. 3) is presented as a horizontal dotted line.
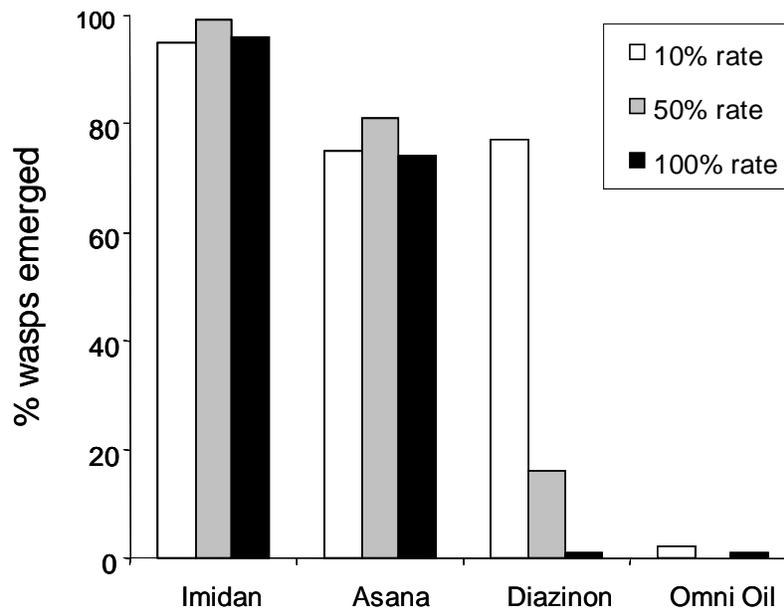

Fig. 5. The percentage of wasps emerging from aphid mummies treated with different low rates of insecticides, with emergence corrected for control mortality using Abbots formula.