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, we discovered a new white morph of *C. juglandicola* for the first time in 2003. 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 nine orchards selected for sampling in 2004-06 showed a failure of control, indicating that walnut aphid outbreaks are not consistent from year to year in a given orchard. When aphid populations remained under the control of *T. pallidus*, the patterns of aphid abundance were similar to that reported in the early seventies following the initial introduction of the parasitoid: a first small peak in spring, following by a summer decline and a build up again in late summer. The compatibility of currently registered products used against other walnut pests with the biological control of the walnut aphid was investigated in the laboratory. In general, the hyperparasitoid *Syrphophagus aphidivorus* seemed more susceptible to organophosphates, pyrethroids and spinosyns than *T. pallidus*. Some products that could potentially enhance *T. pallidus* control by reducing hyperparasitoid populations were identified. Life history characteristics of *T. pallidus* were investigated to provide a basis for understanding potential limitations of this parasitoid: longevity, temperature tolerance, thermal requirements and host instar preference. Investigation comparing the two color morphs of the aphid showed a small but significant reproductive advantage for the white morph. *T. pallidus* did not show any preference between white and yellow aphids, but showed a less pronounced preference for larger nymphs 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 new 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, and mummy predation

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

3. To determine the life history characteristics and potential limitations of *T. pallidus*.

4. To compare the biological characteristics and ecological significance of the white form of *C. juglandicola*.

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 and mummy predation

The seasonal activity of the walnut aphid, its parasitoid and antagonists was monitored in three orchards each year. The orchards were selected based on their recent history of high aphid population. In 2004, one orchard was located in San Joaquin Co. and the two others were located in Yuba Co. where white aphids were observed in 2003. In 2005, one orchard was located in Yolo Co. and the two others were located in Butte Co. where white aphids were observed in 2004. In 2006, we monitored aphid seasonal activity in the same orchards as 2005. Every other week from May until September, twelve trees were randomly sampled in each orchard. Number of aphids and mummies were counted on the two subterminal leaflets of 25 leaves per tree (= 50 leaflets per tree). Mummies were examined for emergence holes or predation marks. 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 reexamined for puncture mark. 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 plus the number of predated mummies divided by the number of unmerged mummies plus predated 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 impact of several registered products on *T. pallidus* and *S. aphidivorus* emergence and longevity was tested in the laboratory. Leaflets with 3-5 day-old mummies in the case of *T. pallidus* and 8-11 day-old mummies in the case of *S. aphidivorus* were dipped in insecticide solutions (Table 1). For both parasitoids, the treatment was timed to be 3-5 days before adult emergence from the mummies. Two rates of insecticides were tested: the recommended field rates (100%) and 25% of field rate (Table 1). A total of 13-94 mummies were tested for each product, rate and parasitoid species. Mummies were dipped in water as control. After dipping, mummies were kept in glass vials until emergence. Emerged parasitoids were provided with honey everyday until they died. Two weeks after treatment, unmerged mummies were dissected and examine for the presence of a dead nymph or adult wasp.

Objective 3. To determine the life history characteristics and potential limitations of T. pallidus

Life history characteristics of *T. pallidus* were investigated to provide a basis for understanding potential limitations of this parasitoid: longevity, temperature tolerance, thermal requirement and host instar preference. Field-collected *C. juglandicola* and *T. pallidus* were maintained on walnut seedlings in a greenhouse located on the Oxford Tract of the Berkeley Campus. Longevity and temperature tolerance were assessed by looking at the survival of *T. pallidus* kept at 4 constant temperatures (16, 22, 25 and 30°C). For each temperature, 14-41 freshly emerged *T. pallidus* of each sex were kept in glass vials with water and honey replaced daily. The number of dead individuals was recorded twice a day. The effect of temperature on *T. pallidus* development was asessed by examining the time taken to develop from egg to adult when kept at 4 constant temperatures (15, 22, 25 and 30°C) and a 16h photoperiod.. For each temperature, 12-16 stung fourth instar aphid nymphs were clip-caged on a walnut seedling. Aphids were examined daily and each new mummy was kept separately in a gelatin capsule. Mean development time was recorded for each temperature. Linear regression was used to determine the lower threshold for parasitoid development and the thermal requirement for adult emergence. Host instar preference is presented in the next objective.

Objective 4. To compare the biological characteristics and ecological significance of the white form of C. juglandicola

The significance of the white color morph of the walnut aphid was assessed in the laboratory. The thermal requirements, adult survivorship, progeny production and host instar preference by *T. pallidus* were studied comparatively for the two color forms. Host color preference by *T. pallidus* was also investigated. Field-collected white and yellow aphids were maintained on walnut seedlings in a greenhouse. These cultures were refreshed every year by collecting new individuals in the field during the summer. The thermal requirements for development were established in the laboratory using cohorts of aphid nymphs freshly deposited on walnut seedlings. These seedlings were held at 7 constant temperatures (15, 18.5, 21, 24.5, 26.5, 29 and 31°C), and a 16h photoperiod. For each temperature and each color form, 6-20 cohorts of 1-10 nymphs (average 3.0 ± 1.9 nymphs/cohort) were used. The nymphs were examined daily until adults appeared. The hind tibia length of adults were measured, an indicator of size and thus growth rate, and the developmental time recorded. Linear regression was used to establish the lower threshold temperature for development and the thermal requirement for completion of a generation. Adult survivorship and progeny production were examined on walnut seedlings by clip-caging late 4th instar nymphs singly onto walnut leaflets, 13-15 individuals were tested for each color form. Seedlings were kept at a constant temperature of 24°C and 16h photoperiod. Date of adult appearance, daily progeny production and time of death were recorded.

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 (n = 14 – 18). 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. As a complementary experiment, the suitability for parasitoid development of each aphid instar was investigated. Mated, honey-fed naive 24h-old *T. pallidus* were given access to aphids of the selected instar placed on a fresh walnut leaflet. Each aphid stung was clip-caged on a fresh walnut seedling. After 10 days, mummies were cut out of the leaflets and placed in vials for adult emergence. The proportion of successfully parasitized aphids (i.e. the number of stung aphids that produced an adult parasitoid in relation to the total number of aphids stung) and the proportion of aphids surviving a sting (i.e. number of stung aphids surviving to adulthood in relation to the total number of aphids stung) were calculated. 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 were offered to the female parasitoids 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 and mummy predation.

A failure of control was observed in only one orchard during 2004-06. In 2004, aphids escaped the control of *T. pallidus* in an orchard located in Yuba County (variety Chico). Aphids peaked in mid June, reaching an average of 28.3 aphids per leaflet, covering the leaves with honeydew. A broad spectrum pyrethroid (lambda-cyhalothrin, Warrior®) coupled with a miticide (clofentezine, Apollo®) was applied on July 1, resulting in a dramatic extinction of the aphid population (Fig. 1a). Live aphids were not observed again in the orchard until early August when they remained under the control of *T. pallidus.*

Aphids remained below the economic threshold of 15 aphids per leaflet in the remaining orchards, including those sampled the two following years (Table 2). When aphid populations remained under the control of *T. pallidus*, their patterns of abundance were similar to that reported in the early seventies following the initial introduction of the parasitoid. This is characterized by a first small peak in the spring, following by a summer decline, and a subsequent build up again in late summer (Fig. 1b). 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. Hyperparasitism rates reached a peak of 58% and 56% in San Joaquin and Butte Co. respectively.

The white morph of *C. juglandicola* was by far the most abundant of the two color forms in the Yuba orchard (Chico) where the aphid outbreak was recorded in spring 2004. In general, increases in aphid densities were associated with an increase in the proportion of white morphs, even in orchards where no outbreak was recorded (Table 2).

The relative abundance of hyperparasitoid species and predators in these orchards are presented in Table 3. Six species of hyperparasitoids were reared from *T. pallidus* mummies: *Syrphophagus aphidivorus* (Encyrtidae), *Pachyneuron aphidis*, *Asaphes suspensus* and *Asaphes californicus* (Pteromalidae), *Dendrocerus* sp. (Megaspilidae), and *Alloxysta* sp. (Charipidae). The dominant species was *S. aphidivorus*, followed by *P. aphidis* and *A. suspensus,* except in Yolo Co., where *A. suspensus* and *P. aphidis* were dominant in 2005 and 2006 respectively (Table 3). *Alloxysta* sp. was mainly retrieve from mummies collected in Yolo and Butte Co. The two main groups of predators observed during field sampling in 2005-06 were spiders and coccinellids (5 surveys out of 9; Table 3). However, in the Yuba (2005) and Butte (2006) orchards, the main predators were lacewings larvae (and Reduviidae in the Yuba orchards), while coccinellids were rare or less abundant (Table 3).

Predation marks left on mummies were of two types: punctures holes, caused by lacewing larvae (Hemerobiidae – Chrysopidae), and mummies chewed open by coccinellid larvae and adults. 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 4. Note that predated mummies could have remained on the leaf for several weeks before they were sampled.

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

Organophosphates (Guthion®, Lorsban® and PenncapM®) and pyrethroids (Asana® and Warrior®) significantly reduced *S. aphidivorus* emergence and survival after emergence (Fig 2-3). Organophosphates, with the exception of Guthion®, also reduced *T. pallidus* emergence, although to a lesser extent compared to *S. aphidivorus* (Fig 2). In contrast, pyrethroids did not seem to affect *T. pallidus* emergence (Fig 3). *T. pallidus* survival after emergence was only significantly affected by Guthion®, Lorsban® and Asana® (Fig 2-3). Treatment with Success® resulted in a slight reduction of parasitoid emergence (although not significant), but a significant reduction in hyperparasitoid emergence (Fig 4). No significant effect of Success® on survival after emergence was recorded for either species. Also no effect of Intrepid® or Omite® was recorded for either emergence rates or survival after emergence for the two species (Fig 5-6).

Objective 3. To determine the life history characteristics and potential limitations of T. pallidus

T. pallidus survivorship decreased with increasing temperature, and overall, females had a greater survivorship than males (Fig 7). A small significant difference was detected in parasitoid size (HTL) between temperature categories ($F = 3.39$, 3 and 66 df, $p = 0.02$), but none of the pairwise multiple comparison were significant using the Student-Newman-Keuls method. Males were significantly smaller than females ($F = 30.8$, 1 and 169 df, $p < 0.001$).

The relationship between temperature and development rate was linear in the range of temperature 15 - 30°C. *T. pallidus* developed faster at higher temperatures (Fig 8). The threshold temperature for development and the thermal requirement were estimated to be 3.8°C and 250°D(C) respectively.

Objective 4 To compare the biological characteristics and significance of the white form of C. juglandicola found in Yuba County in 2003

The relationship between temperature and development rate was linear within the range 15 – 26°C: aphids developed faster at higher temperatures (Fig. 9a). No difference in the threshold temperature for development or thermal requirement for completion of a generation was detected between the two color morphs. The threshold temperature for development was estimated to be 8.7°C and 9.0°C, and the thermal requirement was estimated as 131.6°D(C) and 128.2°D(C) for yellow and white morphs respectively. In both cases, aphids raised at higher temperature were smaller (Fig. 9b).

No significant differences were detected in the pattern of daily progeny production of the two color morphs except for the first two days where the progeny production of white morphs was higher (Fig 10). However, no difference in the mean number of nymphs produced daily was detected between the two color morphs: 2.3 ± 0.6 (SD) nymphs (n = 15) *vs.* 2.4 ± 0.6 nymphs (n = 13) for yellow and white morphs respectively (t = 0.38, df = 26, p = 0.71). Also, no difference in the mean lifetime fecundity was detected: 59.3 ± 21.8 (SD) nymphs *vs.* 62.8 ± 16.9 nymphs for yellow and white morph respectively (t = 0.48, df = 26, p = 0.63). Yellow and white aphids showed the same survivorship, with average longevities of 25.5 ± 6.7 days and 26.2 ± 3.5 (SD) days respectively (t = 0.30, df = 26, p = 0.77). There was a slight but significant difference in the size of aphids used in this experiment: yellow aphids were slightly larger (HTL: 0.693 ± 0.032 mm) than white aphids (0.642 ± 0.047 mm) (t = 3.22, df = 22, p = 0.004). However, no significant effect of size on the three parameters investigated (longevity, lifetime fecundity and daily progeny production) was detected over the range of sizes considered here (data not presented), suggesting that this difference in size did not influence the results.

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. 11, 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*. Although *T. pallidus* preferred to attack large instars, it appears that the second instar in both color morphs is the most suitable for *T. pallidus* development (Fig 12a). A low proportion of stung aphids survived stinging by the parasitoid in both color morphs (Fig 12b).

Based on these results, fourth instar aphids were used to evaluate host color preference in choice tests with both color morphs 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 aphids stung by each of the 12 *T. pallidus* female tested was 48 ± 8%.

DISCUSSION

Walnut aphids escaped the control of *T. pallidus* in a number of orchards sampled during a preliminary survey in 2003, with extremely large numbers of aphids (35 to 64 aphids per leaflet) recorded in September. Leaves were covered with honeydew and aphid exuviae and we estimated that 1 to 9% of the nuts were shriveled. More extensive surveys undertaken in commercial walnut orchards in 2004-06 showed, however, that walnut aphid outbreaks are difficult to predict or not consistent from one year to another. 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 had been 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 such that egg laying aphids have a low reproductive rate at the end of the year and 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.

In 1985 and 1986, efforts were made to isolate and release *T. pallidus* biotypes resistant to azinphos-methyl (Guthion®) a standard spray treatment for codling moth. Some strains successfully established in walnut orchards and interbred with native field biotypes to increase the level of Guthion® tolerance. Cross-resistance to other organophosphate insecticides has been observed in these azinphos-methyl resistant *T. pallidus,* possibly explaining their greater tolerance, compared to *S. aphidivorus*, to Lorsban® and PenncapM® in our recent assays. Both Guthion® and Warrior® appear to be compatible with *T. pallidus*: they both reduce hyperparasitoid populations without affecting primary parasitoid emergence and survival, therefore enhancing the biological control. Asana® and Success® could also be compatible with *T. pallidus*, but to a lesser extent. These products induce a significant reduction in hyperparasitoid emergence and survival, but they also result in slightly lower emergence and survival of *T. pallidus*. Intrepid®, an insect growth regulator effective against many species of lepidopteran insects, and Omite®, a miticide, are both pest-specific products and are compatible with biological control of the walnut aphid.

Investigations in the laboratory comparing the two color morphs of the walnut aphid showed a small yet significant reproductive advantage of the white morph over the yellow morph. Also 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 a stage-structured model of an aphid-parasitoid interaction, it can be shown that parasitoid preference for later aphid instars results in a greater reduction of the population growth rate of the aphid. These two characteristics of white walnut aphids, their slight reproductive advantage and the less pronounced preference of *T. pallidus* for larger instars, could at least partly explain why white aphids were more abundant during the outbreak observed in Yuba 1 in 2004.

Table 1. Commercial name, active ingredient and field rate of products tested in Objective 2

Product	Active ingredient	Field rate (100%)
Lorsban®	Chlorpyrifos (OP)	16 fl oz/acre
Penncap-M®	Methyl parathion (OP)	6 pt/acre
Guthion®	Azinphos-methyl (OP)	0.75 lb/acre
Warrior®	Cyalothrin lambda (pyrethroid)	2.5 fl oz/acre
Asana®	Esfenvalerate (pyrethroid)	8 fl oz/acre
Success®	Spinosad (spinosyns)	3.2 fl oz/acre
Intrepid®	Methoxyfenozide (IGR)	18 fl oz/acre
Omite®	Propargite (miticide)	10 lb/acre

Table 2. Aphid densities, parasitism by *T. pallidus* (% primary), hyperparasitism (% secondary) and proportion of white morphs (% white) recorded in the field in 2004-06. Aphid densities during the first sampling date of the season (1st sampling), the first peak (1st peak) and second peak (2nd peak) peak of aphid abundance, if any are given. Similarly, proportions of white morphs during the first sampling/first peak/second peak of aphid abundance are given, as well as minimum and maximum over the entire season.

	Mean aphid density ± SE			% primary	% secondary	% white
Location (Variety)	1st sampling (date)	1st peak (date)	2nd peak (date)	Mean ± SE (min – max)	Location (Variety)	1st sampling (date)
2004						
San Joaquin (Vina)	0.1 ± 0.0 (May 18)	4.8 ± 0.2 (July 15)	11.0 ± 0.6 (Sept 8)	30 ± 9 (4 – 89)	47 ± 9 (0 – 83)	-
Yuba1* (Chico)	0.1 ± 0.0 (May 25)	28.3 ± 1.2 (June 22)	-	62 ± 15 (4 – 100)	30 ± 8 (0 – 50)	72/83/- (44 – 83)
Yuba2 (Chandler)	0.1 ± 0.0 (May 25)	1.7 ± 0.1 (June 22)	7.5 ± 0.3 (Aug 16)	43 ± 12 (5 – 98)	35 ± 11 (0 – 84)	65/74/85 (47 – 85)
2005						
Yolo (Tulare)	5.8 ± 0.2 (May 20)	5.8 ± 0.2 (May 20)	5.0 ± 0.3 (June 30)	36 ± 15 (1 – 96)	42 ± 8 (0 -65)	-
Butte1 (Chandler)	1.6 ± 0.1 (May 26)	1.6 ± 0.1 (May 26)	1.5 ± 0.1 (Aug 8)	32 ± 8 (4 – 77)	16 ± 6 (0 – 50)	31/31/42 (17 – 52)
Butte2 (Chandler)	1.6 ± 0.1 (May 26)	1.6 ± 0.1 (May 26)	3.4 ± 0.3 (Sept 27)	24 ± 4 (1 – 38)	49 ± 8 (15 – 100)	65/65/84 (62 – 84)
2006						
Yolo (Tulare)	5.5 ± 0.2 (May 22)	5.5 ± 0.2 (May 22)	-	58 ± 18 (1 – 100)	40 ± 22 (0 – 100)	-
Butte1 (Chandler)	0.0 ± 0.0 (May 24)	0.4 ± 0.1 (July 6)	0.3 ± 0.1 (Aug 16)	38 ± 9 (13 – 75)	57 ± 9 (0 – 57)	0/22/30 (0 – 30)
Butte2* (Chandler)	0.1 ± 0.0 (May 24)	2.3 ± 0.2 (July 19)	2.5 ± 0.2 (Aug 16)	31 ± 10 (9 – 82)	52 ± 12 (0 – 100)	40/37/55 (34 – 55)

*See also Fig 1

Table 3. Relative abundance (%) of hyperparasitoid species and predators in orchards sampled in 2004-06

Year	2004	2005					2006		
Location	San J.	Yuba1	Yuba2	Yolo*	Butte1	Butte2	Yolo	Butte1	Butte2
HYPERPARASITOIDS (n)	(164)	(31)	(79)	(200)	(109)	(475)	(41)	(41)	(119)
Syrphophagus aphidivorus	74	61	65	11	44	63	34	83	57
Pachyneuron sp.	16	7	4	18	15	15	37	15	27
Asaphes suspensus	9	27	18	52	30	16	10	0	11
Asaphes californicus	0	0	8	8	11	6	5	0	4
Dendrocerus sp.	0	5	6	1	0	0	2	0	0
Alloxysta sp.	0	0	0	11	0	0	12	2	1
PREDATORS (n)	(68)	(18)	(69)	(212)	(34)	(78)	(121)	(11)	(38)
Hemerobiidae - Chrysopidae	16	50	35	16	18	17	9	81	34
Coccinellidae	34	0	3	37	29	44	62	0	23
Spiders	44	17	22	41	47	36	29	0	29
Reduviidae	1	28	23	3	0	4	0	0	3
Orius sp.	1	6	16	2	0	0	0	9	3
Earwings	0	0	0	0	6	0	0	0	8
Chamaemyiidae - Syrphidae	0	0	0	1	0	0	0	0	0
Ants	3	0	0	0	0	0	0	0	0

*Sampling interrupted from 7/28 to 9/22

Table 4. Percentage of mummies punctured (lacewing larvae and assassin bugs) and chewed open (coccinellid larvae and adults) recorded in 2005-06

	Yolo				Butte1				Butte2			
Sampling dates	% punctured		% chewed		% punctured		% chewed		% punctured		% chewed	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
May 20 - 26	6	17	6	0	6	0	0	50	0	44	0	0
June 1 – 9	65	19	0	77	17	0	0		12	64	0	7
June 16 - 23	50	36	1	23	64	0	5	67	62	71	0	5
June 29 - July 7	43	41	8	57	60	11	17	32	50	77	8	15
July 13 - 21	35	44	49	53	74	12	11	33	63	52	8	16
July 27 – Aug 4	29	56	68	44	76	4	15	18	80	65	24	18
Aug 10 – 16		25		75	65	6	16	16	80	60	11	15
August 24 -25		63		38	86		17		70		17	
Sept. 7 – 13		38		51	81	11	15	21	85	86	13	7
Sept 22 - 27	41	64	53	36	87		6		71		7	
TOTAL	39	37	40	48	70	7	12	23	74	68	13	13

Fig 1. Seasonal variation in aphid density, parasitism, and hyperparasitism in (a) a Yuba orchard (Yuba 1) in 2004, and (b) a Butte orchard (Butte 2) in 2006. Warrior® + Apollo® was applied on July 1 and Success® + Omite® on August 17. Average, minimum and maximum temperatures are presented for each location.

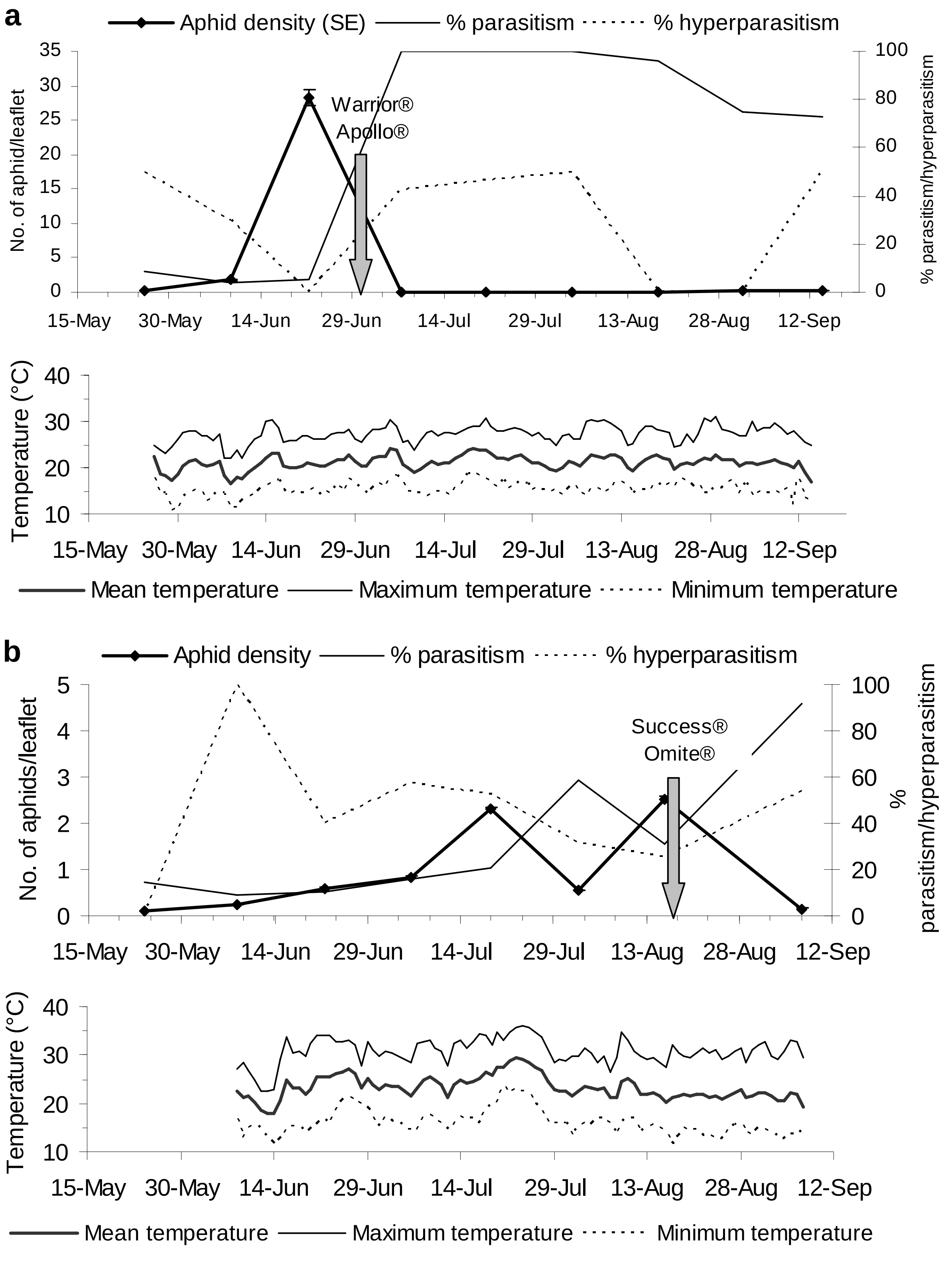
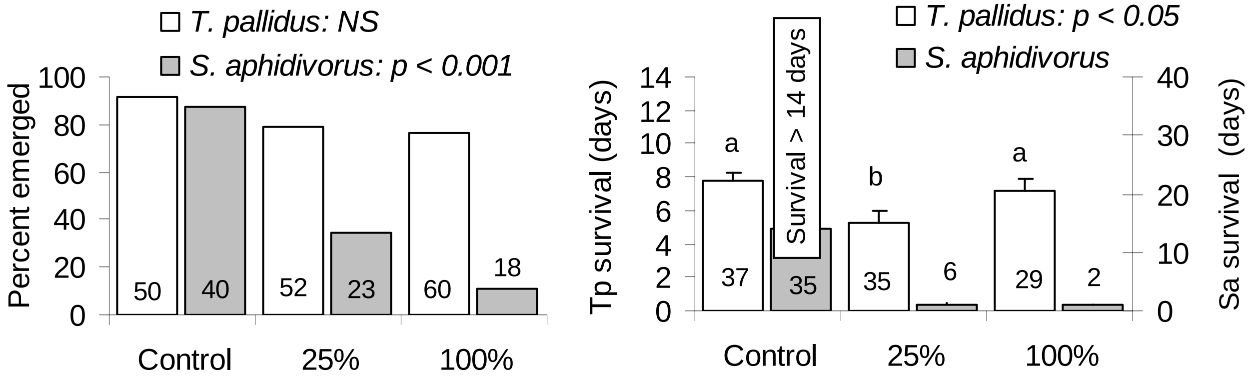
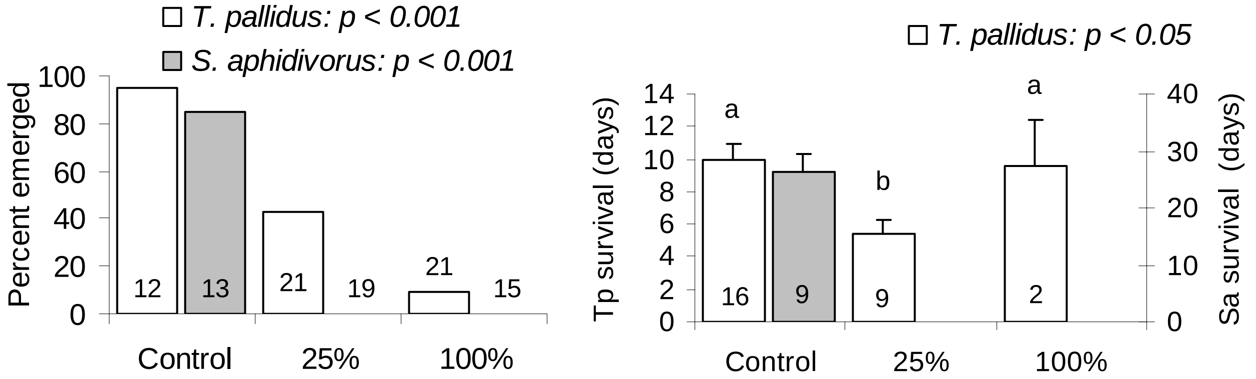


Fig 2. Percentage emergence (left) and mean survival (right) of *T. pallidus* and *S. aphidivorus* emerging from mummies dipped in organophosphate solutions: Guthion®, Lorsban® and PenncapM®. Two rates, 25% and 100% of the field rate, were tested (see Table 1). The data for survival of Guthion®-treated *S. aphidivorus* is preliminary. The effect of treatment on percent emergence and mean survival was analyzed using Chi-square and One-Way ANOVA respectively (columns with same letter do not differ significantly)

Guthion®



Lorsban®



PenncapM®

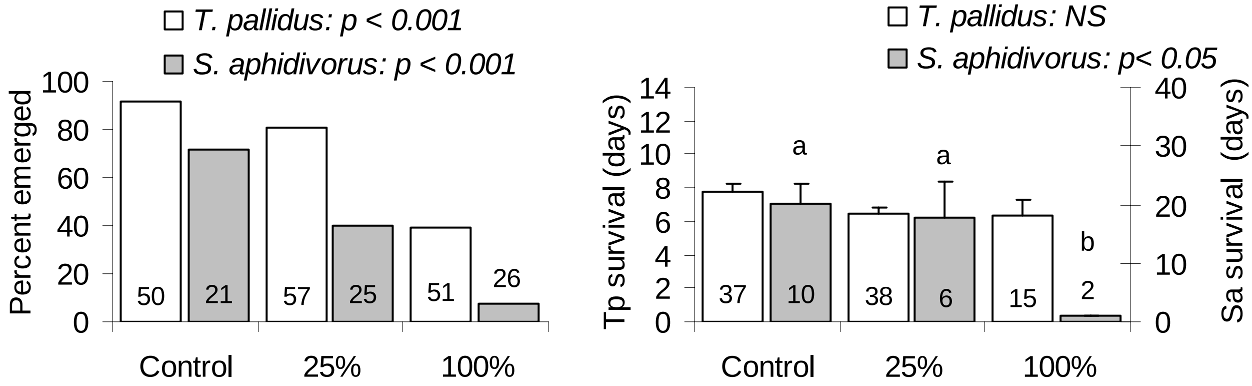
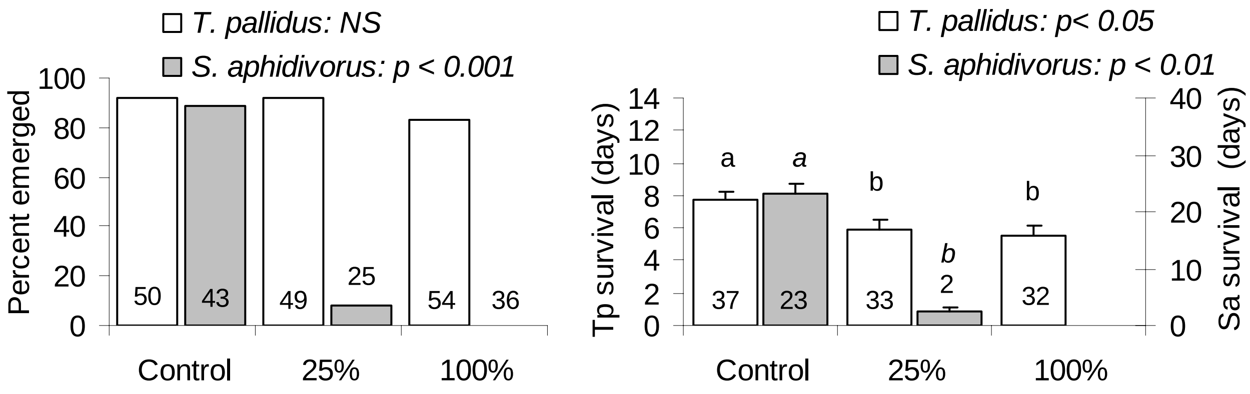
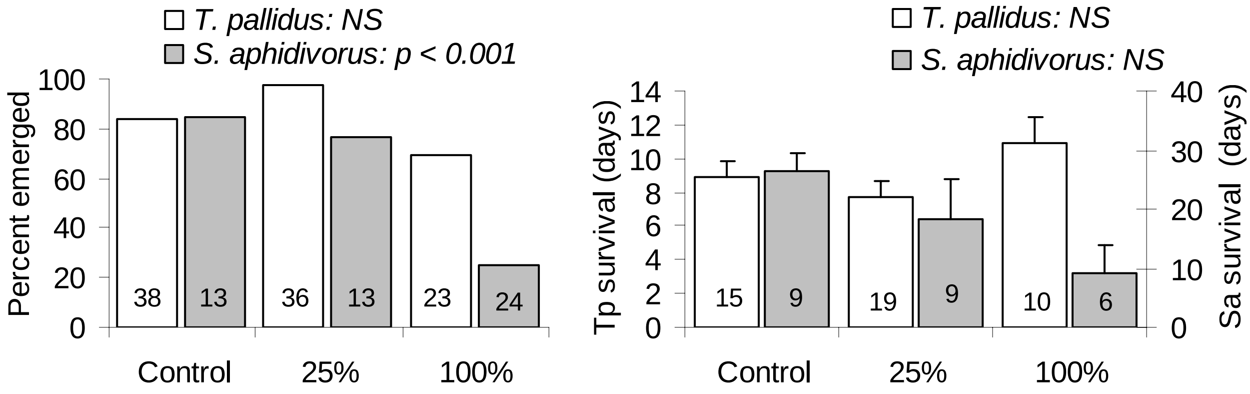


Fig 3. Percentage emergence (left) and mean survival (right) of *T. pallidus* and *S. aphidivorus* emerging from mummies dipped in pyrethroid solutions: Asana® and Warrior®. See Fig 2 for notations.

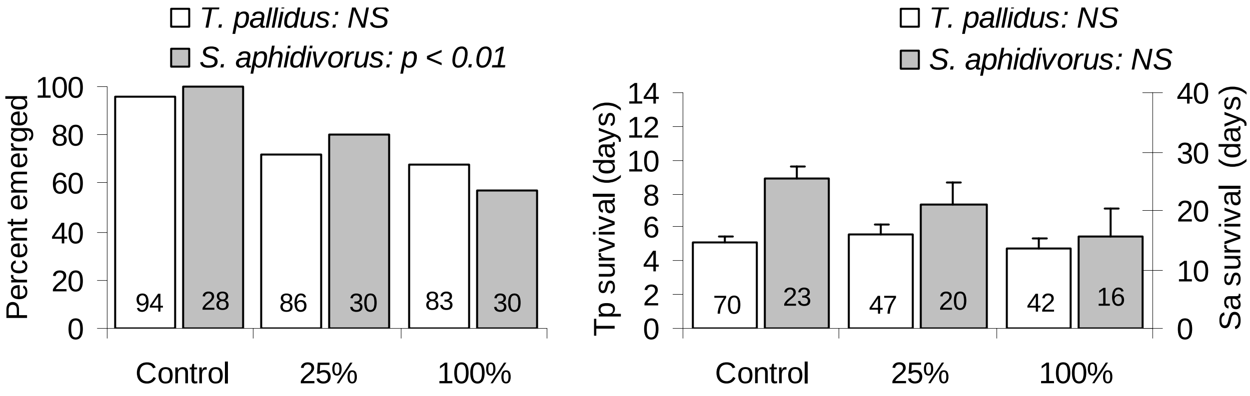


Asana®



Warrior®

Fig 4. Percentage emergence (left) and mean survival (right) of *T. pallidus* and *S. aphidivorus* emerging from mummies dipped in Success® solution. See Fig 2 for notations.



Success®

Fig 5. Percentage emergence (left) and mean survival (right) of *T. pallidus* and *S. aphidivorus* emerging from mummies dipped in Intrepid® solution. See Fig 2 for notations.

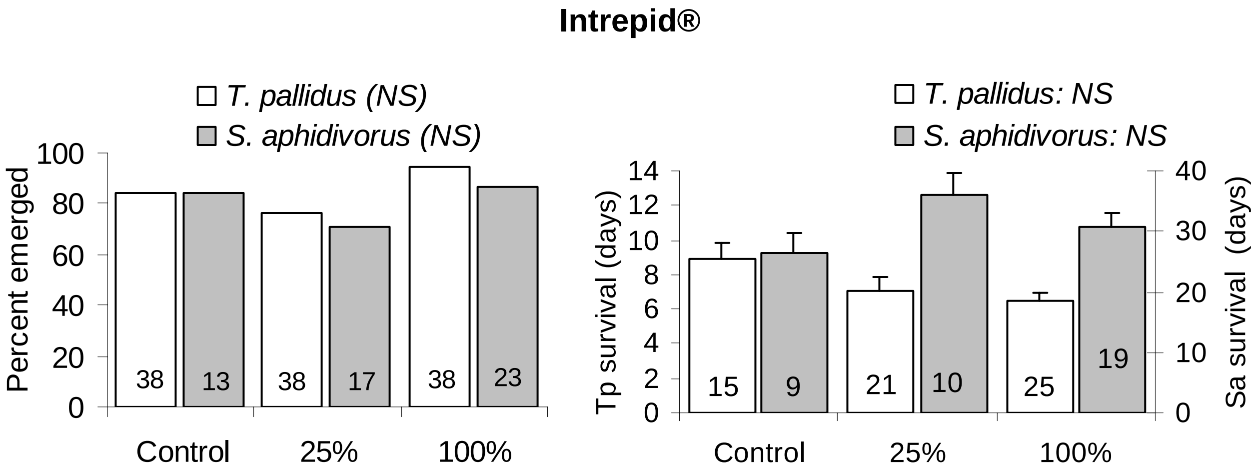


Fig 6. Percentage emergence (left) and mean survival (right) of *T. pallidus* and *S. aphidivorus* emerging from mummies dipped in Omite® solution. See Fig 2 for notations.

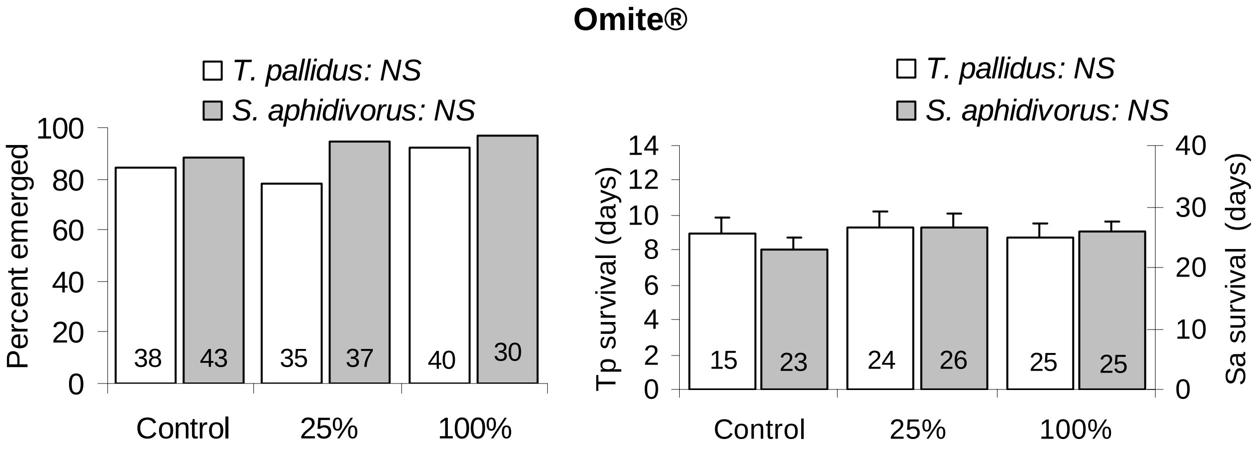


Fig 7. Average longevity (±SE) of *T. pallidus* males and females kept at 4 constant temperatures

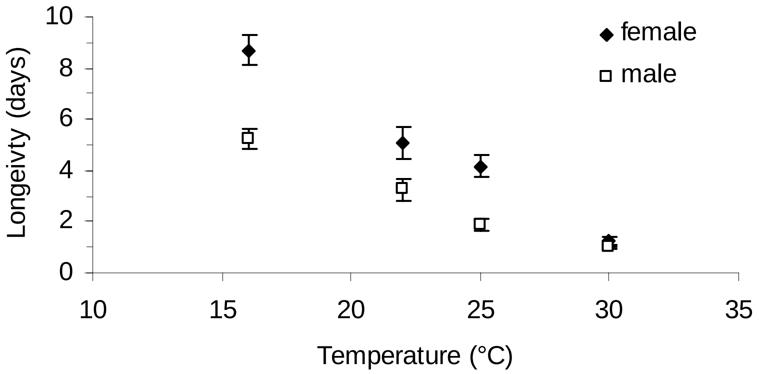


Fig 8. Relationship between temperature and development rate for *T. pallidus* ($y = 0.004x - 0{,}015$; $R^2 = 0.87$; $p < 0.001$)

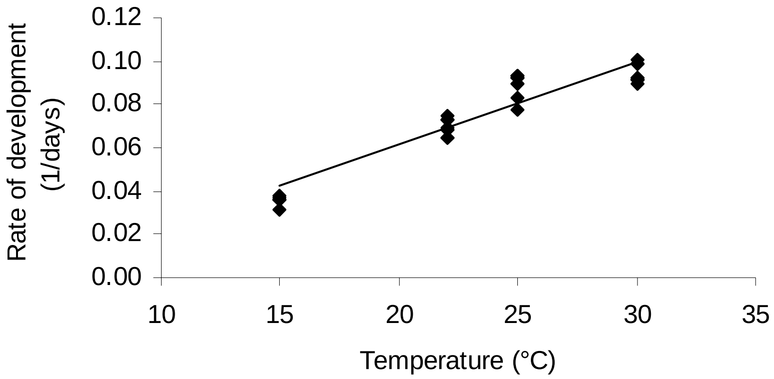


Fig 9. Relationship between (a) temperature and development rate and (b) temperature and hind tibia length (HTL) for the two color morphs of *C. juglandicola*. (a) Yellow: $y = 0.008x - 0.066$, $R^2 = 0.93$, $p < 0.001$; White: $y = 0.008x - 0.070$, $R^2 = 0.95$, $p < 0.001$ (b) Yellow: $y = -0.013x + 1.02$, $R^2 = 0.86$, $p = 0.02$; White: $y = -0.016x + 1.063$, $R^2 = 0.90$, $p = 0.01$. Values for the yellow morph have been slightly moved to the right for better clarity.

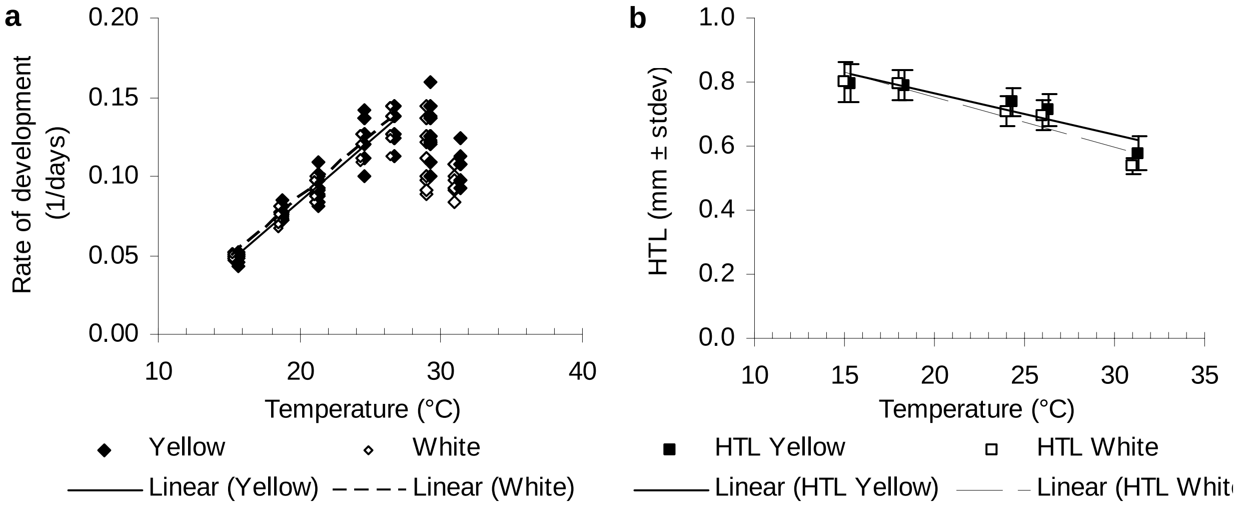


Fig 10. Average daily progeny production (±SE) of yellow and white morphs of *C. juglandicola*. Values for the yellow morph have been slightly moved to the right for better clarity

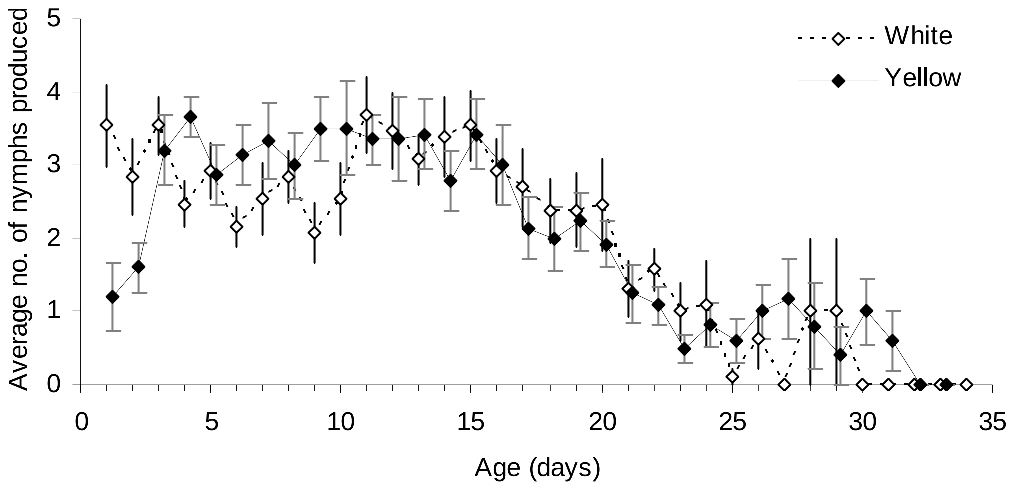


Fig 11. Mean relative preference (±SE) of *Trioxys pallidus* for different instars of yellow (upper) or white (lower) *Chromaphis juglandicola*. Columns with same letter do not differ significantly (One-way ANOVA, p = 0.05)

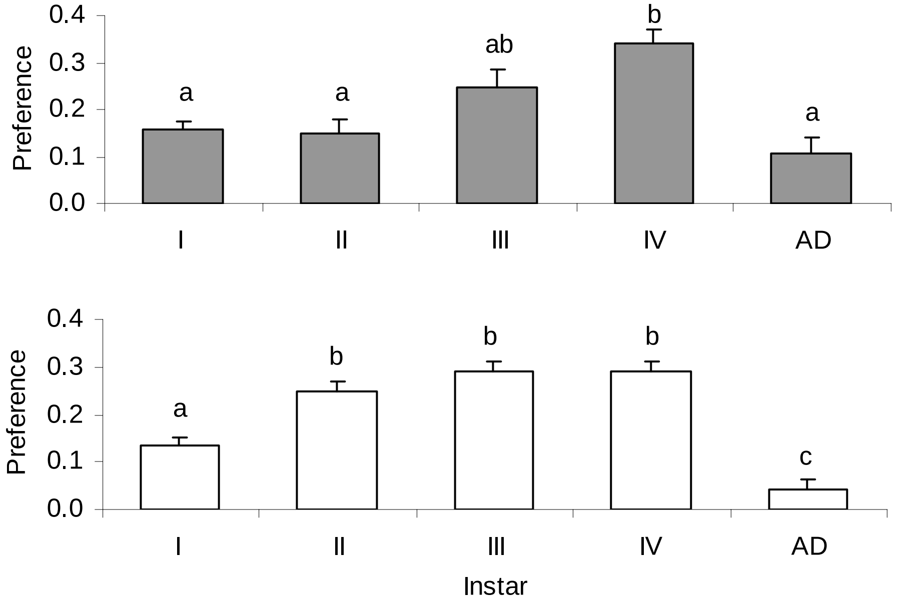


Fig 12. Instar suitability for parasitoid development in yellow (grey bars) and white (white bars) morphs of the walnut aphid. (a) Proportion of stung aphids that produced an adult parasitoid, and (b) proportion of stung aphids that survived to adulthood. Numbers above bars are the numbers of aphids stung within each instar.