UNDERSTANDING AND MEDIATING RECENT FAILURES IN THE BIOLOGICAL CONTROL OF THE WALNUT APHID

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ABSTRACT

The walnut aphid, *Chromaphis juglandicola* is a pest known in California for more than 100 years. The introduction of the parasitic wasp *Trioxys pallidus* from Iran in 1969 led to a dramatic success in the biological control of walnut aphid populations in California, and has provided sustained control of this devastating pest for the past 35 years. However, we are currently facing new walnut aphid outbreaks and in season insecticide treatments are being used more frequently in recent years due to loss of control by *T. pallidus*. Moreover, a new white morph of *C. juglandicola* has recently been reported. This white morph of *C. juglandicola* has never been described before in the literature, and its origin and significance are unknown. In this project we aim to better understand why *T. pallidus* has failed to provide efficient control of the walnut aphid in an increasing number of orchards in recent years and how to enhance the level of biological control in these orchards. In order to improve our understanding of the *Chromaphis/Trioxys* system, we monitored the seasonal activity of *C. juglandicola* and its parasitoid *T. pallidus* in walnut orchards in relation to temperature, hyperparasitism, and mummy predation. Only one of the six orchards selected for sampling in 2004 and 2005 showed a failure of control. Walnut aphid outbreaks are not consistent from year to year in a given orchard, suggesting the occurrence of a see-saw effect. When aphid populations remained under the control of *T. pallidus*, the dynamics of the control was similar to that reported in the early seventies following the introduction of *T. pallidus*: a first small peak in the spring, following by a summer decrease. Then aphid populations built up again in late summer. The compatibility of Success® and GF-120®, two products used against husk fly, with the biological control of the walnut aphid was tested in the field in 2005. Hyperparasitoids seemed more susceptible to Success® than *T. pallidus*, therefore increasing *T. pallidus* control by reducing hyperparasitoid populations. Investigations undertaken in 2004 comparing the two color morphs of the aphid showed a small but significant reproductive advantage for the white morph. Investigations in the laboratory in 2005 indicate that *T. pallidus* did not show any preference between white and yellow aphids. However, a less pronounced preference for larger nymphs was observed in the host instar preference tests for *T. pallidus* in the case of the white morph, suggesting that it may not have as great an impact on white aphids as it does on yellow aphid populations.

INTRODUCTION

Damage from walnut aphid, *Chromaphis juglandicola* (Kaltenbach) varies according to the time of the year that aphids are abundant. When present in large numbers in spring, aphid feeding reduces tree vigor, nut size and quality. During the summer, however, aphids can induce a shriveling of the kernels before harvest. Extremely high populations of aphids may lead to leaf drop, exposing nuts to sunburn. In addition, the honeydew excreted by aphids blackens the husk surface and favors the development of sooty mold, increasing the risk of sunburn on exposed nuts. *Chromaphis juglandicola* is specific to the walnut *Juglans regia* L. and all varieties are susceptible. The introduction of the parasitic wasp *Trioxys pallidus* (Halliday) from Iran in 1969 led to a dramatic success in the biological control of walnut aphid populations in California, and has provided sustained control of this devastating pest for the past 35 years. However, we are currently facing new walnut aphid outbreaks and in season spray treatments are being used more frequently in recent years due to loss of control by *T. pallidus* in mid to late summer. Moreover, a preliminary survey undertaken in Yuba County in 2003 revealed the occurrence of a white morph of *C. juglandicola*, accounting for 36% to 88% of the walnut aphid population present on a tree. This white morph of *C. juglandicola* has never been described before in the literature, and its origin and significance are unknown.

OBJECTIVES

The general objective is to better understand why *T. pallidus* has failed to provide efficient control of the walnut aphid in an increasing number of orchards in recent years and how to enhance the level of biological control in these orchards. The specific objectives are:

1. To analyze changes in the seasonal activity of *C. juglandicola* and its parasitoid *T. pallidus* in California walnut orchards in relation to temperature, hyperparasitism, mummy predation and host plant quality.

2. To determine the compatibility of currently registered products used against other walnut pests with *T. pallidus*.

3. To compare the biological characteristics and significance of the white morph of *C. juglandicola* found in Yuba County in 2003.

PROCEDURES

Objective 1. To analyze changes in the seasonal activity of C. juglandicola and its parasitoid T. pallidus in California walnut orchards in relation to temperature, hyperparasitism, mummy predation and host plant quality.

The seasonal activity of the walnut aphid, its parasitoid and antagonists was monitored in three orchards, selected based on their recent history of high aphid population. One orchard was located in Yolo County and the two others were located in Butte County where white aphids were observed in 2004. Every other week from May until September, twelve trees were randomly sampled in each orchard. Aphid densities and parasitism were assessed by counting aphids and unemerged mummies on the two subterminal leaflets of 25 leaves per tree. Unemerged mummies were brought back to the lab and held individually in gelatin capsules until their emergence. Mummies from which no *Trioxys* or hyperparasitoids emerged were examined for puncture marks from predation to assess the importance of predator induced mortality in the field. If no predation mark were observed, mummies were dissected and the content examined for the presence of primary or secondary parasitoids, if identifiable. Parasitism rate was calculated as the number of unemerged mummies divided by the number of unmerged mummies plus live aphids. Percent hyperparasitism was calculated as the number of emerged plus unmerged secondary parasitoids divided by the number of emerged plus unemerged parasitoids (primary and secondary). Temperature was recorded continuously in each orchard with Hobo data loggers.

Objective 2. To determine the compatibility of currently registered products used against other walnut pests with T. pallidus.

The effect of Success® and GF-120®, two products used against walnut husk fly, on *T. pallidus* mummy emergence was tested in three walnut orchards located near Escalon, CA, that were being used for spray trials by R. A. Van Steenwyk. Each orchard was divided into plots, 3 acres each, and each plot treated with a different rate of Success® or GF-120®. Two different rates of Success® (1.6 and 3.2 oz per acre) and a blank bait control (3 pt NuLure per acre) were tested on July 18 in Escalon 1 and August 3 in Escalon 2. Success® was applied using an air-blast sprayer with a final spray volume of 25 gallons per acre. Three different concentrations of GF-120® (20 oz. per acre, diluted to 1:1.5, 1:4 and 1:9) plus a blank bait control were tested on August 3 in Escalon 3. GF-120® was applied using a modified spot sprayer mounted on an ATV. In each orchard, mummies were collected 2 days after insecticide applications. Four trees were selected in each plot and numbers of aphids counted on 50 leaflets. As aphid populations were extremely low [0.7 ± 0.2 (SE) aphids / leaflet on control trees, $n = 12$), an effort was made to collect as many unmerged mummies as possible from these and adjacent trees to evaluate impacts of the spray treatments on parasitoid emergence (see Fig. 3A for sample size). Mummies were brought back to the lab for parasitoid and hyperparasitoid emergence. Unemerged mummies were checked for predation marks and if no predation mark was observed, dissected and examined for the presence of a dead larva, dead nymph or dead adult wasp.

Objectives 3. To compare the biological characteristics and significance of the white morph of C. juglandicola found in Yuba County in 2003.

Previous investigations undertaken in 2004 comparing the two color morphs did not show any difference in the developmental characteristics of white and yellow aphids, but a slight reproductive advantage for white aphids in reproductive characteristics. Although white morphs did not show greater longevity, total progeny production, or daily progeny production compared to yellow morphs, the reproductive rate of white aphids was greater over the first two days of adult life. As the longevity of adult aphids under field conditions is likely to be much shorter than in the lab, this small advantage early in the adult life of the white morph can translate to more rapid population growth than for the yellow morph. In 2005, after refreshing the colonies with yellow and white aphids collected in Butte Co., the host instar preference of *T. pallidus* for each color morph, and *T. pallidus* host color preference were investigated in the laboratory.

Host instar preference was investigated using mated *T. pallidus* females, 48h old, honey-fed and given prior oviposition experience. Females were released individually in Petri dishes containing a walnut leaflet with 30-130 yellow or white aphids, with variable proportions of instars. Females were observed under a microscope until either 10 aphids had been parasitized or the parasitoids had been inactive for 5 minutes. The relative instar preference was calculated as the ratio of the number of aphids attacked in each instar to the number of each instar present, then standardized so that the sum of preference values equals 1. These preferences were averaged among trials. A preliminary experiment was undertaken to establish the relationship between body size and instar in *C. juglandicola*. The results showed that 3rd, 4th and adult instars are easily identifiable, due to the presence of a variable number of dorsal black spots, wing pads and wings respectively. In contrast, 1st and 2nd instars looked the same and could be distinguished only based on their size. We arbitrarily set up the following range: 1st instar = 0.5 mm and 2nd instar > 0.5 mm in body length).

Host color preference by *T. pallidus* was tested using mated, honey-fed naïve 24h old *T. pallidus*. Ten white and ten yellow aphids of the preferred instars (see above) were offered to the females and the color of the first five aphids stung was recorded.

RESULTS

Objective 1. To analyze changes in the seasonal activity of C. juglandicola and its parasitoid T. pallidus in California walnut orchards in relation to temperature, hyperparasitism, mummy predation and host plant quality.

In 2005, aphid densities remained below the economic threshold of 15 aphids per leaflet in the orchards selected for sampling (Fig. 1-2). In Butte 1 (Fig. 1A), parasitism rates varied between 3% and 30% throughout the season (mean ± SE: 13 ± 3%) which is relatively low compared to other situations where *C. juglandicola* has been reported to be under the control of *T. pallidus*. For instance, data collected by van den Bosch and colleagues in 1972 in two orchards located in San Joaquin and Butte Co. showed that parasitism rates by *T. pallidus* varied between 0 and 79% (mean: 39 ± 17%) and between 1 and 100% (mean: 56 ± 26%) respectively. In Butte 1, hyperparasitism rates were also extremely low, averaging 16 ± 6%, compared to 58% and 56% recorded in 1972 in San Joaquin and Butte Co. respectively. Two applications of Success® (Spinosad) were undertaken against walnut husk fly in this orchard (Fig. 1A). Hyperparasitism rate dropped dramatically after the first application (July 21), suggesting that hyperparasitoids were greatly affected by this treatment (see also Objective 2 - Compatibility of currently registered products used against other walnut pests with *T. pallidus*). In contrast, walnut aphid and *T. pallidus* did not seem to have been affected by the first spray of Success®. The decrease in aphid density after the second application (August 4) could either result from this treatment, or, more likely, result from higher parasitism by *T. pallidus*.

In Butte 2 (Fig 1B), parasitism by *T. pallidus* was also low (range: 0 to 22%, mean 9 ± 2%) but hyperparasitism rates were greater than in Butte 1, reaching reached 49 ± 7%. Hyperparasitism peaked early in the season, resulting in low parasitism rates but did not compromise the biological control of the walnut aphid later in the season. On September 9, an organophosphate (Lorsban®) plus bait was applied against husk fly. Three weeks later, aphid numbers and parasitism rates were only slightly higher than observed before the spray, suggesting either that this treatment did not affect aphids and primary parasitoid populations, or that the orchard was rapidly recolonized from neighboring trees.

The mean (±SE) proportion of white morphs observed in Butte 1 and 2 was 31 ± 3% and 70 ± 2% respectively, and these proportions were fairly constant throughout the season. In 2004, we observed that increases in aphid densities were associated with increases in the proportion of white morphs, but no outbreak was recorded in these orchards in 2005.

In the Yolo Co. orchard (Fig. 2A), overall parasitism and hyperparasitism rates were 14 ± 7% and 42 ± 8% respectively. Malathion® plus a molasses-based bait was applied against husk fly on July 28 (Fig. 2A). Because of the significant drop in aphid number, we interrupted our sampling for 8 weeks. In between, a second spray treatment against mites (Onager®) was applied on August 11. Sampling was resumed on September 22 and showed extremely low aphid numbers with a moderate level of parasitism (24%), and no hyperparasitism. A combination of factors, such as parasitism by *T. pallidus*, insecticide control and high temperatures could be responsible for the low aphid population at the end of the season.

The relative abundance of hyperparasitoid species and predators in these orchards are presented in Table 1. Six species of hyperparasitoids were reared from *T. pallidus* mummies: *Syrphophagus aphidivorus* (Encyrtidae), *Pachyneuron* sp., *Asaphes suspensus* and *Asaphes californicus* (Pteromalidae), *Dendrocerus* sp. (Megaspilidae), and *Alloxysta* sp. (Charipidae). The two dominant species were *S. aphidivorus* and *A. suspensus*. *A. suspensus* is an early species, being more abundant during the first part of the season, while *S. aphidivorus* is a late species. This explains why in Yolo Co. where sampling was interrupted mid season, *A. suspensus* is the most abundant species instead of *S. aphidivorus*.

Data from 2004 are provided in Table 1 as well to allow comparisons between years. In 2004, a walnut aphid outbreak was observed in one of the orchards sampled (Yuba 1). Aphids escaped the control of *T. pallidus* in mid June, reaching an average density of 28.3 aphids per leaflet, covering the foliage with honeydew and requiring the application of a broad spectrum pyrethroid (Warrior®). The insecticide was applied on July 1 and live aphids were not observed in the orchard until early August. From August onwards, they remained under the control of *T. pallidus*. No significant difference in the relative abundance of hyperparasitoids was observed between years

In 2005, the two main groups of predators observed in the orchards were spiders and coccinellids. In contrast, in 2004 the main groups of predators were lacewings and reduviids, while coccinellids were rare (Table 1). Predation marks left on mummies were of two types: punctures holes, most probably caused by lacewing larvae (Hemerobiidae – Chrysopidae), and mummies chewed open, most probably by coccinellid larvae and adults (Table 2). Multiple holes in mummies, suspected to be evidence of ant predation, were rare. The seasonal variation in the percentage of mummies predated (punctured or chewed open) is presented in Table 2. Note that predated mummies could have remained on the leaf for several weeks before they were sampled.

* Sampling interrupted from 7/28 to 9/22

Table 1. Relative abundance (%) of hyperparasitoid species and predators in orchards sampled in 2004 and 2005

County	Yolo *	Butte 1	Butte 2	Yuba 1	Yuba 2
Year	2005	2005	2005	2004	2004
Walnut variety	Tulare	Chandler	Chandler	Chico	Chandler
Level of control	Sustained	Sustained	Sustained	Lost	Sustained
HYPERPARASITOIDS					
Syrphophagus aphidivorus	10	40	68	61	65
Asaphes suspensus	56	34	11	27	18
Pachyneuron sp.	16	17	15	7	4
Asaphes californicus	7	9	5	0	8
Dendrocerus sp.	0	0	0	5	6
Alloxysta sp.	11	0	1	0	0
PREDATORS					
Hemerobiidae – Chrysopidae	16	18	17	50	35
Coccinellidae	37	29	44	0	3
Spiders	41	47	36	17	22
Reduviidae	3	0	4	28	23
Orius sp.	2	0	0	6	16
Earwings	0	6	0	0	0
Chamaemyiidae - Syrphidae	1	0	0	0	0
Ants	0	0	0	0	0

.

Table 2. Percentage of mummies punctured (lacewing larvae and assassin bugs) and chewed open (coccinellid larvae and adults) recorded in 2005

Sampling date	Yolo		Butte 1		Butte 2	
	% punct.	% chewed	% punct.	% chewed	% punct.	% chewed
May 20 - 26	6	6	6	0	0	0
June 2 - 9	65	0	17	0	12	0
June 16 - 23	50	1	64	5	62	0
June 30 - July 7	43	8	60	17	50	8
July 14 - 21	35	49	74	11	63	8
July 28 - August 4	29	68	76	15	80	24
August 11			65	16	80	11
August 25			86	17	70	17
Sept. 8			81	15	85	13
Sept 22 - 27	41	53	87	6	71	7
TOTAL	39	40	70	12	74	13

Objective 2. To determine the compatibility of currently registered products used against other walnut pests with T. pallidus.

Success® had a small but interesting effect on the emergence of parasitoids from mummies (Fig 3A). Higher rates of Success® resulted in slightly lower parasitoid emergence, but more importantly, it significantly reduced the emergence of hyperparasitoids which seemed more susceptible than *T. pallidus*. Increasing concentrations of GF-120® had no consistent effect on mummy emergence (Fig. 3A), probably because this product provides a very uneven coverage of material in the canopy of the trees. In addition, the ratio of primary parasitoids to hyperparasitoids did not vary between treatments of GF-120® suggesting that, in contrast to the Success® treatments, neither *T. pallidus* nor its hyperparasitoids were affected. Unemerged mummies contained mainly dead adults or pupae (Fig 3B).

Objective 3. To compare the biological characteristics and significance of the white form of C. juglandicola found in Yuba County in 2003

When offered yellow morphs of *C. juglandicola*, *T. pallidus* females preferred to attack third and fourth instars, with a higher but not significant preference for the fourth instar (Fig. 4, n = 18). When offered white morphs of the aphid, *T. pallidus* attacked second, third and fourth instars with equal preference (n = 14). Adults of both color morphs are quite mobile and often escaped attack by *T. pallidus*.

Based on these results, fourth instar aphids were used to evaluate host color preference in choice tests with both present. No preference was observed: in total, 12 wasps were tested, and 29 white and 29 yellow aphids were stung (one wasp flew away after stinging 3 aphids). The mean (±SE) proportion of white aphids stung by each of the 12 *T. pallidus* female tested was 48 ± 8%.

DISCUSSION

Field sampling undertaken in 2004 and 2005 showed that walnut aphid outbreaks are not consistent from year to year in a given orchard. Orchards were selected for sampling based on their recent history (the preceding year) of aphid outbreaks. However, no outbreaks were observed the following year (except for Yuba 1, where aphids were abundant at least two consecutive years). Tree-dwelling aphid populations have been suggested to show a “see-saw” effect, i.e. when a high aphid population in one year is usually followed by a low population the following year. Alternating years of higher abundance can in some cases be driven by the aphid’s effect on host plant quality, but more generally it has been suggested to be caused by the aphid itself. High aphid abundance early in a year results in the production of small aphids later in the year and egg laying forms with a low reproductive rate that generate a low abundance the following year. A more indirect process could also operate, such as increased mortality from natural enemies. A better knowledge of walnut aphid population fluctuations from one year to another, and the impact of parasitism, would help to establish whether such alternating years of abundance occur in walnut orchards.

Success® appears to be a product that could be compatible with the biological control of walnut aphid. Moreover, it could enhance control by *T. pallidus* by reducing hyperparasitoid populations. Not only was this effect seen in the direct evaluation trials of Objective 2, it was also evident from the data collected during the field sampling under Objective 1. Success® was applied in one of the orchards selected for sampling in 2005 (Butte 1, Fig 1A) and hyperparasitism rate dropped dramatically after the first application (July 21) while both aphids and *T. pallidus* were not affected. Butte 2, an orchard very similar to Butte 1 (Chandler variety, no insecticide application at least before July 21, same sprinkler irrigation system) and located less than a mile apart, was not treated with Success®. In that orchard, hyperparasitism rate continued to increase after July 21, resulting in lower parasitism rates and slightly higher aphid densities compared to Butte 1 (Fig 1B). Additional documentation of this effect is needed to confirm the compatibility and anti-hyperparasitism effect of Success®.

Previous investigations in the laboratory in 2004 comparing the two color morphs of the walnut aphid showed a small yet significant reproductive advantage of the white morph over the yellow morph. Experiments undertaken in 2005 showed that inexperienced *T. pallidus* did not show any color preference between white and yellow aphids. However, a slight divergence was observed in the host instar preference of *T. pallidus* for each color morph. When offered yellow aphids, *T. pallidus* preferred to attack large-sized (fourth instar) aphids. When offered white aphids, *T. pallidus* equally attack 2nd, 3rd and 4th instar. Through use of an age-structured model of an aphid-parasitoid interaction, it has been shown by others that parasitoid preference for larger hosts results in a greater reduction of the population growth rate of an aphid. These two characteristics of white walnut aphids, their slight reproductive advantage and the less pronounced preference of *T. pallidus* for larger white aphid instars, could at least partly explain why white aphids were more abundant during the outbreak observed in Yuba 1 in 2004.

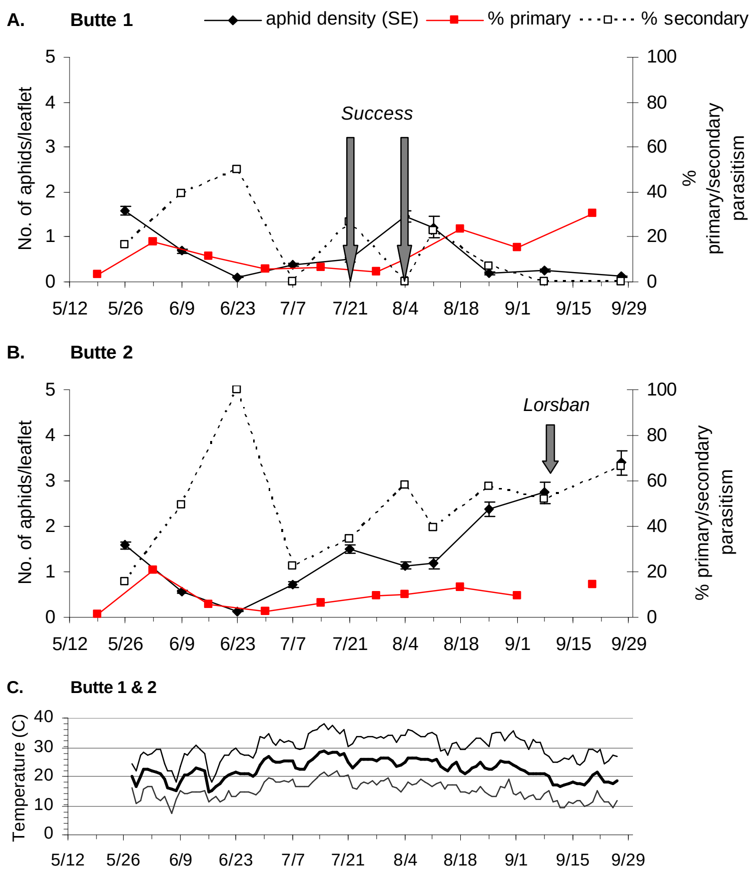

Fig. 1. **A.** Seasonal variation of aphid density, parasitism by *T. pallidus* (% primary) and hyperparasitism (% secondary) in Butte 1. **B.** Seasonal variation of aphid density, parasitism by *T. pallidus* (% primary) and hyperparasitism (% secondary) in Butte 2. Twelve walnut trees (variety Chandler) were sampled in most cases (only 8 trees on 8/11 and 9/27), 50 leaflets per

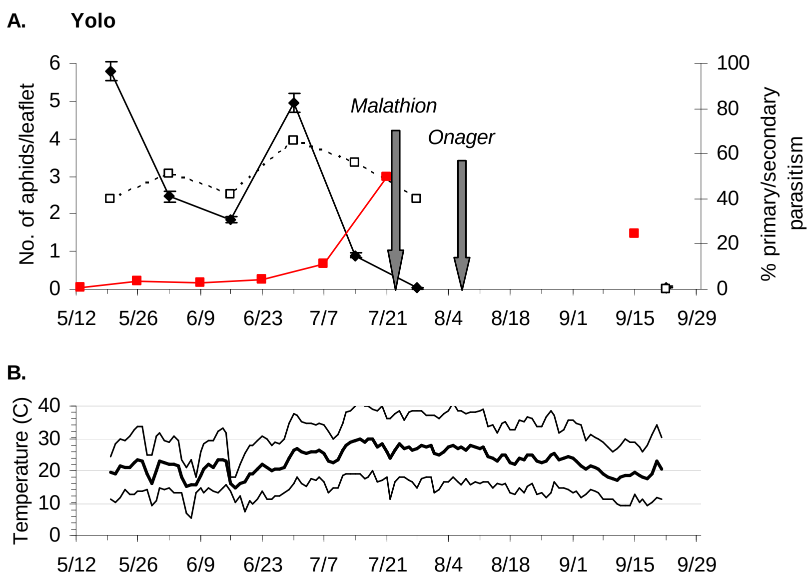

Fig. 2. **A.** Seasonal variation of aphid density, parasitism by *T. pallidus* (% primary) and hyperparasitism (% secondary) in a Yolo orchard. Twelve walnut trees (variety Tulare) were sampled in each case, 50 leaflets per tree. Malathion® was applied against husk fly and Onager® against mites. Sampling was interrupted and resumed 9/22. **B.** Mean daily temperature

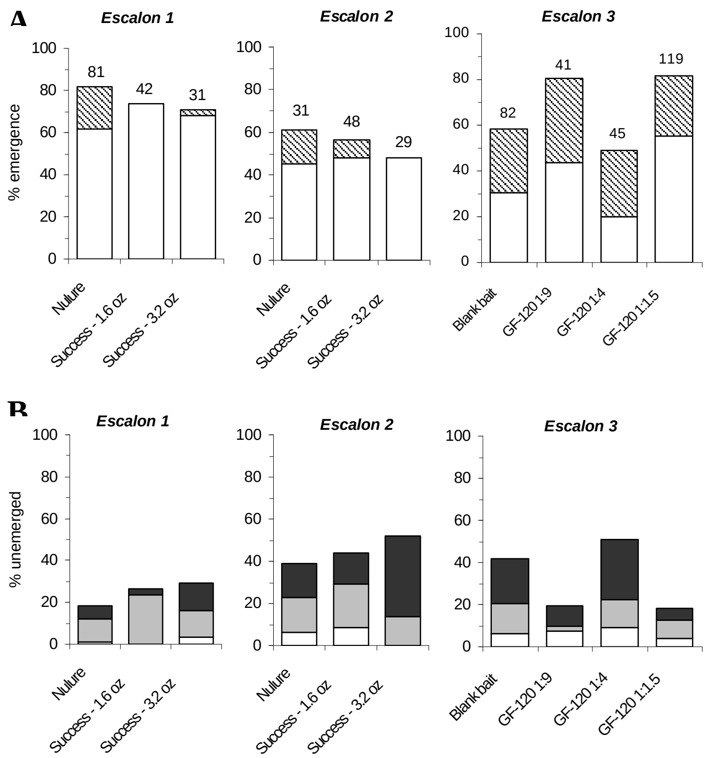

Fig 3 A. Percentage emergence and proportion of *T. pallidus* (white) and hyperparasitoids (shaded columns) emerging from mummies collected on trees treated with different insecticides.

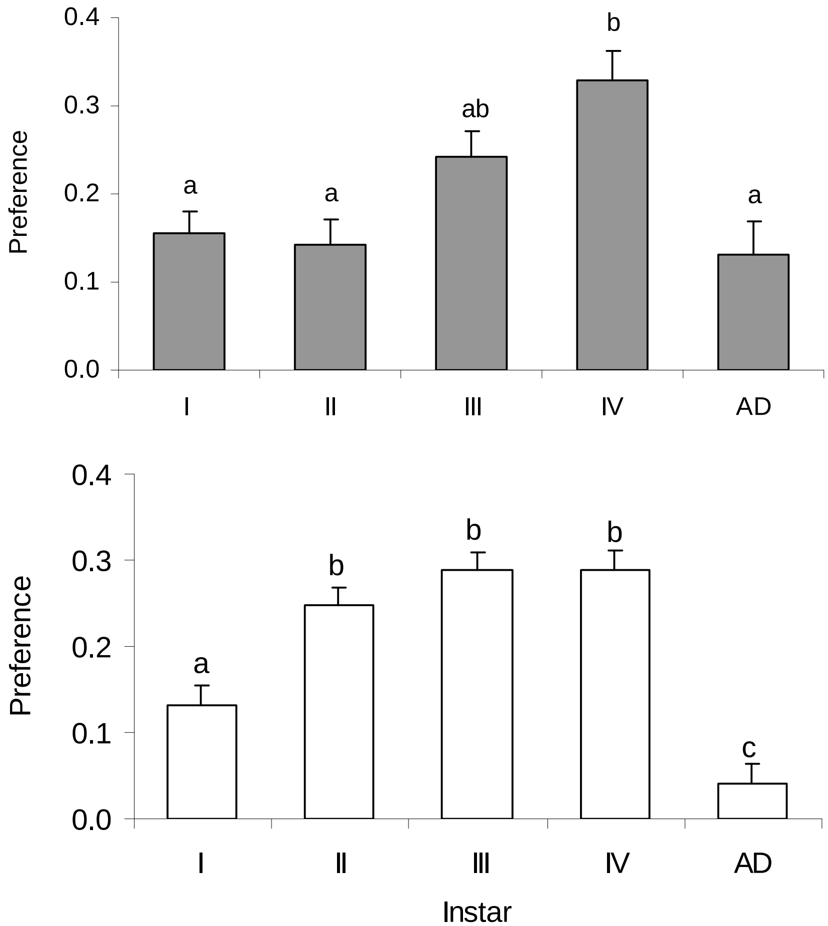

Fig. 4. Mean relative preference of *Trioxys pallidus* for different instars of yellow (shaded columns) or white (white columns) *Chromaphis juglandicola*. Bars denote standard errors. Columns with same letter do not differ significantly