WALNUT MARKETING BOARD
Research Proposal Cover Page

CONTRACTOR		AUTHORIZED INSTITUTION REPRESENTATIVE	
Company:	Walnut Marketing Board	Institution:	The Regents of the University of California
Contact:	David Stefani	Contact:	Lynne Hollyer
Address:	1540 River Park Dr., Ste. 203	Address:	Industry Alliances Office
	Sacramento, CA 95815		2150 Shattuck Ave, Ste 950
			Berkeley, CA 94704-6701
Phone:	(916) 922-5888	Phone:	(510) 642-5758
Fax:	(916) 923-2548	Fax:	(510) 642-4566
E-mail:	<dstefani@walnuts.org>	E-mail:	lhollyer@berkeley.edu

Project Title:	Selective pesticides and biological control in walnut pest management
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Budget Amount:	$20,931
Principal Investigator:	Nicholas J Mills
Mailing Address:	Environmental Science, Policy and Management
	Mulford Hall, University of California, Berkeley CA 94720-3114
Phone:	(510) 642-1711
Fax:	(510) 643-5438
E-mail:	nmills@nature.berkeley.edu
Cooperating Personnel:	C. Pickel, J. Grant, S. Wulfert

Signature of Principal Investigator:	N. J. Mills
Signature of Authorized Institution Rep:	Lynne Hollyer

University of California
Division of Agricultural Sciences

PROJECT PLAN/RESEARCH GRANT PROPOSAL

Project Year: 2009 Anticipated Duration of Project: 3 years

Project Leader: N.J. Mills Location: UC Berkeley

Cooperating Personnel: C. Pickel, J. Grant, S.Wulfert

Project Title: Selective pesticides and biological control in walnut pest management
Keywords: biological control, selective pesticides, walnut aphid, *Trioxys pallidus*

Commodity(s) Walnut Relevant AES/CE Project No.

Problem and its Significance:

The walnut aphid, *Chromaphis juglandicola* (Kaltenbach) has been known in California for more than 100 years. Damage to the crop varies according to the time of the year that the aphids are abundant (Olson and Buchner, 2002). When present in large number in the spring, aphid feeding reduces tree vigor, nut size and quality (Michelbacher and Ortega, 1958). During the summer, it induces a shriveling of the kernels before harvest (Barnes and Moffiti, 1978; Sibbett et al., 1982). Extremely high populations of aphids may lead to leaf drop, exposing nuts to sunburn. In addition, the honeydew excreted by the aphids can blacken the husk surface due to the development of sooty mold, increasing the risk of sunburn on exposed nuts. *C. juglandicola* is specific to the walnut *Juglans regia* L. and all varieties are susceptible. However, populations on late walnut varieties appear not to build as much as those on earlier varieties (Sluss, 1967). 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 38 years. However, both growers and PCAs have noted that aphid outbreaks, though not consistent, are of increasing concern in the Central Valley requiring in season spray treatments on a more regular basis. As pest management practices in walnuts change to both reduce costs and make use of new pesticides, it is important to retain the benefits of the long term biological control of walnut aphid provided by the introduction of *T. pallidus*.

In addition, we discovered a new white morph of the walnut aphid in 2003 which has since spread to many parts of the eastern side of the Sacramento Valley and seems likely to spread southward into the San Joaquin Valley over the next few years. Our recent research indicates that this white morph differs from the typical yellow morph in two important ways; it has an early reproductive advantage, and it is less susceptible to attack in its later instars (Mills et al., 2005). These two characteristics indicate that the white morph is more likely to develop outbreak populations than the typical yellow morph, and that the importance of effective aphid management is likely to become of greater importance in the future. In addition, it is possible that the new white morph is less susceptible to insecticides than the yellow morph, and could thus present an even more serious threat to walnut pest management in the future.

While the fraction of orchards requiring in season aphid sprays is still small, failure to understand the reasons for the changing effectiveness of walnut aphid control by *T. pallidus* in these orchards could readily lead to an increasing problem in the future. Surveys undertaken in commercial walnut orchards in the Sacramento Valley from 2004-06 have shown that walnut aphid outbreaks are both difficult to predict and not consistent from one year to another in the same orchard. However, walnut aphid densities exceeded the economic threshold of 15 aphids per leaflet in several of the orchards sampled, and in 2006 several orchards in the San Joaquin Valley (e.g., Woodford, Hanford and Tulare) also experienced greater aphid densities than usual. Our recent research has shown that *T. pallidus* has very limited tolerance of several of the newer products used as codling moth and husk fly treatments, and that *T. pallidus* and its hyperparasitoids show differential tolerance to the same products.

The goal of this project is to screen new pesticides to identify selective products that will enhance the level of biological control of walnut aphid in orchards. Pesticides that are currently or likely to be registered for use in walnuts in the near future will be bioassayed using a standard tiered assay approach to determine their impact on walnut aphid, *T. pallidus*, and its dominant hyperparasitoid *Syrphophagus aphidivorus*.

Objectives:

1. To compare the susceptibility of white and yellow walnut aphids to walnut pest management products using simple laboratory bioassays.
2. To assess the differential susceptibility of *T. pallidus* and *S. aphidivorus* to walnut pest management products using simple laboratory bioassays.
3. To use a demographic bioassay to test a subset of pest management products for their compatibility with and enhancement of biological control of walnut aphid.

Background:

The walnut aphid is an introduced species from Eurasia. This small yellow aphid is found in California from March until early December (Davidson, 1914; Nowierski, 1979; Nowierski and Gutierrez, 1986a). Before *T. pallidus* was introduced, aphids multiplied rapidly in spring and peaked in late June/early July. Aphid densities could reach an average of 100 aphids/leaflet (Sluss, 1967) with a consequent reduction of nut size and quality (Michelbacher and Ortega, 1958). This early peak was followed by a rapid decrease during the summer and then a smaller late summer increase (Sluss, 1967; Frazer and van den Bosch, 1973). *T. pallidus* is a solitary endoparasitoid of *C. juglandicola*. The adult female lays an egg inside the aphid nymph, and the hatching larva consumes the aphid which becomes "mummified" as the wasp pupates. After pupation, a new adult wasp emerges from the aphid mummy. *T. pallidus* was imported from Iran and released in 1968. Two years later, the parasitic wasp was established in all major walnut-growing areas and successfully controlled the aphid populations (Frazer and van den Bosch, 1973; van den Bosch et al., 1979). A study undertaken in 1977 in Tracy showed that parasitism rates by *T. pallidus* were over 50% throughout most of the season and reached 94% in late season. Following establishment of *T. pallidus*, the peak periods of *C. juglandicola* activity have changed from spring to mid or late summer (Frazer and van den Bosch, 1973; van den Bosch et al., 1979). However, summer outbreaks of walnut aphids can still considerably reduce walnut quality and yield (Barnes and Moffiti, 1978; Sibbett et al., 1982).

A new white morph of *C. juglandicola* was first discovered by us in the Sacramento Valley in 2003,

and has since spread throughout the east side of the Sacramento Valley with a strong likelihood that it will soon move down into the San Joaquin Valley also. In many orchards where it is present, both white and yellow forms of the aphid feed side by side on the same leaflets. More generally, aphids can occur in a variable range of color morphs, with a seasonal shift from green to red being the most common. The mechanisms for such color changes remain largely unknown, but could include nutritional or environmental (such as light and temperature) factors, or the action of bacterial symbionts (Jenkins et al., 1999). The consequences of color changes in aphids can be significant, however, with novel color morphs experiencing reduced rates of parasitism and predation (Ankersmit et al., 1981; Losey et al., 1997), and reduced susceptibility to insecticides (Harlow & Lampert, 1990; Kerns et al., 1998). In earlier research, we found that in general the white and yellow morphs of the walnut aphid are very similar in terms of rates of development, lifetime fecundity, and size. However, the two color morphs are significantly different in two subtle, yet important life history characteristics. Firstly, the age of first reproduction for the white morph is the first day of adult life as compared to the second day for the yellow morph. Then secondly, *T. pallidus* preferred to attack the later instars (3rd and 4th) of the yellow morph, but attacks 2nd to 4th instars equally for the white morph. Both of these characteristics confer an advantage to the white morph over the yellow morph, suggesting that populations of white walnut aphids are likely to grow more rapidly than those of yellow walnut aphids (Hougardy & Mills 2008). Whether the white morph of the walnut aphid is more resistant to insecticides than the yellow morph is currently unknown, but if so, could pose a more serious threat to the future of walnut pest management.

Aphids differ from other insect pests in that biological control by insect parasitoids can be compromised by hyperparasitism. High rates of hyperparasitism are common in aphid systems and five hyperparasitoid species have been reared from *T. pallidus* during our recent surveys: *Syrphophagus aphidivorus* (Encyrtidae), *Pachyneuron* sp. *(aphidis?), Asaphes suspensus, Asaphes californicus* (Pteromalidae) and *Dendrocerus* sp. (Megaspilidae; in 2004 only). The relative importance of these species varied with location and time, but *Syrphophagus aphidivorus* was the dominant species (51 – 100%) as in earlier studies (98%; Frazer & van den Bosch, 1973; van den Bosch et al., 1979). Thus enhancement of biological control of walnut aphid must take into account what effect walnut pest management products have on the walnut aphid itself, its effective parasitoid *T. pallidus*, and its dominant hyperparasitoid *S. aphidivorus*.

The susceptibility of walnut aphid to pesticides that currently used for the management of codling moth, walnut husk fly and spider mites is unknown and to our knowledge has never been tested due to the success of biological control. In contrast, the tolerance of *T. pallidus* to several of the older pesticides has received greater attention. In 1985 and 1986, efforts were made to isolate and release *T. pallidus* strains resistant to azinphos-methyl, until recently the standard spray treatment for codling moth (Hoy and Cave, 1988; Hoy et al., 1989; Hoy et al., 1990; Caprio et al., 1991; Hoy et al., 1991; Edwards and Hoy, 1995). Some strains successfully established in walnut orchards and interbred with native field biotypes to increase the level of pesticide tolerance. Cross-resistance to other organophosphate insecticides (such as chlorpyrifos, endosulfan, methidathion and phosalone) was observed in these azinphos-methyl resistant *T. pallidus* (Hoy and Cave, 1989; Brown et al., 1992). Other products have also been tested against *T. pallidus* (diflubenzuron, alsystin, CGA 112913 and thuringiensin) and no toxicity has been detected (Purcell and Granett, 1985). In contrast, pyrethroids used against husk fly cause heavy mortality of *T. pallidus* (Hislop et al., 1981). More recent bioassays carried out in our laboratory suggest that in the mummy stage, *T. pallidus* currently has little or

no resistance to chlorpyrifos, but is quite tolerant of pyrethroids (Mills et al., 2006), results that are in broad agreement with studies on other aphid parasitoid species (Longley, 1999). However, a greater understanding of the differential tolerance of *T. pallidus* to pesticides in the adult stage of its life cycle is clearly needed if compatible pest management products for use in walnuts are to be determined.

Far less is known of the influence of pesticides on aphid hyperparasitoids. They typically live longer and have a greater rate of search than primary aphid parasitoids, perhaps due to the lower levels of abundance of their hosts (Chua, 1979). This suggests that as adults they may come into greater contact with pesticide residues than primary parasitoids, but there have been few studies that document such interactions. However, adults of the specialist aphid hyperparasitoid *Alloxysta curvicornis* were found to be less tolerant of five aphicides than its primary parasitoid host (Wiackowski & Herman, 1968). In addition, the laboratory searching behavior of the specialist aphid hyperparasitoid *Dendrocerus carpenteri* was found to be disrupted in the presence of deltamethrin (Longley & Jepson, 1996). In recent tests in our laboratory it also appears that the generalist hyperparasitoid *Syrphophagus aphidivorus* is less tolerant than *T. pallidus* to topical application of a range of insecticides to parasitized aphid mummies (Mills et al., 2006). Thus this raises the possibility of utilizing the differential tolerance to different classes of pesticide to achieve insecticide selectivity in walnuts through choice of products that are simultaneously compatible with *T. pallidus*, but detrimental to hyperparasitoids.

A standard set of protocols have been developed in Europe by the International Organization for Biological Control for testing the compatibility of pest management products with natural enemies (Vogt 2003). These protocols consist of a three tier testing system that begins with simple tier I laboratory bioassays and finishes with full tier III field tests. However, pesticide toxicity ratings from tier I bioassays are often sufficient, as has recently been validated for natural enemies of grape pests in Australia (Thompson & Hoffmann 2006). In contrast, Stark et al. (2004, 2007) argue that for newer generation pesticides that are likely to have chronic and sub-lethal effects rather than acute mortality, a demographic bioassay based on life table response experiments is needed to ensure that products that pass the tier I acute toxicity ratings are fully compatible.

Plans and Procedures:

1. *To compare the susceptibility of white and yellow walnut aphids to walnut pest management products using simple laboratory bioassays*

The most effective way to compare the susceptibility of aphids to a variety of pest management products is to screen them through standard laboratory bioassays. In a comparison of bioassay techniques Lowery & Smirle (2003) and Lowery et al. (2006) found that rearing 3rd instar aphids on treated leaf disks provided the most accurate and useful information on susceptibility of tree dwelling aphids to a variety of insecticides. Following this approach, 20mm diameter walnut leaf disks will be treated by being dipped for 3 secs into one of a series of 5 serial dilutions of each insecticide and water as a control. A set of 4 treated leaf disks will then be placed into a ventilated plastic Petri dish (45 mm diameter) together with 10 3rd instar aphids of one of the two color morphs, and 5 separate dishes will be used as replicates for each dilution and the control. The Petri dishes will be held upside down on moist paper towel at 22°C for 2 days. The survival of each color morph after 48h will be used to determine the LC_{50} for each

product, or lethal concentration needed to kill 50% of the test aphids, using probit analysis. Isofemale lines of individual clones of both color morphs of the walnut aphid from a single orchard in the Sacramento Valley and of the yellow morph from a single orchard in the San Joaquin Valley will be maintained in a glasshouse at UC Berkeley on potted walnut seedlings. Initial products to be tested will include those currently registered in walnuts either for codling moth, husk fly or aphids including Guthion (as a historical baseline), Lorsban, Imidan, Asana, Intrepid, and Success, and, and will subsequently include newer and organic products such as Altacor, Delegate, Brigade, Warrior and Omni oil.

2. *To assess the differential susceptibility of T. pallidus and S. aphidivorus to walnut pest management products in simple laboratory bioassays*

Laboratory assays will be used to determine the effect of pest management products on the emergence and survival of *Trioxys pallidus* and *Syrphophagus aphidivorus*. In addition to the products used for the aphid bioassays, products such as Omite and Agrimek (miticides), and Kocide and Maneb (fungicides) will be included as they could also affect these parasitoids. Bioassays will target both the pupal (4 days before adult emergence within the aphid mummy) and adult stage of both parasitoids and the significance of the impacts will be assessed based on the standard IOBC toxicity ratings developed in Europe (Vogt 2000). These protocols suggest that any product causing less than 79% mortality of *T. pallidus* should be considered compatible, and greater than 79% mortality of *S. aphidivorus* should be considered selective.

For the pupal stage bioassay, walnut leaflets with 3-day old (*T. pallidus*) or 10-day old (*S. aphidivorus*) aphid mummies will be collected from laboratory cultures and dipped in the pesticide solutions. Three rates will be tested for each product: the recommended field rate (100%), 25% of the recommended field rate, and 0% as a control. A minimum of 30 mummies will be tested for each product and rate. After dipping the leaves will be kept in plastic boxes, the stem tightly wrapped in wet cotton and inserted in a vial full of water. This method has proven to keep the leaflet fresh until the emergence of parasitoids (approximately 3-5 days later). Leaflets will be naturally covered with honeydew, but additional honey will be provided in each plastic box every other day until the parasitoids die. Success of adult emergence, sex ratio of emerging adults, and adult survivorship of each parasitoid will be recorded at 22°C. Two weeks after treatment, unmerged mummies will be dissected and examined for the presence of a dead nymph or adult.

For adult bioassays, a combination of oral and residual routes of exposure will be used, and three rates of each product will be tested as for the pupal stage bioassays. Glass vials (8 x 2.5 cm) will be will filled with insecticide solutions and then drained and dried to provide a consistent residue on the inner surface. Similarly small droplets of a honey-sugar-agar mixture (10-1-1 ratio) will be used as food for the adult parasitoids and treated by dipping in insecticide solutions to gain a surface coating of residue. Three 1-2 day old adult female parasitoids will be placed into a treated glass vial, provided with treated food that will be changed every 2 days, and held at 22°C. Ten replicate vials will be used for each parasitoid species and each product rate, and the duration of adult survivorship will be monitored daily.

3. *To use a demographic bioassay to test a subset of pest management products for their compatibility with and enhancement of biological control of walnut aphid*

For the subset of pest management products that prove compatible for the tier I laboratory bioassays, an additional ecologically relevant bioassay will be conducted on adults of both *T. pallidus* and *S. aphidivorus* following Stark et al. (2004, 2007). As in the tier I adult bioassays, glass vials and honey-sugar-agar droplets will be treated with the full recommended field rate of the pesticide to be assayed. Individual male–female pairs of parasitoids will be held in the treated vials in environmental chambers at 22°C and offered 50 hosts daily on excised walnut leaflets until death of the females for *T. pallidus* and 7 days only for *S. aphidivorus*. Twelve replicate pairs of parasitoids will be held in treated vials and compared to 12 pairs in control vials. Following exposure to the parasitoids, the hosts will be held at 22°C in plastic boxes to await adult emergence, with the stem of the leaflets tightly wrapped in wet cotton and inserted in a vial full of water. Emerging offspring adults will be counted and their sex determined to estimate the sex ratio (=proportion of females). Additionally to monitor survivorship of parasitoid larvae, three replicates of 20 parasitized hosts will be dissected at intervals of 5, 7, 9, and 11 days after exposure to parasitism.

The life table data collected from these demography bioassays will be used to parameterize a Leslie matrix that will be used to estimate the rate of population growth of *T. pallidus* and *S. aphidivorus* for both treatments and controls. Following Stark et al. (2007) we will use an index of population recovery to assess compatibility with and enhancement of biological control of walnut aphid. The index of population recovery is determined from the Leslie matrix and is based on the delay in population growth of the treatment population in comparison to the control population. The product is deemed compatible with *T. pallidus* if the delay in population growth is less than one generation time interval. Similarly, the product is deemed selective and able to enhance the biological control of walnut aphid if the delay in population growth of *S. aphidivorus* is greater than one generation time interval.

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BUDGET REQUEST

Budget Year 2009

Funding Source Walnut Marketing Board

Salaries and Benefits			
Postdocs/RA's			
SRA's			
Lab/Field Assistance	12 mo @ 60%		17,209
Subtotal		Sub 2	17,209
Employee benefits		Sub 6	1,222
		TOTAL	18,431
Supplies and Expenses		Sub 3	2,500
Equipment		Sub 4	
Operating Expenses and Equipment Travel (Davis campus only)		Sub 5	
Travel		Sub 7	
		TOTAL	20,931

Department account number

(continued)

Additional funding sources:

This CWB project forms an integral part a larger project in the western region entitled 'Enhancing biological control to stabilize western orchard IPM systems' that is supported through a grant from the USDA-SCRI. The funding from the CWB will serve as matching funds for the USDA-SCRI project.

Originator's Signature — Date 10/7/08

COOPERATIVE EXTENSION — County Director — Date

Program Director — Date

AGRICULTURAL EXPERIMENT STATION — Department Chair — Date 10/7/08

LIAISON OFFICER — Date

D2454-2 (1/84)