PUPATION BIOLOGY OF *FRANKLINOTHRIPS ORIZABENSIS* (THYSANOPTERA: AEOLOTHRIPIDAE) AND HARVESTING AND SHIPPING OF THIS PREDATOR

MARK S. HODDLE, KIKUO OISHI, AND DAVID MORGAN
Department of Entomology, University of California, Riverside, CA 92521, USA

ABSTRACT

In the laboratory, 82-99% of late second instar *Franklinothrips orizabensis* Johansen larvae abandoned avocado branches and artificial branches constructed of wooden dowels and were recovered below branches trapped on tangle foot coated plastic sheets, suggesting a preference by this life stage for selection of pupation sites beneath host plants. Of three media tested (coarse and fine vermiculite, and parafilm cones) for harvesting *F. orizabensis* pupae in cocoons, parafilm cones were most easily harvestable from colonies, and 44% of deployed late second stage larvae that were recovered used parafilm cones for pupation in experimental cages. Harvesting and shipping trials using aspirated adult *F. orizabensis* or pupae in parafilm cones showed significant differences in survivorship when held in the laboratory or shipped round trip from Riverside, California to Amherst, Massachusetts. Survivorship of aspirated adults was reduced on average by 41% following shipping, and mortality was highest for adult males. Transit survivorship was increased by 53% if *F. orizabensis* were shipped as cocoons in parafilm cones. Inclusion of ice packs in polystyrene boxes did not significantly increase survivorship rates for *F. orizabensis* adults or pupae that were either retained in the laboratory or shipped. This result may have been an artifact resulting from the time of year (i.e., May and temperatures were moderately cool) when shipping trials were conducted.

Key Words: *Franklinothrips*, pupation biology, harvesting, shipping

RESUMEN

En el laboratorio, 82-99% de las larvas en el segundo instar de *Franklinothrips orizabensis* abandonaron las ramas de aguacate y las ramas artificiales construidas de clavijas de madera y fueron recuperadas bajo ramas atrapadas en hojas plásticas con capa pegajosa "tangle foot", sugiriendo una preferencia de selección de lugares de pupación debajo de plantas huéspedes por esta etapa de vida. De les tres medios probados (vermiculita fina y ordinaria, y conos de parafina) para cosechar pupas de *Franklinothrips orizabensis* en capullos, conos de parafina fueron los mas fáciles de cosechar de las colonias, y el 44% de larvas de segunda etapa que fueron recuperadas usaron conos de parafina para pupación en jaulas experimentales. Ensayos de cosecha y embarque usando adultos aspirados de *Franklinothrips orizabensis* o pupas en conos de parafina demostraron diferencias significativas en supervivencia al ser llevadas a cabo en el laboratorio o embarcadas ida y vuelta desde Riverside, California hasta Amherst, Massachussets. Supervivencia de adultos aspirados fue reducida un promedio de 41% después de embarque, y la mortalidad fue mayor entre machos adultos. Supervivencia de transporte fue incrementada por 53% si *Franklinothrips orizabensis* eran embarcadas como capullos en conos de parafina. La inclusión de bolsas de hielo en cajas de poliestireno no incrementó significativamente las cantidades de supervivencia para adultos o pupas de *Franklinothrips orizabensis* que fueron retenidas en el laboratorio o embarcadas. Este resultado puede haber sido un artefacto resultando por el tiempo del año (es decir, Mayo y las temperaturas eran moderadamente frías) cuando los ensayos de embarque fueron llevados a cabo.

Inoculative, augmentative, and inundative biological control programs targeting pestiferous thrips in perennial outdoor crops have seldom been successful (Parker & Skinner 1997). The major limiting factors that have been identified as constraints on successful thrips biological control programs for outdoor crops are: (1) lack of effective resident natural enemies that respond in a rapid density dependent manner to increasing thrips densities. (2) Thrips phenology and life cycle characteristics can result in long periods of low thrips densities. In some instances this may facilitate rapid pest outbreaks as thrips natural enemy densities have declined because of lack of prey. (3) Incompatibility of thrips natural enemies with broad-spectrum insecticides used to control other crop pests. (4) The high cost of insectary-reared natural enemies make large-scale field releases for thrips control into outdoor crops of low value unfeasible economically (Grafton-Cardwell & Ouyang 1995a; Parker & Skinner 1997; Parrella & Lewis 1997). Natural enemies that have been commonly observed with phytophagous thrips in perennial tree crops are phy-

toseiid mites and predatory thrips (Parker & Skinner 1997, Parrella & Lewis 1997).

In some instances, thrips biological control by Type IV phytoseiid mites (i.e., specialized pollen feeders that exhibit generalist predatory activity [McMurtry & Croft 1997]) can be enhanced by pollen bearing wind break trees (Grout & Richards 1990, 1992a,b) or cover crops in orchards (Grafton-Cardwell et al. 1999a). Crop management practices such as pruning that enhances succulent leaf material can also promote increased densities of Type IV phytoseiids (Grafton-Cardwell & Ouyang 1995b; Grafton-Cardwell 1997). Augmentative releases of insectary-reared phytoseiids for thrips control have been shown to significantly reduce densities of *Scirtothrips citri* Moulton (Thysanoptera: Thripidae) on citrus (*Citrus* spp.), but, at present, this technology is not cost effective (Grafton-Cardwell & Ouyang 1995a; Grafton-Cardwell et al. 1999b).

Scirtothrips perseae Nakahara (Thysanoptera: Thripidae) was first discovered damaging avocado foliage and fruit in southern California orchards in 1996, and at time of discovery was a species new to science (Nakahara 1997). In 1998, crop losses due to down-graded fruit and increased production costs due to *S. perseae* feeding damage were estimated to have cost California growers (US) $7-$13 million (Hoddle et al. 1998, 1999). Foreign exploration efforts to determine the native range of *S. perseae* indicate that this pest is of Central American origin (Hoddle et al. 1999) and associated natural enemies collected concurrently from avocados in Latin America have included *Franklinothrips* spp. (Hoddle, unpublished).

Surveys in southern California avocado orchards for indigenous natural enemies associated with *S. perseae* have revealed that an undescribed species of *Franklinothrips orizabensis* Johanson (Thysanoptera: Aeolothripidae) is the dominant predator where this pest has attained high densities (>10 *S. perseae* larvae per leaf) (Hoddle, unpublished). Female *Franklinothrips* spp. lay eggs directly into plant tissue. Developing larvae pass through two instars (second instars are distinguished from firsts by red hypodermal pigments), before pupating within silk cocoons which are spun from secretions produced from the anal region (Arakaki & Okajima 1998).

Unlike *Euseius tularensis* Congdon (Acari: Phytoseiidae), which in some instances can regulate citrus thrips, *S. citri* (Moulton) (Grafton-Cardwell & Ouyang 1995b), *E. hibisci* (Chant) a common Type IV phytoseiid in southern California avocado orchards, has not been observed to respond in a significant density dependent manner to increasing *S. perseae* populations (Hoddle unpublished). Because of industry interest, the potential for using *F. orizabensis* for augmentative releases against *S. perseae* in avocado orchards is being investigated. Optimal temperature requirements and diets for mass rearing this predator have been evaluated (Hoddle et al. 2000, 2001) and limited field trials evaluating releases of *F. orizabensis* against *S. perseae* on avocados have been conducted (Silvers 2000).

Augmentative field releases of mass-reared *F. orizabensis* onto avocado trees failed to significantly suppress *S. perseae* populations in California. This may have been due more to the poor quality of adults which suffered high mortality (>50%) after shipping from an insectary in Europe rather than the inherent ineffectiveness of *F. orizabensis* (Silvers, 2000). In order to fully explore the potential of augmentative releases of *F. orizabensis* for controlling of *S. perseae* in California avocado orchards, low impact techniques for harvesting and distribution that minimize transit mortality of this predator are required. One possible approach would be to collect and transport *F. orizabensis* when larvae are pupating within protective silk cocoons as opposed to aspirating and shipping adults. The purpose of this study was to investigate the pupation biology and behavior of *F. orizabensis*, and to develop techniques based on an understanding of pupation behavior for collecting and shipping pupating larvae and compare survivorship rates to currently employed methods for collecting and shipping adult predators.

Materials And Methods

Franklinothrips orizabensis Colony

Franklinothrips orizabensis colonies were maintained in cages in a temperature controlled room (25°C, 60% RH, L:D 14:10) on lima beans (*Phaseolus lunatus* Linnaeus variety "Baby Fordhook" [plants are needed for oviposition of eggs by female *F. orizabensis*]) at the University of California Riverside, California, USA. Colony reared *F. orizabensis* were fed irradiated *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs (supplied by Beneficial Insectaries, Oak Run, CA, USA) which were liberally deposited on upper surfaces of horizontal bean leaves. Our colony was initiated with adult *F. orizabensis* collected from an avocado orchard infested with *S. perseae* in Fallbrook, California, USA. Adult progeny produced by field collected *F. orizabensis* used to initiate the colony were deposited with the Systematic Entomology Laboratory, USDA-ARS, Beltsville, Maryland, USA and identified as *F. orizabensis* by Dr. S. Nakahara.

Source of Second Instar *Franklinothrips orizabensis* Larvae for Experiments

Undersides of mature 'Hass' avocado leaves collected from the Biological Control Grove (*F. orizabensis* has not been collected here) at the University of California, Riverside were pre-

sented to colony-reared female *F. orizabensis* for oviposition (Hoddle et al. 2000). Adult females were confined with male *F. orizabensis* within modified Munger cells (Munger 1942, Morse et al. 1986), fed irradiated *E. kuehniella* eggs, and left to oviposit in temperature cabinets (25°C, L:D 14:10 h).

At the end of the 24 h oviposition period, the leaf area enclosed by the Munger cell which was exposed to ovipositing females was excised from the avocado leaf. Trimmed leaves were labeled, placed on water-saturated foam pads in stainless steel trays, and incubated at 25°C. Leaves were examined daily for emerged *F. orizabensis* larvae. Emerged first instar larvae were collected with a fine camel hair brush, placed individually in 1 dram glass shell vials with irradiated *E. kuehniella* eggs as food, and vials were sealed with parafilm® (American National Can, WI). Larvae were reared in temperature controlled cabinets (25°C, L:D 14:10 h) in shell vials until second instars which were then used for experiments. Late second instar larvae select pupation sites and spin pupation cocoons within which propupal and pupal stages occur.

Pupation Behavior

Pupation behavior experiments were conducted in the laboratory at 25°C and ambient light to determine the proportion of *F. orizabensis* larvae that pupate on avocado branches and artificial wooden branches.

Pupation on avocado branches. Individual branches were selected on small (1.5-2 m tall) Hass avocado trees planted in 20 liter plastic pots. Tanglefoot was applied at the base of branches at trunk attachment to prevent larvae leaving experimental branches. Clear plastic sheets (60 cm × 40 cm) covered with tanglefoot were placed under each experimental branch and the outline of the overhanging branch was drawn in the tanglefoot. Ten late second instar *F. orizabensis* larvae of known age were placed on avocado leaves and supplied with irradiated *E. kuehniella* eggs as food. Larvae were left for three days and then numbers recovered from tanglefoot covered plastic sheets were recorded, and the perpendicular distance jumped from branches was determined by measuring distances from larvae to drawn branch outlines in tanglefoot. Avocado branches were removed from trees and examined under a dissecting microscope and numbers of larvae pupating on branches were recorded. This experiment was replicated 10 times.

Pupation on artificial branches. Artificial branches were constructed of wooden dowels 90cm long and 0.75 cm wide and bouquets of avocado leaves wrapped in moist paper towels were attached to distal ends of dowels with parafilm. Proximal ends of artificial branches were attached to a 1.2 m ringstand with a versatile clamp. A second versatile clamp was used to hold a clear sheet of plastic (100cm × 30 cm) covered with tanglefoot under the artificial branch. Proximal ends of artificial branches were coated with tanglefoot to prevent larval escape. Outlines of artificial branches were drawn in tanglefoot. Two types of artificial branches were used: (1) wooden dowels without pupation refugia, and (2) wooden dowels with pupation refugia. Refugia consisted of four 3mm diameter holes drilled equidistant around the dowel's circumference at 20 cm intervals from the distal end. Artificial pupation sites were provided to determine the propensity of *F. orizabensis* larvae to stay on branches to pupate when abundant sites were available in comparison to artificial branches without refugia, or naturally occurring pupation sites on avocado branches. Ten late second instar *F. orizabensis* larvae of known age were placed on avocado leaf bouquets attached to artificial branches and supplied with irradiated *E. kuehniella* eggs as food. Larvae were left for three days and numbers of larvae on tanglefoot covered plastic sheets were recorded. The perpendicular distance jumped from branches was determined by measuring distances from larvae to drawn branch outlines in tanglefoot. Artificial branches were examined under a dissecting microscope and numbers of larvae pupating on wooden dowels were recorded. Experiments were replicated 10 times for artificial branches with and without refugia.

Pupal Harvesting Techniques

The pupation behavior study outlined above indicated that >80% of late second instar *F. orizabensis* larvae would fall or jump from natural and artificial branches to seek pupation sites below branches (see Results Section). Since late second instar larvae spin silk cocoons we sought to identify a media within which larvae would pupate that could allow easy harvesting of *F. orizabensis* in cocoons. Evaluations of pupation media were conducted in temperature controlled cabinets (25°C, L:D 14:10). Five late second instar *F. orizabensis* larvae were placed with pupation media in glass Petri dishes (9.5 cm diameter) with lids which were sealed with parafilm and left for three days in temperature controlled cabinets. Cocoons were then located in Petri dishes and numbers of adult *F. orizabensis* that successfully emerged following harvesting were recorded daily.

Vermiculite. Vermiculite (Therm-o-Rock West Inc., Chandler, AZ) was sieved into two grades coarse (all granules that were not retained by 20 mesh USA Standard Testing Sieve) and fine (all granules not retained by 35 mesh USA Standard Testing Sieve). Five ml of each material was placed into glass Petri dishes and the ability to harvest cocoons spun onto vermiculite granules was assessed after three days. Each vermiculite

treatment was replicated 10 times. Collection of cocoons was attempted by sieving vermiculite with a 20 mesh sieve based on the assumption that cocoons encased with vermiculite would be too large to pass through and would be retained.

Parafilm cones. Parafilm cones (diameter 3 mm, height 5 mm) (Fig. 1) were constructed by wrapping strips of parafilm (4 mm wide, 15 mm long) around the tapered end of a camel hair brush. Five parafilm cones were placed in each Petri dish, and this treatment was replicated 10 times. Cone utilization for pupation by *F. orizabensis* larvae was visually assessed after three days.

Parafilm cones and fine vermiculite. Five parafilm cones and 5 mls of fine vermiculite were combined in a glass Petri dish. This treatment was replicated 6 times, and utilization of either cones or vermiculite for pupation was assessed after three days.

Selection of Pupation Sites in Small Rearing Cages

Thirty individually-reared late second instar *F. orizabensis* were hand placed on four lima bean plants contained within rearing cages (30 cm × 30 cm × 37.5 cm), fed irradiated *E. kuehniella* eggs which were liberally deposited on upper surfaces of horizontal bean leaves, and maintained in a temperature controlled room (25°C, 60% RH, L:D 14:10) for three days. Cages were supplied with 50 parafilm cones which were distributed on potting media and cage floors. After three days, numbers of *F. orizabensis* cocoons in parafilm cones, in cage corners and sleeves, on bean plants, and in vermiculite in which bean plants were growing (plants were destructively sampled) were determined. After inspection for cocoons, experimental cages were examined every two days for 10 days for adults that emerged from cocoons that were not detected during initial cage inspection. This treatment was replicated 10 times.

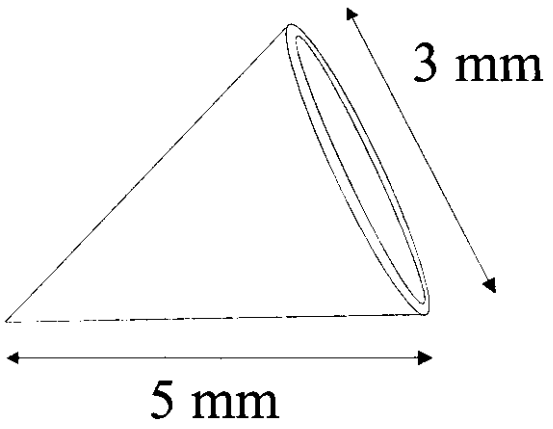

Fig. 1. Schematic of a parafilm cone used for harvesting *Franklinothrips orizabensis* pupae.

Harvesting of *Franklinothrips orizabensis* Pupae from Laboratory Colonies

Parafilm cones were readily used as pupation sites by *F. orizabensis* larvae in glass Petri dishes and small rearing cages (see Results Section). The efficacy of using parafilm cones to harvest *F. orizabensis* pupae was determined in laboratory colonies. *F. orizabensis* colonies used for this experiment were fed irradiated *E. kuehniella* eggs and maintained in cages (75 cm × 40 cm × 45 cm) with 9-12 lima bean plants. Plants were raised 3 cm from the cage floor on metal pipes fixed to cage walls. Under these metal supports a clear plastic sheet (70 cm × 30 cm) was positioned holding 100 parafilm cones (Fig. 2). Parafilm cones were left beneath plants for three days before the plastic sheet with cones was removed. Cones containing pupae were counted, held in Petri dishes in a temperature controlled cabinet (25°C, L:D 14:10), and number and sex of emerging adult *F. orizabensis* was recorded daily. This trial was replicated 10 times and approximately 300 adult *F. orizabensis* were present in mass-rearing cages each time.

Shipping Adult and Pupal *Franklinothrips orizabensis*

Thirty individually-reared adult *F. orizabensis* (15 male and 15 female; all were 1-2 days of age) were aspirated into three dram glass shell vials, supplied with irradiated *E. kuehniella* eggs as food, and sealed with a wad of cotton wool. Thirty *F. orizabensis* pupae (1-2 days of age) in parafilm cones were placed in three dram shell vials and sealed with cotton wool. Aspirated adults and pupae in parafilm cones were each subjected to one of the following treatments:

Aspirated adults and cocoons retained in the laboratory. The purpose of this experiment was to quantify predator mortality in the absence of shipping stress. Aspirated adults and pupae in parafilm cones were placed in sealed polystyrene foam boxes (20.5 cm × 16.5 cm × 15.5 cm) with or without ice packs. Temperatures and humidities in boxes were recorded every 10 mins with Hobo data loggers (Onset Computer Corp., Pocasset, MA). Survivorship of aspirated adults was recorded after 48 h and pupae in parafilm cones were removed from boxes after 48 h and reared in a temperature controlled cabinet (25°C, L:D 14:10) to determine emergence rates and sex. This treatment was replicated five times.

Aspirated adults and cocoons shipped to Massachusetts. The purpose of this experiment was to quantify predator mortality due to shipping stress. Adult and pupal *F. orizabensis* were collected, prepared, and shipped in polystyrene foam boxes with or without ice-packs with Hobo data loggers as described for the laboratory retention study. Boxes were shipped Federal Express prior-

Fig. 2. Parafilm cones on a clear plastic sheet that is placed beneath elevated bean plants in a *Franklinothrips orizabensis* rearing cage. *Franklinothrips orizabensis* pupae are harvested from the laboratory colony in parafilm cones.

ity overnight to Amherst, Massachusetts and then returned immediately the next day by Federal Express priority overnight to Riverside, California. Upon receipt in Riverside, proportion of adults surviving was determined, and pupae in parafilm cones were removed from boxes and reared in a temperature controlled cabinet (25°C, L:D 14:10) to determine emergence rates and sex. Round trip transit time from California to Riverside was approximately 48 h. This treatment was replicated five times.

Analysis of collecting and shipping data. Analysis of adult and cocoon survivorship data was performed on logit transformed data (ln live/dead) after weighing for sample size. The effects of shipping, cooling, life stage, and sex on survivorship of *F. orizabensis* were tested for significance using Chi-square analysis and pair-wise T-test comparisons (0.025 level of significance).

Analysis of temperature and humidity data. Temperature and humidity recorded for each day of the two day period for both laboratory held and shipped polystyrene foam boxes was analyzed using ANOVA and Tukey's Studentized range test for means separation (0.05 level of significance).

RESULTS

Pupation Behavior

In all three treatments (i.e., natural avocado branches and artificial branches with and without pupation refugia) >90% of deployed late second instar *F. orizabensis* larvae were recovered (Table 1). On avocado branches and artificial branches with refugia <20% of recovered larvae pupated on branches, and the majority were recovered from tanglefoot coated plastic below branches. This result indicated that late second instar *F. orizabensis* larvae may prefer to pupate below trees, or suitable sites for pupation on branches were either not available or located. Artificial branches without pupation refugia resulted in 100% branch abandonment by larvae (Table 1). Of larvae recovered from plastic sheets, 80-98% were found within 0-2.5 cm of branches, indicating that larvae fall rather than actively jump away from branches when searching for pupation sites below host plants (Table 1). High rates of branch abandonment may make it possible to collect *F. orizabensis* below host plants if suitable pupation sites for cocoon construction can be provided that allow for easy harvesting of this life stage.

Pupal Harvesting Techniques

In coarse vermiculite, 80% of deployed late second instar *F. orizabensis* larvae pupated successfully (Table 2). Use of this media for harvesting cocoons was unsuitable as larvae spun silk cocoons between vermiculite particles and the bottoms and sides of Petri dishes, or crawled into cracks in vermiculite particles making detection difficult. Consequently, a technique for harvesting pupal *F. orizabensis* based on sieving for cocoons encased in vermiculite would not be prac-

TABLE 1. MEAN PERCENTAGE RECOVERY (± SE) OF *FRANKLINOTHRIPS ORIZABENSIS* LARVAE RELEASED ONTO AVOCADO BRANCHES, ARTIFICIAL BRANCHES LACKING PUPATION SITES, AND ARTIFICIAL BRANCHES WITH PUPATION SITES.

	Avocado branches	Artificial branches without pupation refugia	Artificial branches with pupation refugia
% Total larval recovery	93.00 ± 2.13	98.00 ± 1.33	95.00 ± 1.67
% Larvae pupating on branch	1.11 ± 0.01	0.00 ± 0.00	17.51 ± 0.01
% Larvae recovered from sticky traps 0-2.5cm from branch	97.78 ± 0.01	90.89 ± 0.01	80.38 ± 0.01
% Larvae recovered from sticky traps 2.5-5.0 cm from branch	1.11 ± 0.01	9.11 ± 0.07	2.11 ± 0.01
% Larvae recovered from sticky traps >5.0 cm from branch	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

tical. The use of fine vermiculite resulted in 100% mortality of larvae as this grade probably acted as an abrasive desiccant (Table 2).

In Petri dishes, parafilm cones were utilized as pupation sites by 80% of deployed late second instar *F. orizabensis* larvae of which 72% emerged successfully. All recovered larvae had spun cocoons inside parafilm cones (Table 2). Parafilm cones combined with fine vermiculite resulted in 67% successful pupation and all recovered pupae were found inside parafilm cones. Provision of parafilm cocoons mitigated the adverse effects of fine vermiculite on pupation success rates (Table 2). Parafilm cones were the most successful media tested for harvesting *F. orizabensis* pupae.

Selection of Pupation Sites in Small Rearing Cages

Of the 300 second instar *F. orizabensis* larvae released into small rearing cages 58% were recovered. The highest numbers of recovered pupae were found inside parafilm cones (Table 3). No pupating larvae were found on bean leaves or stems. Approximately 10% of pupae were found inside seed coats attached to cotyledons, and a similar percentage of cocoons were found in vermiculite potting mix down to a depth of 2 cm (Table 3).

Harvesting of *Franklinothrips orizabensis* Pupae from Laboratory Colonies

Over a three day period, 48% of parafilm cones in cages were used by pupating *F. orizabensis* larvae (Table 4). Greater numbers of females were harvested using parafilm cones, and 0-67% (mean 35% ± 6.50 [SE]) of cones would have more than one pupating larva. Adult emergence rates from parafilm cones placed in colony cages was high, >97% (Table 4).

Shipping Adult and Pupal *Franklinothrips orizabensis*

Handling and shipping. Survivorship of adult *F. orizabensis* was significantly affected by shipping ($\chi^2 = 44.39$, df = 1, $p < 0.0005$) with survivorship rates of adults being reduced on average by 41% in comparison to *F. orizabensis* that were held in the laboratory and not subjected to shipping stress (Fig. 3). Adult male *F. orizabensis* suffered significantly higher mortality rates than females ($\chi^2 = 34.18$, df = 1, $p < 0.0005$) with survival being reduced on average by 24% (Fig. 3). Survivorship of *F. orizabensis* was significantly increased when predators were shipped as cocoons ($\chi^2 = 185.10$, df = 1, $p < 0.0005$) and transit survivorship was increased on average by 53%. (Fig. 3). Inclusion of icepacks in polystyrene foam boxes that were either retained in the laboratory or shipped roundtrip from California to Massachusetts did not significantly alter survivorship rates in comparison to the same treatments without icepacks ($\chi^2 = 0.62$, df = 1, $p = 0.43$).

Temperature and humidity. Significant differences in temperature ($F = 2627$, df = 3, 1448, $p < 0.0005$) (Fig. 4A) and humidity ($F = 173$, df = 3, 1448, $p < 0.0005$) (Fig. 4B) existed between poly-

TABLE 2. MEAN PERCENTAGE (± SE) OF *FRANKLINOTHRIPS ORIZABENSIS* IN EACH OF FOUR PUPATION MEDIA CONTAINED IN PETRI DISHES THAT EITHER PUPATED SUCCESSFULLY AND EMERGED AS ADULTS, DIED AS LARVAE OR PUPAE, OR WERE NOT RECOVERED.

Pupation Media	% Emerged	% Dead	% Unrecovered
Coarse vermiculite	80.00 ± 5.96	6.00 ± 3.06	14.00 ± 6.70
Fine vermiculite	0.00 ± 0.00	100 ± 0.00	0.00 ± 0.00
Parafilm cones	72.00 ± 4.42	8.00 ± 4.42	20.00 ± 5.16
Parafilm cones & fine vermiculite	66.67 ± 4.22	16.67 ± 6.15	16.67 ± 6.15

TABLE 3. MEAN PERCENTAGE (± SE) RECOVERY OF *FRANKLINOTHRIPS ORIZABENSIS* COCOONS FROM DIFFERENT LOCATIONS IN SMALL REARING CAGES.

Location of Cocoons in Cage	% Recovered
Cage sleeves	10.40 ± 4.34
Cage corners	7.01 ± 1.49
Parafilm cones on potting media & cage floor	44.02 ± 6.86
Seed coat attached to cotyledons	9.61 ± 5.53
Potting soil	
0-1cm deep	7.22 ± 3.52
1-2cm deep	3.89 ± 1.24
>2 cm deep	0.00 ± 0.00
Dead larvae & pupae	3.51 ± 1.24
Adults emerged into cages after inspection for cocoons	13.58 ± 3.10

styrene foam boxes that were retained in the laboratory. Boxes with ice-packs were significantly cooler and had higher humidity rates, particularly on the second day, presumably resulting from condensation on ice packs. The mean temperature also increased in boxes with ice packs on the second day (Fig. 4A). Significant differences in temperature ($F = 1422$, $df = 3, 2672$, $p < 0.0005$) (Fig. 4C) and humidity ($F = 513$, $df = 3, 2672$, $p < 0.005$) (Fig. 4D) existed between polystyrene foam boxes that were shipped round trip from California to Massachusetts. Boxes with ice-packs were significantly cooler, especially on the first day, and had higher humidity rates, particularly on the second day, probably from condensation on ice packs. Humidity in boxes without ice packs was constant during the transit period (Fig. 4B).

DISCUSSION

In the laboratory, the majority (82-99%) of recovered late second instar *F. orizabensis* larvae actively abandoned natural and artificial wooden dowel branches to search for pupation sites below host plants. Predatory and phytophagous thrips have been captured emerging from the ground beneath citrus trees (Childers et al. 1994), and pupation beneath host plants is common for pestiferous phytophagous thrips (Grout et al. 1986; Harrison 1963; Okada 1981; Reed & Rich 1975; Schweizer & Morse 1996, 1997; Tsuchida 1997). Host plant abandonment in the field has been inferred from analysis of leaf duff samples from commercial avocado orchards in southern California where *F. orizabensis* specimens have been recovered to a depth of 2.5 cm into the soil (Hoddle et al. 1998).

Host plant abandonment rates by *F. orizabensis* larvae prior to pupation in avocado orchards have not been quantified. Our estimates of larval abandonment on young avocado trees in the laboratory may have been over-estimated as young trees have fewer bark fissures that can be used as pupation refugia in comparison to mature trees in orchards. This suggestion is supported by higher recovery rates of *F. orizabensis* pupae on artificial branches with pupation refugia. In comparison to phytophagous thrips, there is little published information on pupation behavior and site selection by predatory thrips in field situations and this area would benefit from more research.

Coarse and fine vermiculite were unsuitable pupation substrates for harvesting *F. orizabensis* pupae. In Petri dishes with coarse vermiculite, pupating larvae constructed cocoons by attaching silk to walls and floors of dishes, and coarse vermiculite particles were then attached to cocoon surfaces not adhered to the Petri dish. Pupating *F. orizabensis* could not be harvested in this media as cocoons were destroyed (i.e., cocoons were ripped open exposing pupae) when removal of vermiculite granules was attempted. Fine vermiculite was an unsuitable pupation substrate as it resulted in mortality of 100% of second instar

TABLE 4. UTILIZATION OF PARAFILM CONES BY *FRANKLINOTHRIPS ORIZABENSIS* IN MASS REARING CAGES.

Measured Variable	Mean ± SE
Parafilm cones utilized by pupating larvae	48.33% ± 3.43
Male *Franklinothrips orizabensis* emerging from cones	39.49% ± 1.79
Female *Franklinothrips orizabensis* emerging from cones	60.51% ± 1.79
No. adult *Franklinothrips orizabensis* emerging per cone	1.31 ± 0.07
Pupal mortality in parafilm cones	2.35% ± 0.35

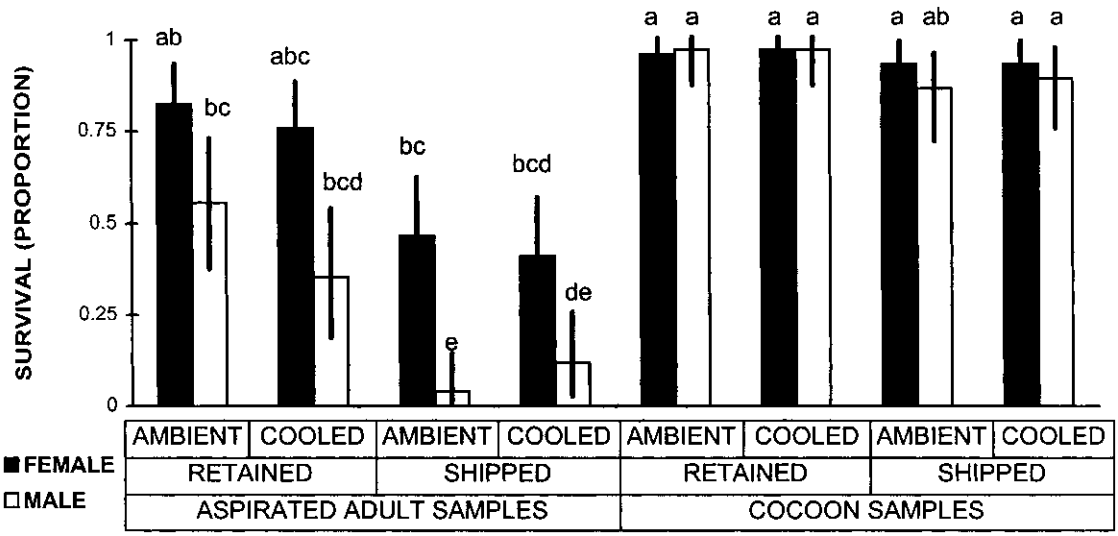

Fig. 3. Survivorship of aspirated adult male and female *Franklinothrips orizabensis* and pupae in parafilm cones either retained in the laboratory at ambient temperatures or cooled with ice packs, or shipped roundtrip from Riverside, California to Amherst, Massachusetts either with or without ice packs. Mean treatment proportions surviving with the same letters are not significantly different (0.025 level of significance).

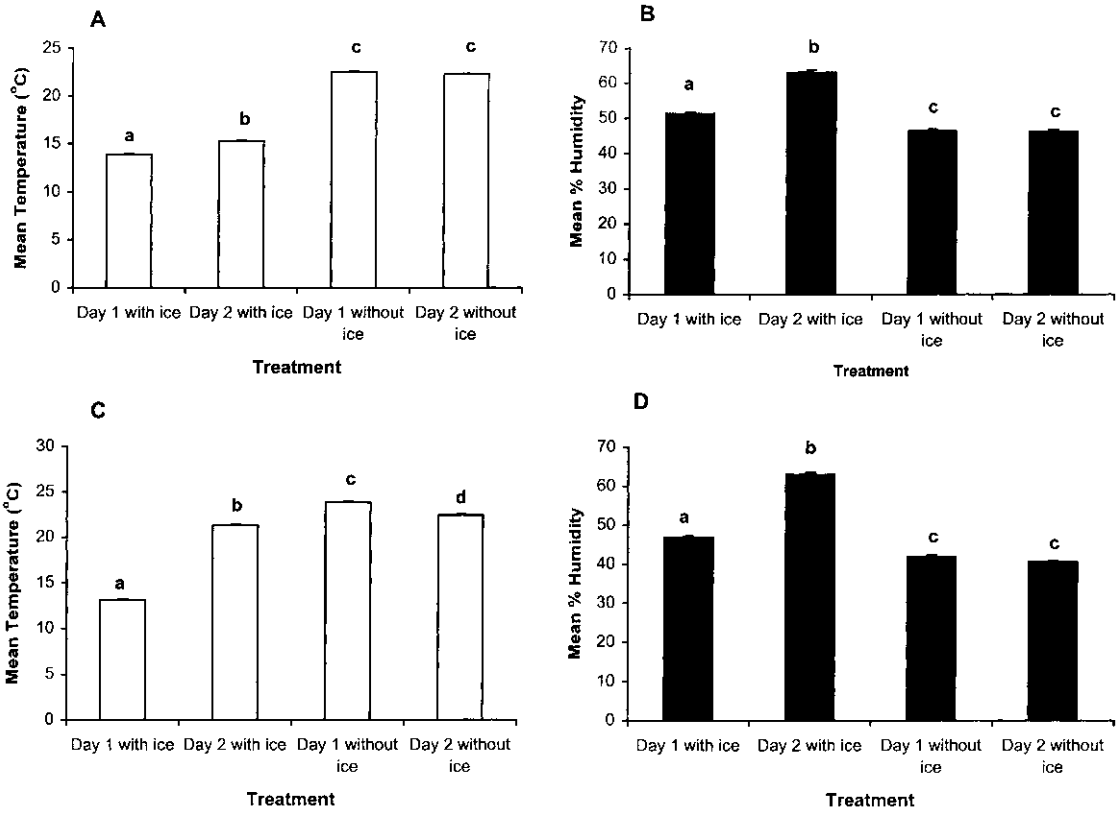

Fig. 4. Mean temperatures (A) and humidities (B) in polystyrene foam boxes with and without ice packs when retained in the laboratory for 48 h, and mean temperatures (C) and humidities (D) when polystyrene boxes were shipped round trip from Riverside, California to Amherst, Massachusetts with or without ice packs. Mean temperatures and humidities followed by the same letters are not significantly different (0.05 level of significance).

F. orizabensis larvae. Placing parafilm cones in Petri dishes with fine vermiculite significantly increased larval and pupal survivorship.

Parafilm cones were utilized by late second instar *F. orizabensis* larvae as pupation sites in Petri dishes, small rearing cages and laboratory colonies. In small rearing cages where known numbers of second instar larvae were deployed, 44% of recovered *F. orizabensis* pupae were found in parafilm cones. Parafilm cones may have been desirable pupation sites because of the conical design (i.e., the tapering nature of the unit and its horizontal position) resulted in larvae being able to find a desirable wall width within the cone to adhere silk strands during cocoon construction. In commercial mass rearing operations, the use of parafilm cones or some other artificial pupation site under host plants (e.g., small, mass produced clear plastic cones) that are easily harvestable may be cost effective for rapidly collecting and shipping *F. orizabensis* pupae. The use of electronic scanning devices could conceivably assist in sorting cones with and without pupae as cones with cocoons turn a blackish color as adults mature. This color change could aid electronic detection and mechanical sorting (Petitt et al. 1996; Smittle et al. 1986; Whitten 1969; Wolf et al. 1972). Alternatively, weight differences between cones with and without pupae may be amenable to air stream separation (Jackson et al. 1996). Mechanical harvesting and sorting of *F. orizabensis* pupae in artificial pupation media could significantly reduce production costs for this predator.

F. orizabensis larvae that pupate in sites other than cones could be a source of adults for sustaining cage colonies and may mitigate the need to set aside portions of the harvested product for colony maintenance. It is possible that harvesting pupae in cones and not returning individuals to colonies that exhibit a preference for cones could select for individuals that do not utilize harvestable media for pupation.

Survivorship of *F. orizabensis* during shipping was greatly enhanced if predators were shipped as pupae in parafilm cocoons. Adult *F. orizabensis*, especially males, were extremely sensitive to aspiration and transit stress, and high mortality resulted when adult males and females were shipped long distances. Transit mortality of adult and pupal *F. orizabensis* was not significantly reduced by inclusion of ice packs in polystyrene foam boxes. This may have been an artifact resulting from the time of year this shipping trial was conducted as shipments were made during May when weather conditions were cool. Inclusion of ice packs with shipments of *F. orizabensis* pupae would be recommended, especially during summer.

Work is currently underway with commercial insectaries in California to implement mass rearing of *F. orizabensis* for field trials against *S. perseae* in avocado orchards.

ACKNOWLEDGMENTS

This work was supported in part by the California Avocado Commission. Dr. Roy Van Driesche, University of Massachusetts at Amherst kindly assisted with the shipping aspects of this trial. We thank Dr. Miwa Takano-Lee for critically evaluating this work.

REFERENCES CITED

ARAKAKI, N., AND S. OKAJIMA. 1998. Notes on the biology and morphology of a predatory thrips, *Franklinothrips vespiformis* (Crawford) (Thysanoptera: Aeolothripidae): First record from Japan. Entomol. Sci. 1: 359-363.

CHILDERS, C. C., S. NAKAHARA, AND R. J. BESHEAR. 1994. Relative abundance of *Frankliniella bispinosa* and other species of Thysanoptera emerging from soil beneath "Navel" orange trees in Florida during spring flowering. J. Entomol. Sci. 29: 318-328.

GRAFTON-CARDWELL, E. E., AND Y. OUYANG. 1995a. Augmentation of *Euseius tularensis* (Acari: Phytoseiidae) in Citrus. Environ. Entomol. 24: 738-747.

GRAFTON-CARDWELL, E. E., AND Y. OUYANG. 1995b. Manipulation of the predacious mite, *Euseius tularensis* (Acari: Phytoseiidae), with pruning for citrus thrips control, pp. 251-254. *In* Thrips Biology and Management. B. L. Parker, M. Skinner and T. Lewis (eds.). Plenum Press, New York.

GRAFTON-CARDWELL, E. 1997. Manipulation of *E. tularensis* for improved thrips control. Citrograph 82: 8.

GRAFTON-CARDWELL, E. E., Y. OUYANG, AND R. L. BUGG. 1999a. Leguminous cover crops to enhance population development of *Euseius tularensis* (Acari: Phytoseiidae) in citrus. Biol. Control 16: 73-80.

GRAFTON-CARDWELL, E. E., Y. OUYANG, AND R. A. STRIGGOW. 1999b. Predacious mites for control of citrus thrips, *Scirtothrips citri* (Thysanoptera: Thripidae) in nursery crops. Biol. Control 14: 29-36.

GROUT, T. G., J. G. MORSE, AND O. L. BRAWNER. 1986. Location of citrus thrips (Thysanoptera: Thripidae) pupation: tree or ground? J. Econ. Entomol. 79: 59-61.

GROUT, T. G., AND G. I. RICHARDS. 1990. The influence of windbreak species on citrus thrips (Thysanoptera: Thripidae) populations and their damage to South African citrus orchards. J. Entomol. Soc. South Africa 53: 151-157.

GROUT, T. G., AND G. I. RICHARDS. 1992a. The dietary effects of windbreak pollens on longevity and fecundity of a predacious mite *Euseius addoensis addoensis* (Acari: Phytoseiidae) found in citrus orchards in South Africa. Bull. Entomol. Res. 82: 317-320.

GROUT, T. G., AND G. I. RICHARDS. 1992b. *Euseius addoensis addoensis*, an effective predator of citrus thrips, *Scirtothrips aurantii*, in the eastern Cape Province of South Africa. Experimental and Applied Acarology 15: 1-13.

HARRISON, J. O. 1963. Notes on the biology of banana flower thrips, *Frankliniella parvula,* in the Dominican Republic (Thysanoptera; Thripidae). Ann. Entomol. Soc. America 56: 664-666.

HODDLE, M. S., J. G. MORSE, P. PHILLIPS, AND B. FABER. 1998. Progress on the management of avocado thrips. California Avocado Society Yearbook 82: 87-100.

HODDLE, M. S., J. G. MORSE, P. PHILLIPS, B. FABER, Y. WEE, AND S. PEIRCE. 1999. Avocado thrips update. Citrograph 84: 13-14.

HODDLE, M. S., L. ROBINSON, K. DRESCHER, AND J. JONES. 2000. Developmental and reproductive biology of a predatory *Franklinothrips orizabensis* (Thysanoptera: Aeolothripidae). Biol. Control 18: 27-38.

HODDLE, M. S. J. JONES, K. OISHI, D. MORGAN, AND L. ROBINSON. 2001. Evaluation of diets for the development and reproduction of *Franklinothrips orizabensis* (Thysanoptera: Aeolothripidae). Bull. Entomol. Res. (in press).

JACKSON C. G., H. T. CHAN JR., M. H. TANIGUCHI, D. B. CHURCHILL, AND D. M. BILSLAND. 1996. Pneumatic air separation for the sorting of parasitized and unparasitized fruit fly (Diptera; Tephritidae) puparia. J. Econ. Entomol. 89: 353-358.

MCMURTRY, J. A., AND B. A. CROFT. 1997. Life-styles of phytoseiid mites and their roles in biological control. Ann. Rev. of Entomol. 42: 291-321.

MORSE, J. G., T. S. BELLOWS JR., AND Y. IWATA. 1986. Technique for evaluating residual toxicity of pesticides to motile insects. J. Econ. Entomol. 79: 281-283.

MUNGER, F. 1942. A method for rearing citrus thrips in the laboratory. J. Econ. Entomol. 35: 373-375.

NAKAHARA, S. 1997. *Scirtothrips perseae* (Thysanoptera: Thripidae), a new species infesting avocado in southern California. Insecta Mundi 11: 189-191.

OKADA, T. 1981. Searching behavior for pupation place by late second instar larvae of *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae). Japanese Soc. Applied Entomol. and Zool. 25: 10-16.

PARKER, B. L., AND M. SKINNER. 1997. Integrated Pest Management (IPM) in Tree Crops, pp. 615-638. *In* Thrips as Crop Pests. T. Lewis (ed.). CAB International, Wallingford.

PARRELLA, M. P., AND T. LEWIS. 1997. Integrated Pest Management (IPM) in Field Crops, pp. 595-614. *In* Thrips as Crop Pests. T. Lewis (ed.). CAB International, Wallingford.

PETITT, F. L., D. S. SCHUMAN, D. O. WIETLISBACH, AND J. A. COFFELT. 1996. An automated system for collection and counting of parasitized leafminer (Diptera: Agromyzidae) larvae. Florida Entomol. 79: 450-454.

REED, D. K., AND J. R. RICH. 1975. A new survey technique for citrus thrips. J. Econ. Entomol. 68: 739-742.

SCHWEIZER, H., AND J. G. MORSE. 1996. Pupation sites of *Scirtothrips citri* (Thysanoptera: Thripidae) and potential management through increasing mortality of instars on the ground. J. Econ. Entomol. 89: 1438-1445.

SCHWEIZER, H., AND J. G. MORSE. 1997. Factors influencing survival of citrus thrips (Thysanoptera: Thripidae) propupae and pupae on the ground. J. Econ. Entomol. 90: 435-443.

SILVERS, C. 2000. Biological control of *Scirtothrips perseae* Nakahara in California avocados: assessment of two generalist predators. MS Thesis, University of California at Riverside. 103 pp.

SMITTLE, J., J. A. SEAWRIGHT, AND B. K. BIRKY. 1986. Use of a commercial color sorter to separate sexes of stable fly (Diptera: Muscidae) pupae from a genetic sexing strain. J. Econ. Entomol. 79: 877-878.

TSUCHIDA, K. 1997. Adult emerging sites of *Thrips palmi* (Thysanoptera; Thripidae) in an egg plant field with mulching sheet. Applied Entomol. and Zool. 32: 246-249.

WHITTEN, M. J. 1969. Automated sexing of pupae and its usefulness in control by sterile insects. J. Econ. Entomol. 62: 272-273.

WOLF, W. W, M. W. STIMMAN, AND G. K. PARKER. 1972. A mechanism to aid sorting of male and female cabbage looper pupae. J. Econ. Entomol. 65: 1044-1047.