RESEARCH PROJECT REPORT
California Almond Board
1 July 2005

Project Leader: Marshall W. Johnson, Associate Extension Specialist, Dept of Entomology, UC Riverside; Phone: (559) 646-6519, FAX 646-65; Email: mjohnson@uckac.edu

Location: Kearney Agricultural Center, 9240 S. Riverbend Ave, Parlier, CA 93648

Co-PI: Mark Freeman Location: 1720 South Maple Ave., Fresno, CA 93702-4516
Farm Advisor, University of CA Cooperative Extension (UCCE)
Mailing Address: 1720 South Maple Ave., Fresno, CA 93702-4516
Phone: 559-456-7265 FAX: 559-456-7575 E-mail: mwfreeman@ucdavis.edu

Co-PI: Richard Coviello Location: 1720 South Maple Ave., Fresno, CA 93702-4516
Farm Advisor, University of CA Cooperative Extension (UCCE)
Mailing Address: 1720 South Maple Ave., Fresno, CA 93702-4516
Phone: 559- 456-7549 FAX: 559-456-7575 E-mail: rlcoviello@earthlink.net

Cooperating Personnel: Walter Bentley, Regional IPM Entomologist, UCCE, Kearney Ag Center
Frank Zalom, Entomology, UC Davis
Michael McKenry, Plant Patholgy, UC Riverside
Michael Klein, USDA-ARS, Wooster, OH
Albrecht Koppenhofer, Entomology, Rutgers Univ., New Brunswick, NJ
Harry Kaya, Entomology, UC Davis

Project Title: *Biology and Management of Tenlined June Beetle in Almonds*

Current Report Period: 1 May 2004 to 1 July 2005

Objectives:

1. Develop, conduct, and analyze a formal survey of growers and Pest Control Advisors regarding Tenlined June Beetle (TLJB).
2. Evaluate new soil insecticides available for scarab grub control.
3. Evaluate the potential of using insect pathogens for TLJB control.
4. Determine horticultural factors (such as rootstocks, water stress management) that may influence TLJB damage.
5. Develop grower usable sampling methods for TLJB detection.

General Progress:

TLJB studies were initiated in mid-June 2003 and have continued to date. The first funded year of this project was completed in August 2004. Studies to date have included a) establishment of light trap surveys to detect and estimate TLJB infestations as indicated by numbers of adult beetles; b) development of methods to detect and sample for the immature stages of TLJB within the soil substrate of almond orchards; c) efforts to observe the scoliid wasp *Campsomeris pilipes* (Saussure) that parasitizes the beetle grubs; d) efforts to locate naturally occurring nematodes that attack and reproduce on TLJB grubs; e) development of techniques to test insecticides and insect pathogens for control of TLJB grubs; and f) studies to determine the impact of TLJB grub feeding on almond plant growth and root development. Due to the importance of sampling techniques to estimate TLJB grub and adult densities for biology and control studies, the goals of Objective 5 were the initial focus of the work to date. Experimental units were designed to conduct laboratory tests on various aspects of grub biology (Obj. 4) and susceptibility to soil pesticides (Obj. 2) and insect pathogens (Obj. 3). Fresno County Farm Advisor Mark Freeman provided valuable assistance in regards to locating grower cooperators; carrying out the light trap surveys; and other work on the project. Detailed information follows relative to the various objectives.

Two part-time laboratory assistants (Luis Rodriguez and Martha Gerik) were hired on the project in March 2004 to assist with the work for Spring and Summer 2004. Mr. Rodriguez previously helped on the project when employed by Mark Freeman, and he was carried on the project through Fall 2004 and part of Winter 2005.. Unfortunately, the services of Mr. Rodriguez were lost in February 2005, and we were only permitted to search for a replacement as of 1 June 2005. No replacement has been found as of the writing of this report.

One challenge that was not anticipated at the beginning of this project was the difficulty in obtaining sufficient numbers of beetle grubs to conduct tests. Given that the TLJB has a two-year developmental cycle (from egg to adult), one cannot quickly and easily grow the beetle in the laboratory to obtain the numbers of grubs needed for various tests. It is not unusual to need as many as 500 to 1,000 individual grubs for testing pesticides, insect pathogens, or various aspects of the insect's biology (e.g., effects of various rootstocks on grub development, influence of soil composition on grub survival, etc.). We have been collecting grubs from infested orchards, bringing them back to the laboratory, and then holding them in soil (and feeding them carrots) until we need them. Digging for the grubs is laborious, and we developed more effective collection techniques. It was found that running the soil through sieves helped to quickly locate 2nd and 3rd-instar grubs in the soil. However, we have not encountered too many 1st-instars nor pupae. To obtain TLJB eggs and 1st-instars, we have to collect fertilized females and hold them in the laboratory for egg production. Once in the laboratory, it was found that we could feed the grubs organic carrots.

Although this project has not been funded for 2005-2006, we will continue to work on the objectives below and will submit a final report in 2006.

Specific Progress:

Objective 1. We have no new progress to report on this objective. As we gained more information from the field, our questions for the growers have changed. We intend to conduct the survey this summer.

Objective 2. We have refined our testing techniques and grub collection methods and will be conducting the work on this objective during this summer. Halofenozide (Mach 2 / Grub-X), imidacloprid (Merit / Marathon), Thiamethoxam (Meridian), Oxamyl (Vydate), Carbaryl (Sevin), and Chlorpyrifos (Lorsban) are being evaluated for their ability to kill the grub stages of the

TLJB. Given the difficulty in accurately locating TLJB infestations in orchards, the spotty distribution of the grubs within the orchards, and the amount of work needed to find and collect the grubs after a field treatment, it was decided to conduct the initial screening work in the laboratory. To date, we have developed a 6-inch diameter "soil column" to test the efficacy of the various insecticides after penetrating various soil depths (e.g., 3, 6, 9, 12, 15, 18, 21 inches). The columns are made from PVC irrigation pipe. The columns are filled with sandy soil (about 90% sand in composition and purchased from a local supplier) and the "test" insecticides are dripped through the soil using various application techniques to determine the optimum chemical movement method. Field collected grubs are used in the studies. "Test" grubs are placed at the bottom of the column to determine insecticide penetration through the soil. Once effective compounds and application techniques are identified, we will conduct field tests.

Objective 3. Work with the insect pathogens was also dependent on construction of the soil columns (see Obj. 2) to test soil penetration. Dr. Robert Fritz, Certis Corporation (Columbia, MD), provided the entomopathogenic nematode (attacks insects) known as *Steinernema riobrave* Cabanillas, Poinar & Raulston, which is commercially sold for augmentative purposes. This species was suggested by Dr. Larry Duncan, University of Florida at Gainesville, and is used in Florida to control the citrus root weevil, *Diaprepes abbreviatus*, which is found in sandy soil. Preliminary tests with *Steinernema riobrave* indicated it would attack TLJB grubs. Unfortunately, continuation of our testing was disrupted because Certis Corporation limited distribution of the nematode to consumers during specific times of the year. Later, the company completely stopped distributing the nematode. We are now seeking nematodes from Becker Underwood, the producer that originally supplied *S. riobrave* to Certis Corporation.

We plan also to examine the entomopathogenic nematode *Heterorhabditis bacteriophora* that is indigenous to California and produced in southern California by Ricon-Vitova Insectaries, Inc., Ventura, CA. Testing is underway now. A grower reported some success with this species in an almond orchard east of Madera.

Field collection of TLJB 3rd instar grubs from a cherry orchard near Carruthers revealed that a nematode was naturally infecting and killing grubs in that area. We are unsure whether the nematode found was a "true" entomopathogenic nematode (i.e., one that requires insect hosts to reproduce and survive) or a "facultative" natural enemy (i.e., one that may only attack stressed insects and is not a highly effective natural enemy). We have also seen this nematode in TLJB individuals that we have held in the laboratory for significant lengths of time. Attempts to establish a laboratory colony of the nematode species were unsuccessful, but we will attempt to recover the nematode again from the orchard of origin.

Objective 4. Fifteen special testing units, resembling giant ant farms, were constructed from plastic sheets to conduct experiments for this objective. We have initiated tests on the preference of TLJB grubs for various rootstocks (provided by Dave Wilson Nursery, Reedley). These run for 60 days at a time. For each test, 4 different rootstocks were established in the soil in the test unit and 20 third instar grubs were introduced to each unit. Nonpareil scion on Nemaguard rootstock served as a control standard. In the initial test, Hansen, Atlas, and Viking rootstocks (with Nonpareil scions) were compared to the Nemaguard control. Amounts of damage to the rootstocks were determined based on loss of root dry weight compared to control plants that were not exposed to the grubs. Potential changes in the above soil stems were also evaluated by comparing stem and leaf growth on exposed and unexposed plants.

There was significant variation in dry root weight among the rootstock varieties (Two Way ANOVA, $F_{3,24} = 16.13$, $P < 0.0001$) with the roots of Atlas and Nemaguard being significantly smaller than those of Hansen (the largest) and Viking varieties (Fig. 1). Rootstocks that were exposed to 3rd instar TLJB grubs weighed significantly less (dry root weight) than those not exposed (Two Way ANOVA, $F_{1,24} = 4.45$, $P = 0.045$) (Fig. 1). On average, reduction in root dry weight by grub feeding for all rootstocks was 37.26%. The roots of Hansen rootstock were reduced about 26.2% compared to 65.2% for the Atlas rootstock. There was no interaction between rootstock variety and exposure to TLJB. This means that no preference of TLJB grubs for one rootstock over another was apparent.

There was significant variation in dry weight of stems and leaves of the rootstock varieties (Two Way ANOVA, $F_{3,24} = 20.02$, $P < 0.0001$). However, exposure of the plants' roots to TLJB grub feeding did not significantly impact the growth of the above ground plant structures over the 60 day test period (Two Way ANOVA, $F_{1,24} = 1.21$, $P = 0.282$). This suggests that TLJB grubs can greatly reduce root capacity without symptoms of plant damage immediately showing up on the above ground plant structures. If this trend occurs in the field, it could have tremendous consequences for growers because large amounts of subsurface damage could occur before a tree would exhibit any significant changes in above surface growth and development. We plan to continue these studies if funding can be found to support the work.

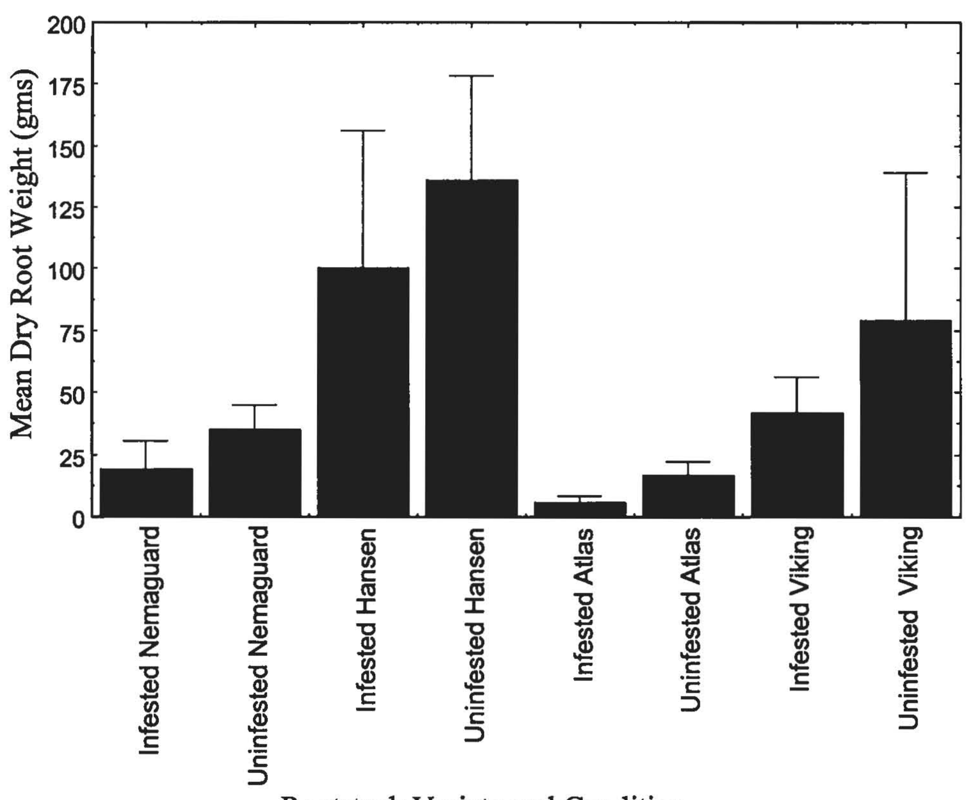

Fig. 1. Comparison of dry root weights of infested and uninfested almond rootstocks exposed to 3rd instar tenlined June beetles for 60 days.

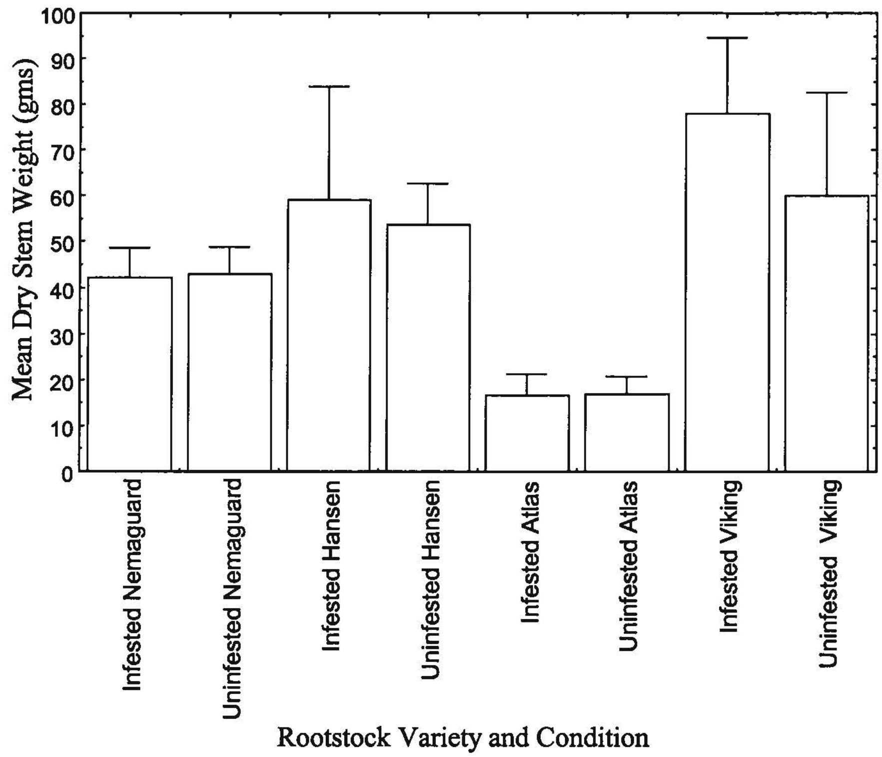

Fig. 2. Comparison of above ground dry stem weights of infested and uninfested almond rootstocks exposed to 3rd instar tenlined June beetles for 60 days.

Objective 5. In 2003, light traps were maintained adjacent to four almond orchards in the Carruthers area (southwest of Fresno) and adult beetles were collected at all sites with high variation in trap counts among sites (Fig. 3). The highest nightly trap catch at one site was 629 total adults. Light traps appear to be an effective way to monitor for male adult beetles, but ratios of male to female beetles within traps were predominantly male-biased. Mean ratios of male to female individuals trapped were between 406 to 1 and 1 to 1 (males to females) among the 4 sites monitored, suggesting that the females do not readily fly at night or that they are not attracted to traps. Peak trap counts for the summer survey period varied among sites with peaks occurring during the 1st and 2nd weeks of July 2003. Checking traps on a weekly basis resulted in traps in some areas being "swamped" with beetles and other insects, thereby leading to less accurate beetle counts because at some point the trap filled up and no more beetles were trapped. During Summer 2004, we again trapped at various locations that included the Carruthers area, Cobb Ranch (NW Fresno area) and the UC Kearney Agricultural Center (west of Parlier) (Fig. 4). Overall, the trap catches in 2004 were less than recorded in 2003 with the highest nightly trap count being 89 total adults per night (Carruthers area). Peak trap counts in Summer 2004 were more variable than in 2003 and were observed during mid-June (NW Fresno) through July (Carruthers and Parlier areas). Of special interest was that light traps established at some distance from almond orchards at the Parlier site had a smaller male to female ratio in the trap counts than

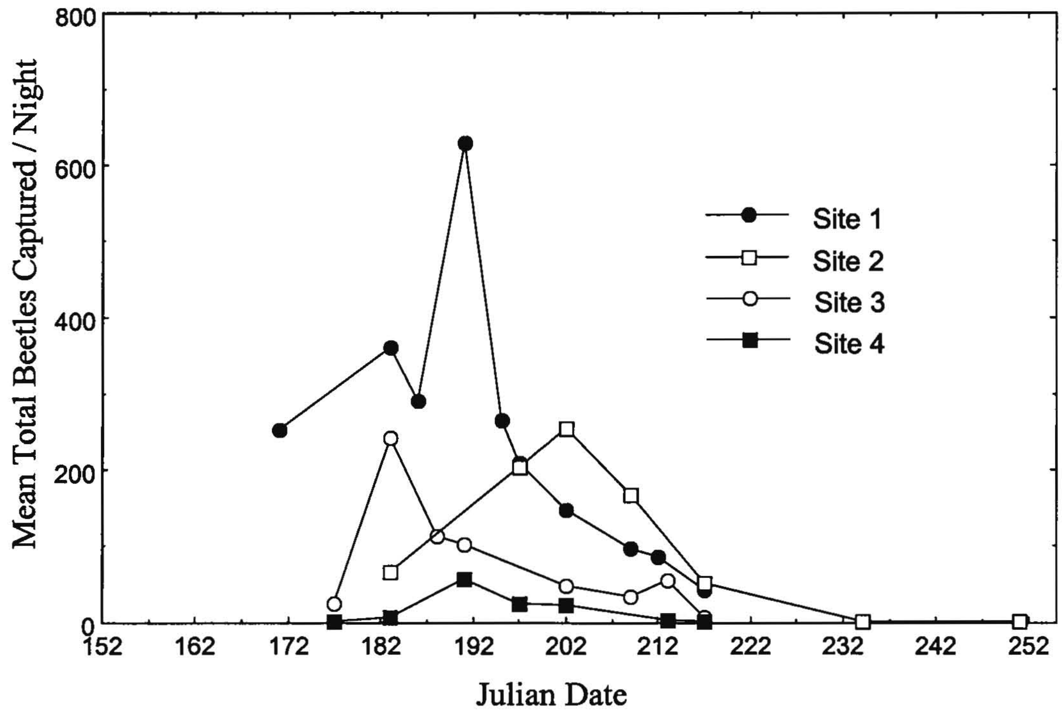

Fig. 3. Mean numbers of total tenlined June beetles captured per night in light traps at four sites southwest of Fresno, CA, during summer 2003.

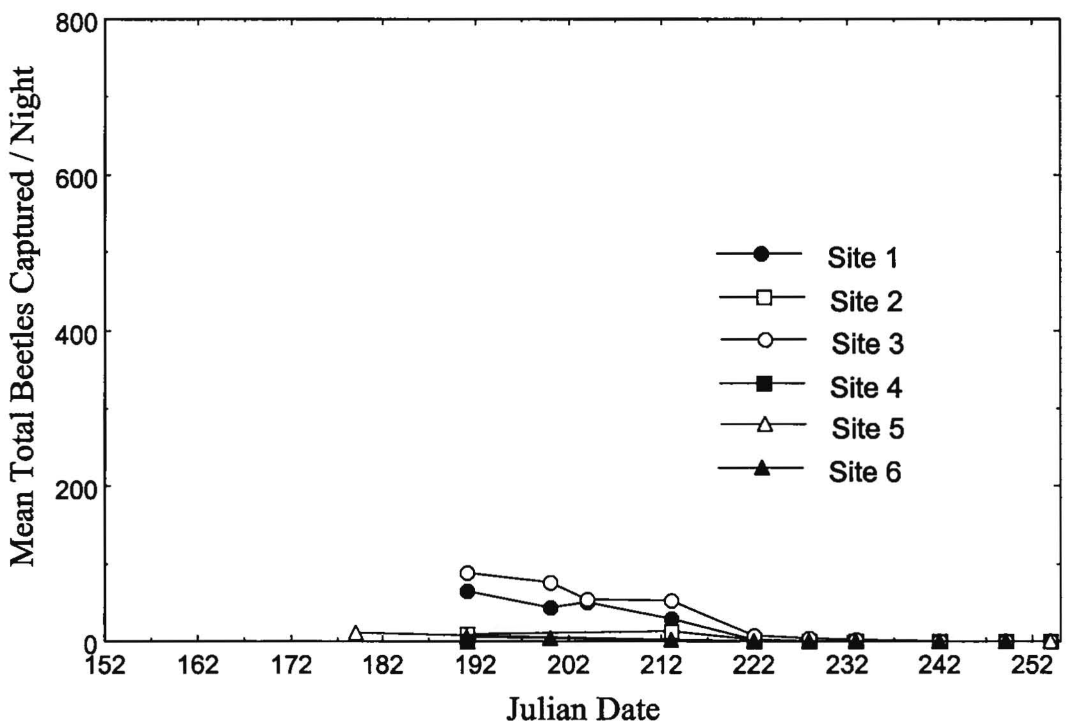

Fig. 4. Mean numbers of total tenlined June beetles captured per night in light traps at six sites south of Fresno, CA, during summer 2004.

light traps established within orchards. Maximum male to female ratios at Parlier were 6 to 1 and 18 to 1 (males to females) at Sites 1 and 2, respectively, compared to male to females ratios of 36 – 96 to 1 in the Carruthers area. We hypothesize that within orchards more males may be present due to the phermones given off by calling females. Between orchards the light traps may pick up more females dispersing among orchards. More work is needed to determine this.

During the day, adult females are underground. Adult male beetles tend to hide in leaf litter and under objects (e.g., boards, rocks) within or near almond orchards during the daylight hours. Male beetles may be found among tree foliage in some stone fruit trees, but this is an uncommon event.

We found that 2nd and 3rd instar grubs are usually found adjacent to tree roots. Grubs may be found in the absence of emergence holes produced by adult beetles when leaving the soil substrate. Random soil samples using soil cores and a power auger rarely produced grubs because tree roots could not be included in the sample. Surveying for beetle grubs was physically demanding and time consuming work. Attempts to use organic (i.e., pesticide free) carrots inserted perpendicularly into the soil to detect grub feeding within the orchard were unsuccessful. Standardized soil sampling methods need to be developed so grub densities within and among orchards can be accurately compared. Adult beetles and pupae were rarely found in soil samples, except in early summer. We will continue to develop standardized soil sampling methods during Summer 2005. To reduce the amount of physical labor while sampling / collecting grubs, we have employed a raisin shaker to help sift through the soil while looking for the grubs. The shaker has made it easier to collect 2nd and 3rd instar grubs as well as female and male adults and pupal coccoons of the scoliid parasitoid *Campsomeris pilipes* (Saussure). Because the parasitoid cocoons are "soil colored", they were often missed when soil was not sieved.

Campsomeris pilipes female adults can be easily found searching for beetle grubs during the early morning hours in highly TLJB infested orchards. Adult wasps locate potentially grub-infested sites and quickly tunneled down through the soil to the grubs to parasitize them. More information is needed on the impact of the parasitoid on TLJB densities. It was discovered that adult *C. pilipes* males will enter into holes (about 2 inches diameter and 6 inches deep) within the orchard floor and are unable to escape if the sides of the hole are smooth. Efforts will be made to utilize this knowledge to design a monitoring method to estimate wasp densities among almond orchards.