# BIOLOGICAL CONTROL OF *LYGUS HESPERUS* WITH INUNDATIVE RELEASES OF *ANAPHES IOLE* IN A HIGH CASH VALUE CROP

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## ABSTRACT

The western tarnished plant bug (WTPB) *Lygus hesperus* Knight is a major pest in strawberries on the central coast in California. Nymphs and adults feed on the fruits, and as a result, the fruits are distorted or 'cat-faced', and are not acceptable in the fresh market. We evaluated the potential for inundative biological control of WTPB with the native *Anaphes iole* Girault in strawberries on the central coast in California. Being a high cash value crop, inputs into strawberry production are high, and an expensive control strategy such as inundative biological control has a high likelihood of being adopted if the program is effective. In a trial conducted in strawberry fields, we released 15,000 *A. iole* in acre-sized plots every week at three sites, and observed a 64 % suppression of WTPB. In comparison, with the application of insecticides, growers achieved 44.7 % reduction in WTPB. In a separate trial that was conducted at four sites the following year, we modified the release strategy and doubled the release frequency to determine if parasitism levels could be increased substantially. While we achieved 51.1 % reduction in WTPB with weekly releases, the semiweekly release provided only an additional 9.5 % reduction. Given that earlier research has shown that plant-related factors affect the performance of *A. iole* in strawberries, further modification of release numbers or timings are not likely to increase the level of suppression. Since the release program appears to be provide 64 % or less suppression, we need to integrate inundative biological control of WTPB in strawberries with additional tactics aimed at reducing nymph densities. Integration with selective insecticides is an option if parasitoid release timings can be adjusted to reduce the negative impacts of insecticide residues.

## INTRODUCTION

The native western tarnished plant bug (WTPB), *Lygus hesperus* Knight, is the most predominant and damaging species of *Lygus* in the Southwestern United States (Clancy and Pierce 1966). It is closely related to the tarnished plant bug (TPB), *Lygus lineolaris* Palisot de Beauvois, which is dominant in the east and the south. It is polyphagous and is reported to feed on 117 non-crop and over 25 cultivated plants (Schwartz and Foottit 1998). The adults are often nomadic, moving from one plant to another as each plant species begins to flower (Fleischer and Gaylor 1987). Breeding host plants of the WTPB are primarily species of Asteraceae, Brassicaceae, Chenopodiaceae, Fabaceae and Polygonaceae (Scott 1977; Schwartz and Foottit

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(1998). Among cultivated crops, WTPB is known primarily as a pest of cotton (*Gossypium hirsutum* L.; Malvaceae) and seed alfalfa (*Medicago sativa* L; Fabaceae) (Jones and Jackson 1990). It is, however, also a major pest in strawberries (*Fragaria x ananassa* Duchenese; family Rosaceae) in California (UCIPM 1994).

In the central coastal regions of California, WTPB over-winters in diverse plant species including several that occur as weeds (Romney and Cassidy 1945, Clancy and Pierce 1966, UCIPM 1994). When the rains cease in late spring, adults disperse from senescing weeds to strawberries. Typically WTPB colonizes strawberries in early April, causes extensive damage to the fruits, and rapidly acquires pest status. Two generations develop between April and August in strawberries and hence short day, day neutral and second year strawberries are all susceptible to colonization by this important pest. The strawberry fruit is unusual in that it is an aggregate of several hundred achenes or one-seeded fruitlets around a fleshy receptacle (McGregor 1976). Feeding by WTPB on the achenes prevents growth of the fleshy receptacle and causes the fruits to be distorted or 'cat-faced' (Allen and Gaede 1963; UCIPM 1994). Strawberries do not appear to be a preferred host, and densities of WTPB are lower in strawberries compared with other crops such as alfalfa. However, damage caused by WTPB is very visible and the economic threshold is low since cat-faced fruits are not accepted in the fresh market

We are evaluating the potential for inundative biological control of WTPB in strawberries in the central coast in California. Inundative biological control programs tend to be expensive due to the high numbers of natural enemies that need to be released. However, the strawberry industry in California is one of the few industries that can support such programs. California accounts for more than 80% of fresh market and processed strawberries grown in the United States and almost half of California strawberries are produced on the central coast. The estimated annual value of California strawberries is over $700 million (CASS 2000). Not surprisingly, biological control is already an accepted and established pest management strategy in strawberries in California. For years, augmentative releases of the predatory mite, *Phytoseiulus persimilis* Athias-Henriot, have been made in California strawberries for suppressing populations of the two-spotted spider mite, *Tetranychus urticae* Koch (UCIPM 1994).

The parasitoid with potential for use in an inundative biological control program for WTPB is the native *Anaphes iole* Girault. In the southwestern US, parasitism by *A. iole* has been observed on several native host plants (Romney and Cassidy 1945, Clancy and Pierce 1966, Jackson and Graham 1983, Graham et al. 1986, Gordon et al. 1987). It attacks the eggs of WTPB and hence has the advantage that it can suppress the pest before damage can occur by nymphs feeding on the fruits. To determine the potential for use of *A. iole* for biological control of WTPB in strawberries, an initial cage study was conducted (Norton et al. 1992). When A. *iole* were exposed to strawberry plants infested with WTPB eggs, parasitism levels of up to 80% were observed. Subsequently, in a field study conducted in conventional strawberries (Norton and Welter 1996), a release rate of 5000 *A. iole* / week / acre (0.4 ha) provided 19% WTPB suppression whereas a release rate of 15,000 / week / acre provided a 43 % reduction in WTPB.

In the present study we repeated the field trial, and compared the impact of the parasitoid release with that of standard insecticide control. A second trial was conducted the following year to determine whether an increase in release frequency would result in an increase in WTPB suppression. Norton and Welter (1996) observed that parasitism rates that averaged 60% for the first 48 hours after release dropped to less than 5% after 96 hours, and were indistinguishable from non-release control plots. Potential explanations for this observation might be either that the parasitoids are dispersing from the plots or field, or the parasitoids may have a short longevity under field conditions. If significant parasitism by *A. iole* only occurs during the first 3 days, a change in release frequency might be beneficial. Increasing release frequency from once a week to twice a week, but at half of the original release rate, would functionally maintain the major cost of parasitoid purchase at the original rate, but would increase the effective temporal coverage by the parasitoid.

In this paper we present results of the parasitoid release strategies that we evaluated for WTPB suppression on the central coast in California. We review possible limitations in parasitoid performance in strawberries and the potential for integrating inundative *A. iole* releases with insecticide applications for enhancing suppression of WTPB in strawberries.

## MATERIALS AND METHODS

Inundative biological control of WTPB with *A. iole* was evaluated in conventional fields of strawberries on the central coast in California. Since WTPB eggs are inserted in strawberry tissues, direct estimation of egg parasitism after *A. iole* release was not possible. The impact of the inundative releases was hence determined by comparing densities of early instars of WTPB in parasitoid release and non-release areas using protocols described by Norton and Welter (1996).

In the first trial, the following three treatments were compared:

1. Control: no release of parasitoids; no insecticide sprays for WTPB control.
2. Conventional: no parasitoid releases but application of insecticides by growers following the appearance of WTPB nymphs in the field.
3. Weekly-Release: release of *Anaphes* @15,000 per acre per week.

The experiment was replicated at five sites using a randomized block design. At each site, acre-sized plots were assigned to one of the above three treatments while blocking for site effects. The plots were separated by at least 150 m to minimize the effect of parasitoid movement between plots. One site was located in Salinas, one in Prunedale and three in Watsonville, CA. At all sites an annual planting system was used and day-neutral varieties were planted. Plantings were made in late fall of the previous year and fruiting commenced in March-April. At Salinas, Prunedale, and one site in Watsonville, the fields were planted with the cultivar 'Selva' while at the second site in Watsonville the cultivar planted was 'Commander'.

In treatment and control plots fungicides were sprayed for control of powdery mildew, gray mold, common leaf spot or anthracnose. The Bug-vac, which is a large vacuum device designed to physically remove WTPB (Zalom et al. 1993), was used at the Salinas site and one site in Watsonville but vacuuming was always done prior to release of the parasitoids. Insecticides for WTPB control were applied in conventional plots.

In a second trial conducted the following year, a Semiweekly-Release strategy was also evaluated in addition to the original three treatments described above. In the Semiweekly-Release plots, the parasitoid release rate was maintained at 15,000 / acre / week but the release frequency was doubled. As such, 7,500 adult *A. iole* were released twice a week with an interval of 3-4 days. The experiment was conducted at 4 different sites, one in Salinas, one in Prunedale and two in Watsonville. 'Selva' strawberries were planted at the sites in Salinas and Prunedale, while 'Camarosa' was planted at one site in Watsonville and 'Swede' at the second site in Watsonville. As before, there was one replicate for each treatment at each of the four sites resulting in a total of 16 plots.

*Anaphes iole* used in the study were obtained from the commercial insectary Biotactics, Inc. (Perris, CA), where they were reared on WTPB using the protocols developed by Patana & Debolt (1985) and Jones & Jackson (1990). Parasitoids were received as adults and maintained at 12°C before release. In the first trial, fifteen vials (5.2 x 2.5 cm) containing ca. 1000 parasitoids each (over 90% estimated to be females) were distributed at random locations in each

Weekly-Release plot every week. Each vial was placed in the shade at the base of a plant and supported by the petioles with the opening of the vial pointed upwards. In the first trial, parasitoid releases were made from the beginning of April till the end of August. In the second trial, the additional treatment consisted of 7 vials containing 1000 and one vial containing 500 parasitoids being distributed in the Semiweekly-Release plots twice a week with an interval of 3-4 days. Therefore, the total number of *Anaphes* released every week was kept constant at 15,000 in both the Weekly-Release and Semiweekly-Release plots, but the duration between releases were different. *Anaphes* releases were made from the first week of April for eight weeks. Subsequently, there was a decline in production of *Anaphes*, and adequate numbers were not available for continuing the releases. Hence the experiment was suspended at all four sites.

Impacts of the parasitoid releases were determined by sampling for WTPB nymphs on a weekly basis. In each plot, at 10 random locations, 10 consecutive plants were beaten into a white pan and the numbers of 1-$2^{nd}$ instars were recorded. First and second instars were used since these instars reflect the immediate effects of the parasitoid on egg hatch rates. In both trials sampling commenced during the first week in April but it continued for 20 weeks in the first trial and for eight weeks in the second.

To determine the impacts of the parasitoid releases, densities of 1-2nd instar WTPB nymphs were compared across the plots in each trial. The assumptions of the parametric ANOVA were not met hence the data were analyzed using the nonparametric Friedman's test. The data within each block were assigned ranks and $\chi^2$ was calculated (Zar 1984). A multiple comparison analysis applicable to ranked data in a randomized block, similar to the Tukey multiple comparison test, was used to separate means that differed significantly at the $\alpha = 0.05$ level (Zar 1984). In the first trial, the experiment was suspended at two of the five sites in mid June since the fields were sprayed with insecticides. Data from these sites were not included in the analysis.

## RESULTS

In the first trial, sampling commenced in April but for 8 weeks few WTPB adults were observed. WTPB nymphs started to appear in June and by mid July, an average of 0.42 nymphs /plant was observed in the Control plots (Fig. 1A). When WTPB densities started to rise, the insecticides naled or malathion were sprayed in the Conventional plots. Current recommendations for treatment thresholds of WTPB in strawberries are very low at 0.1 nymph per plant (UCIPM 1994). At two sites in Watsonville, over 1 WTPB nymph / plant was observed in mid July in the Control plots. Due to the extensive damage caused to the fruits, insecticides were applied to the Control plots and the experiment was terminated at these two sites. At the remaining three sites, WTPB densities differed significantly across the treatments ($\chi^2 < 0.001$). There were more WTPB nymphs in the Control plots than in the Conventional and the Weekly-Release plots (Fig. 1B). The Conventional and Weekly-Release plots did not differ in WTPB densities. Overall, there was 64 % reduction in WTPB nymph populations in the Weekly-Release plots compared to the Control plots while the Conventional plots had 44.7 % fewer WTPB compared with the Control plots.

In the second trial, adult WTPB were first observed on April 9, $1^{st}$ instar nymphs were first observed on May 10 and thereafter WTPB populations increased rapidly (Fig. 2A). Analysis of the data indicated that WTPB densities differed significantly across the treatment ($\chi^2 < 0.001$). More WTPB were observed in the Control plots compared with the other three plots that did not differ from each other. Overall, in the Weekly-Release plots, there was 51.1 % reduction in WTPB nymphs compared to the Control plots (Fig 2B). In the Grower plots, where the insecticides naled, malathion or fenpropathrin were sprayed for WTPB control, there was a 58.8

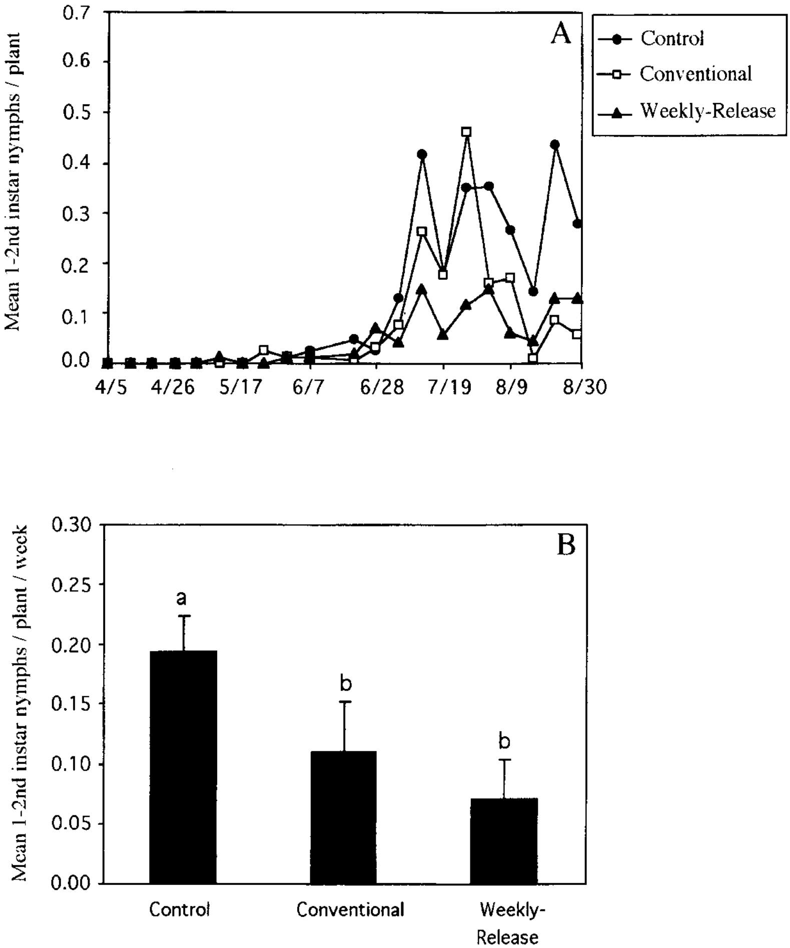



FIG. 1. Comparison of the impacts of inundative releases of *Anaphes iole* with insecticide control of *Lygus hesperus* in strawberries. A. Weekly densities of WTPB in experimental plots. In the Weekly-Release plots, *A. iole* were released @ 15, 000 / acre / week. B. Overall densities of *L. hesperus* nymphs in experimental plots.

% reduction in WTPB nymphs compared to the Control plots. WTPB suppression in the Semiweekly-Release plots was 9.5 % higher than that achieved in the Weekly-Release plots.

## DISCUSSION

Colonization of strawberries by WTPB in the first trial was unusual. Typically WTPB adults migrate to strawberry fields from neighboring weeds in April, and egg hatch occurs in May. This first generation is then followed by a second generation with a higher density compared with the first. In the first trial, we did not observe the typical first generation of WTPB in strawberries. Adult WTPB first appeared in strawberries in April but very few adults were recorded until June. We hypothesize that the extensive rain in that year kept the foliage of the surroundings weedy plant species suitable for WTPB through spring. When the rains ceased and the weeds dried in late spring, WTPB migrated to strawberries. Therefore, the predicted immigration in the spring never really occurred to any great extent. This resulted in delayed colonization of strawberries with a single generation of WTPB. During the second trial conducted the following year the typical pattern of spring WTPB migration to strawberries was observed.

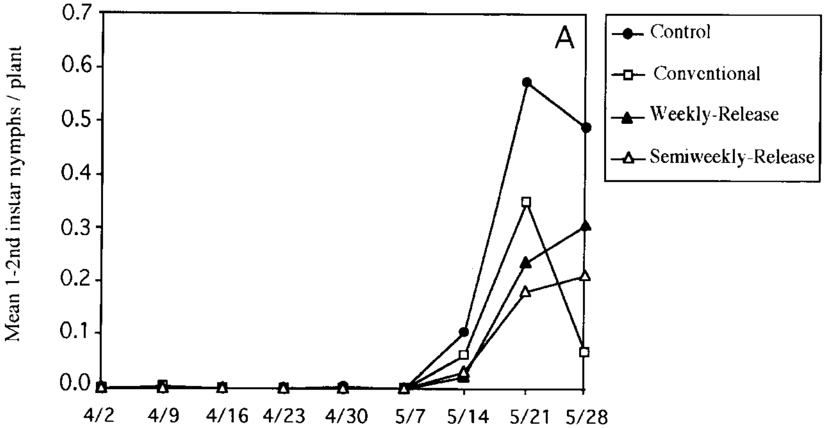
Due to the late migration of WTPB to strawberries during the first trial we expected to observe a reduction in the impact of inundative releases of *A. iole*. In the earlier field trial (Norton and Welter 1996), initial suppression of WTPB by *A. iole* during the first generation was considered to be beneficial for reduction of the pest during the second generation. However, in the present study, despite the absence of the first generation in the first trial, we observed significant reduction in WTPB densities with inundative releases of *A. iole*. WTPB densities in the Weekly-Release plots were comparable with densities in the Conventional plots where standard insecticide application procedures were followed. Densities of WTPB were moderate during this trial at three sites and this may have enhanced the impact of the parasitoid releases. At the remaining two sites, WTPB densities were high. However it was not possible to evaluate the impact of *A. iole* releases under these high WTPB pressure situations at these two sites since entire fields, with all treatment and control plots, were sprayed with insecticides.

Our results indicated that, by doubling the release rate, it was possible to enhance the level of WTPB suppression in strawberries. It may be possible to increase the release frequency even further. However, since doubling the release frequency resulted in only a 9.5 % improvement in WTPB suppression, benefits of further increases in the release frequency are likely to be limited. In addition, the increase in labor costs for placing the parasitoid into the field twice a week would make the program even more expensive.

Instead of increasing the release frequency, one option would be to increase the release rate beyond 15,000 / acre / week. However, it may still not be possible to match the high levels of parasitism observed on other plants. In a survey by Graham et al. (1986), mean monthly parasitism levels up to 100% were observed in certain plants such as guayule (*Parthenium argentatum* Gray), burro weed (*Haplopappus* sp.) and silverleaf nightshade (*Solanum elaeagnifolium* Cav.). Lower levels of parasitism in strawberries could be due to factors associated with the strawberry plant that affect parasitoid performance.

While host associated factors impact parasitoid searching and host location, parasitoids are also known to be influenced by various cues emanating from plants that are used by the parasitoid for host-habitat location (Rabb and Bradley 1968, Read et al. 1970, Price et al. 1980, Vinson 1981, Udayagiri and Jones 1992). Chemical and physical factors are involved in host recognition behavior by *A. iole* (Conti et al. 1996). Since WTPB eggs are inserted in plant tissue with only the operculum and a part of the egg visible above the plant surface, *A. iole* insert their ovipositor through plant tissue to access the egg chorion laterally under the operculum (Conti et al. 1997). Hence we can expect that plant structures in the vicinity of WTPB eggs will influence the performance of *A. iole.*

In strawberries, WTPB lays eggs in various plant structures, but a greater majority (46.5%) are laid in the fruit, between the achenes (seeds) in the fleshy receptacle, compared to other plant structures such as the peduncle (6.2%), calyx (3.7%), petiole (23.3%) or leaflet (20.3%) (Udayagiri and Welter 2000). Strawberry fruits have a complex external structure and the high level of oviposition observed on the fruits is unusual. On other plants such as alfalfa, cotton, beans (*Phaseolus vulgaris* L.) and london rocket (*Sisymbrium trio* L.), WTPB lays its eggs on vegetative structures such as the stem, petiole and the peduncle or reproductive structures with simple external structures such as bean pods (Benedict et al. 1981, Graham and



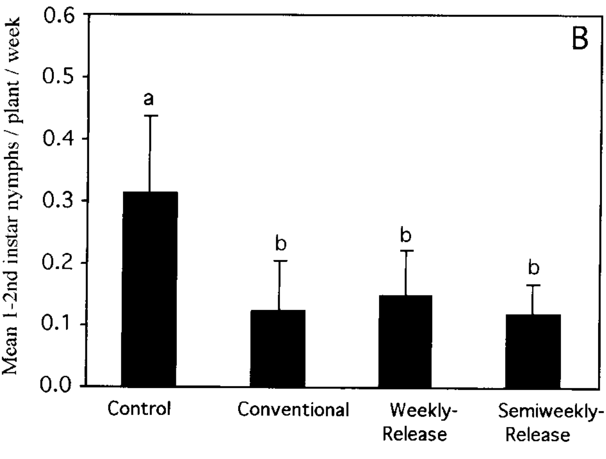




FIG. 2. Comparison of the impacts of weekly and semiweekly releases of *Anaphes iole* with insecticide control of *Lygus hesperus* in strawberries. A. Weekly densities of WTPB in experimental plots. In the Weekly-Release plots, *A. iole* were released @ 15, 000 / acre / week and in the Semiweekly-Release plots 7,500 *A. iole* were release twice in a week with an interval of 3-4 days. B. Overall densities of *L. hesperus* nymphs in experimental plots.

Jackson 1982, Alvarado-Rodriquez et al. 1986). The unusual host egg distribution pattern in strawberries has the potential of having a great impact on parasitism by *A. iole* since host eggs on the fruit experience lower levels of parasitism compared with host eggs on other tissues.

Structural refugia appear to be the basis for the differential parasitism, as the achenes on the fruits appear to hinder parasitoid access to host eggs (Udayagiri and Welter 2000). Hence fruits can provide refugia from parasitism by *A. iole*, and maximum protection can occur on fruits at early stages of development where the achenes are contiguous. Assuming that parasitism on the peduncle and the petiole are similar, that oviposition preference and searching by *A. iole* are random across various plant tissues, and that oviposition behavior remains relatively the same over time, the presence of the refugia can have a major impact on biological control of WTPB in strawberries due to the high numbers of eggs laid in the receptacle of the fruit (Fig. 3). Given .this structural refugium, inundative efforts with *A. iole* may reach a maximal level of parasitism that is below levels that can be achieved on other host plants of WTPB. Cultivars with appropriate changes in fruit structure are needed for enhancing parasitism of WTPB by *A. iole* in strawberries.

Given the potential for escape from parasitism by *A. iole* in strawberries, we need to integrate *A. iole* releases with other strategies for WTPB suppression in strawberries. One possibility is to use natural enemies in over-wintering weed host plants to reduce the numbers of adults colonizing strawberries. Several naturally occurring predators attack WTPB nymphs (UCIPM 1994) but provide minimal suppression of WTPB in strawberries. Earlier efforts to establish the nymph parasitoid *Peristenus stygicus* Loan in southern California met with limited success (Van Steenwyk and Stern 1977). One of the factors that could have been responsible for the low level of establishment was the difference in climatic conditions between the areas where the parasitoids were collected and those where releases were made. A more recent effort by Day (1996) indicated that *P. digoneutis* Loan has potential for suppression of the closely related TPB on the east coast. Further studies are needed to determine the impacts of *Peristenus* spp. collected from climatically appropriate areas in suppression of WTPB on the central coast in California

Another possibility is to integrate *A. iole* releases with insecticide applications for suppression of WTPB nymphs that escape parasitism. *A. iole* releases would be initiated first after the appearance of WTPB adults in strawberries while insecticide applications would be made several weeks later when WTPB nymphs are observed in the fields. However, due to the extended egg laying period of WTPB females (Strong et al. 1970), WTPB eggs are present in strawberry fields even after the appearance of WTPB nymphs. This results in an overlap between the need for insecticide applications against nymphs and releases of *A. iole,* even though the two control strategies target different life stages of the pest. However, one advantage of inundative biological control is that there is a certain level of flexibility in parasitoid release timing and it may be possible to avoid periods of adverse insecticide residues. Hence, integration is possible if there is compatibility between insecticides used for WTPB control and *A. iole*. An earlier study indicated that residues of insecticides used for WTPB control in strawberries have negative impacts on survivorship of *A. iole* (Udayagiri et al. 2000), but the residues vary in their duration of adverse effects. For integration, insecticides with the least toxic effects need to be used. After insecticide application, parasitoid releases need to be adjusted so as to reduce periods of strong negative effects from residues. Based on the toxicity curves that we developed (Fig. 4), integration is possible with insecticides such as naled, where initial toxicity is high but there is rapid decay. If methomyl or malathion is used, a longer period is needed between insecticide application and parasitoid release. Integration is difficult with insecticides such as the pyrethroids bifenthrin and fenpropathrin which have severe negative impacts since there is a limit beyond which the parasitoid releases cannot be delayed.

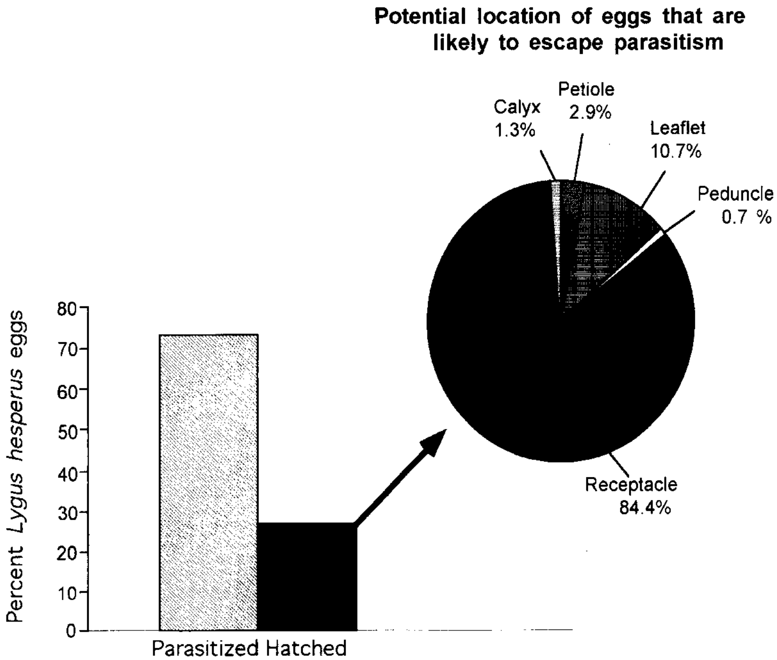



FIG. 3. Diagrammatic representation of the potential limitation in parasitism by *Anaphes iole* in strawberries due to *Lygus hesperus* egg distribution and accessibility on various plant structures. Bars on the left represent percentage of WTPB eggs on various strawberry tissues that were parasitized and those that hatched when exposed to *A. iole* adults for 6 hours under no-choice situation. Pie diagram on the right represents likely location of eggs that can escape parasitism under the assumptions that *A. iole* locates and parasitizes eggs randomly on a strawberry plant, and that oviposition behavior remains relatively the same over time (see text) (Data from Udayagiri and Welter 2000).

In summary, due to the high cash value per acre of strawberries, even low levels of damage may pay for various types of suppression programs, hence the current threshold of 0.1 WTPB nymphs per plant. However, while a high cash value crop may be able to support an inundative biological control program, it may also require an unusually high level of efficacy. Inundative biological control programs may provide reasonably high levels of parasitism (e.g. 64%), but these levels may not be adequate to provide commercially acceptable pest suppression. In strawberries, *A. iole* does provide some WTPB suppression, but parasitism levels are likely to be lower that that achieved on other host plants of WTPB. Additional empirical research using higher release rates, more release points, or more frequent releases are not expected to produce dramatically superior results. Understanding the limitations of biological control of WTPB with *A. iole* in strawberries suggests the need for focusing our efforts on integrating this program with alternative strategies.

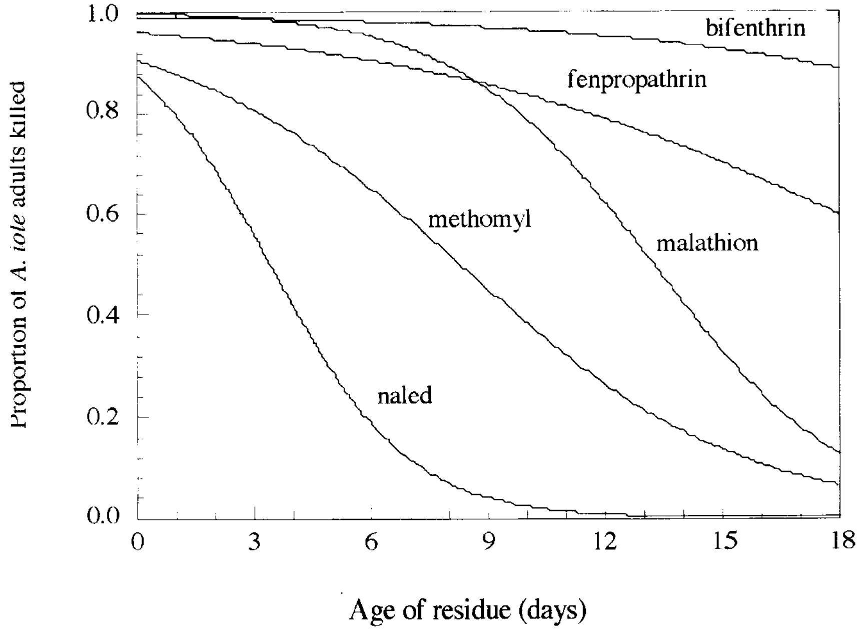



FIG. 4. Estimated toxicity to *Anaphes iole* adults of insecticide residues on strawberry foliage at different times after insecticide application. Lines represent logistic regressions for standard insecticides used for *Lygus hesperus* control in strawberries on the central coast in California. Integration of insecticide application with *A. iole* releases is best with insecticides such as naled with relatively rapid decay. (From Udayagiri et al. 2000).

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