Table 1. Initial and revised rating scales for defoliation damage to Eucalyptus spp. by *Trachymela sloanei* feeding.

	Initial rating scale, *T. sloanei* damage	Revised rating scale, *T. sloanei* damage
0	No damage	Unchanged
1	Slight damage to single leaves only, damage dispersed throughout tree, but not immediately visible.	Unchanged
2	Moderate damage to leaves only, easily visible, throughout tree, but no lost terminals.	Unchanged
3	Heavy damage to leaves, at least one terminal lost, more terminals intact than affected	Moderate to heavy damage to leaves, less than 25% of terminals lost.
4	Heavy damage to leaves, many, but not all terminals lost, more terminals lost than intact, light resprout growth seen on trunk, or major branches	Heavy damage to leaves, up to 50% of terminals lost, no resprouting.
5	Heavy damage throughout tree, most or all terminals lost, heavy resprout growth throughout tree, on trunk and on major branches (ressprout growth being damaged)	Heavy damage to leaves, at least 50% of terminals lost, light resprout growth on trunk and/or major branches.
6	—	Heavy damage throughout tree, 75-100% of terminals lost, heavy resprout growth throughout tree, often with damage to resprout growth.

Definitions:
light damage to leaf = noticeable bite marks, but most of leaf is intact
moderate damage to leaf = leaves chewed to midrib>50% of leaf still intact
heavy leaf damage = leaves chewed to midrib, little or no leaf surface remains intact
lost terminal = terminal void of leaves, petioles remain, leaves on branch are damaged (to rule out loss of terminal for other reasons)
resprout growth = new sprout or terminals emanating from areas other than the ends of branches

Biological Control of a Newly Introduced Pest, the Eucalyptus Tortoise Beetle, *Trachymela sloanei*.

Jocelyn G. Millar, Timothy D. Paine, and Mark Hoddle

INTRODUCTION

Since their introduction into California in the late 19th century, eucalyptus trees have been widely planted in rural and urban settings. They are hardy, attractive, and fast-growing evergreen trees, and are tolerant of poor soils and seasonal drought stress. In southern and central California, they are ubiquitous in urban forests on private lands, and in parks, golf courses, and other landscape settings. Eucalyptus defines the aesthetic beauty of public lands such as Will Rogers State Park in Los Angeles, many college and university campuses, and the Presidio in San Francisco. Furthermore, eucalyptus trees are an essential element for overwintering of our national insect, the Monarch butterfly; Eucalyptus provides roosting sites for the Monarchs along the central California coast.

Eucalyptus in California were virtually free of insect pests until the early 1980's. Since that time, more than a dozen pests of eucalyptus, including two wood-boring beetles, several psyllid species, and a defoliating weevil have been introduced into California. Biological control programs conducted by UC researchers, involving the importation, rearing, and mass release of highly specific parasitoids of these pest insects have been successful in minimizing tree mortality, defoliation, and cosmetic damage to foliage throughout the state.

In February 1998, another Australian pest of eucalyptus, the eucalyptus tortoise beetle *Trachymela sloanei* (Coleoptera: Chrysomelidae), was identified for the first time in the U.S., in southwestern Riverside County (Garrison 1998). Both the larval and adult stages of the beetle feed on a wide variety of eucalyptus (Selman 1985), with preferred hosts including some of the most common species in California (e.g., *Eucalyptus globulus* and *E. viminalis*; Steven &

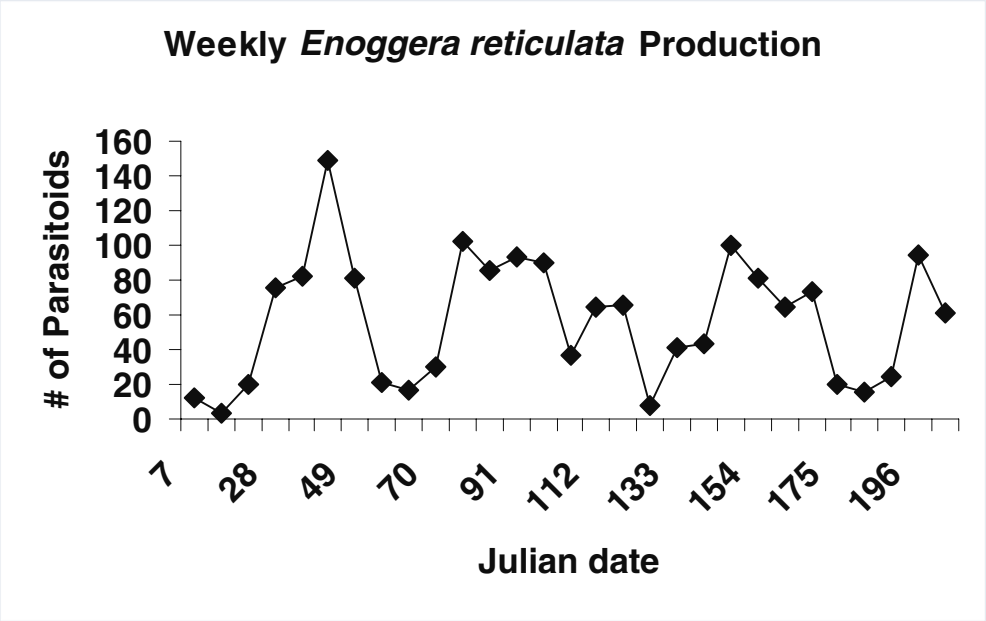

Fig. 1. Production of Enoggera reticulata wasps from the laboratory colony.

Mulvey 1977). Furthermore, the larvae of these beetles secrete irritants, representing a potential hazard to outdoor activities. Documented cases with this and related Australian defoliating beetles have shown that if left unchecked, populations can explode, causing severe damage to eucalyptus plantings (Lawrence & Britton 1991; Selman 1994).

T. sloanei is not normally a pest in Australia presumably because of natural enemies that attack several life stages of the beetle. However, when this species was accidentally introduced into New Zealand in 1976, it caused extensive defoliation and tree damage (Steven & Mulvey 1977; M. Kay, NZ Forest Res. Instit., pers. comm.) before being brought under control with natural enemies (Selman 1994). Another closely related species, *Trachymela tincticollis*, caused extensive damage to eucalyptus in South Africa, but has now been brought under control by a wasp acting as an egg parasitoid, *Enoggera reticulata* (Hymenoptera: Pteromalidae) (Selman 1994; Tribe & Cillie 1997; G. Tribe, pers. comm.), with parasitism rates > 95% reducing populations of the beetle to nondamaging levels.

South African researchers have compiled extensive background data on *E. reticulata*, including host range trials which indicated that the parasitoid only attacks *Trachymela* spp. and a few closely related species in the same subtribe, *Paropsina* (G. Tribe, manuscript in prep.). There are no North American species in this subtribe so no native beetle species should be at risk from this parasitoid. Furthermore, *E. reticulata* searches for host egg masses in bark cracks and fissures of eucalyptus trees, and during the eleven years since the initial releases in South Africa, *E. reticulata* has only been recovered from *Trachymela* eggs (G. Tribe, pers. comm). This wealth of background data expedited obtaining permits to import and release the parasitoid in California. Thus, the objective of this project was to develop a biological control program targeting the eucalyptus tortoise beetle, using the egg parasitoid *E. reticulata* to try and develop an inexpensive and short-term project to establish natural enemies of *T. sloanei* to provide a permanent, effective solution to a serious problem in Eucalyptus plantings statewide.

MATERIALS AND METHODS

1. Rearing of T. sloanei for host egg production.

T. sloanei were reared in the laboratory to provide a constant supply of host eggs for *E. reticulata*. The *T. sloanei* colony, started in 1998 from field-collected individuals, was augmented frequently with adults collected in field surveys (see below). Beetles were reared under long-day conditions (14 hours light,

10 hours dark) and temperatures of 75-80°F, using cages measuring 40 cm X 40 cm X 49 cm. Cages were misted with water daily to maintain high humidity. Insects were provided food in the form of a potted eucalypt and a "bouquet" of new eucalyptus shoots (species which the beetles readily consume, such as *Eucalyptus camaldulensis* and *Eucalyptus grandis*) with the cut ends secured with a foam plug within a water-filled 500-mL Erlenmeyer flask. The foam plug was required to prevent beetles from crawling into the flask and drowning. Bouquets were changed twice weekly to maintain palatability, and potted seedlings were replaced when they were stripped of leaves and shoots. The freshness of the food material, and the presence of new shoots in the bouquets, were crucial for egg production; oviposition rates dropped dramatically when beetles were fed only mature foliage. Because of the beetles' habit of ovipositing into confined spaces, artificial oviposition sites mimicking these tactile requirements were developed, consisting of foam blocks with slits cut along their sides and "cork sandwiches" attached to the blocks using insect mounting pins. These blocks and sandwiches were placed within and between bouquets and potted plants. Egg masses (~4-40 eggs/mass) were removed daily and counted. Bouquets were also checked for egg masses when vegetation was replaced. Any eggs not used the day they were collected were kept in cold storage. Using these methods, several thousand host eggs could be harvested per week.

2. Rearing Procedures for the Parasitoid, Enoggera reticulata.

Parasitoid colonies were started with specimens obtained from Dr. Geoff Tribe, Plant Protection Research Institute, South Africa. The colonies were maintained in the UC Riverside Insectary under long-day conditions at temperatures of ~77°F. Groups of mixed-sex parasitoids were maintained in 9 cm diam X 10 cm high plastic cylinders with honey streaked on the inside for food. A piece of paper towel placed between the container and the lid was moistened with water daily to maintain high humidity. Host egg masses were presented to parasitoids in a ratio of ~ 1 egg mass/female. After 2 days host egg masses were removed, and held in

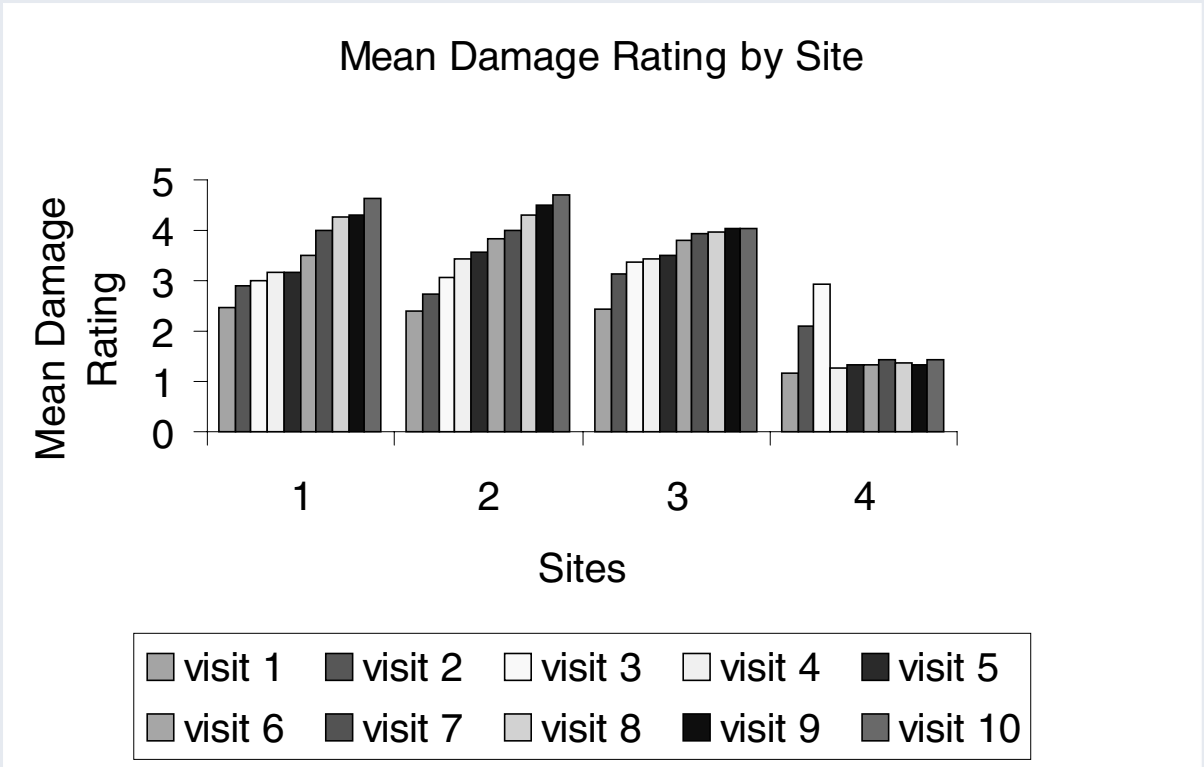

Fig. 2. Tortoise beetle damage at four monitoring sites for the latter part of 1999. The damage scale is described in Table 1.

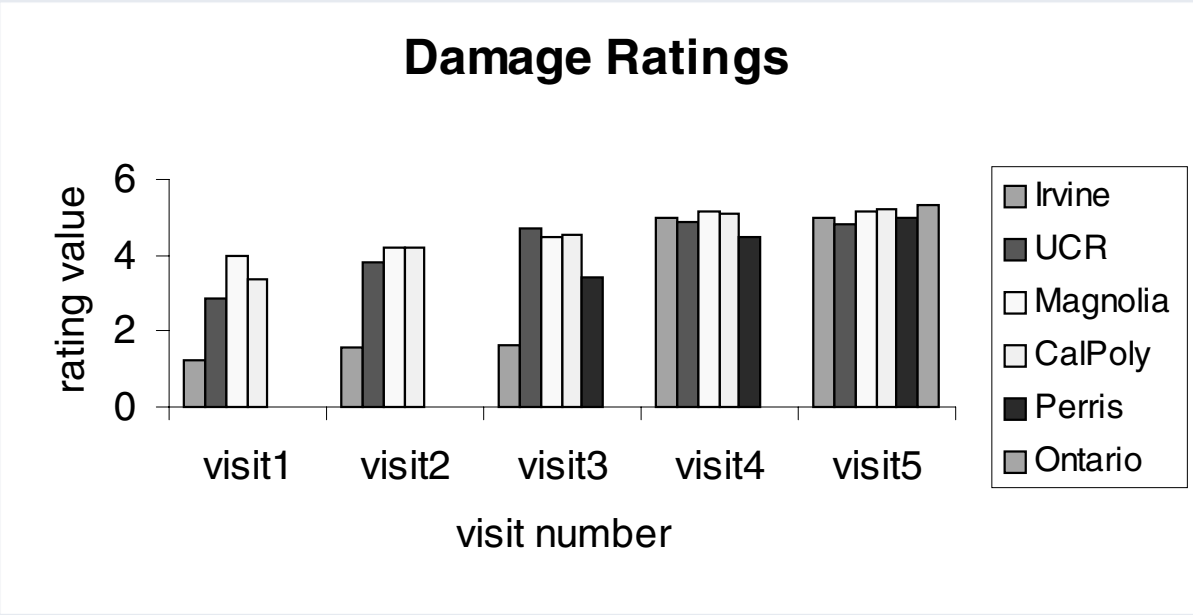

Fig. 3. Tortoise beetle damage at six monitoring sites during the first half of 2000, quantified using the revised damage scale described in Table 1.

self-sealing petri dishes streaked with honey to provide food for the emerging wasps. Dishes were checked daily for 14 days for both neonate beetle larvae and parasitoids. Neonate beetle larvae were killed because of their predilection to cannibalize conspecific eggs, including eggs containing developing parasitoids. Each parasitoid was tracked, and newly emerged adults were added to colony vials but not to containers holding their parents so as to minimize inbreeding. Numbers of neonate beetle larvae, numbers of emerging parasitoids, and numbers of eggs from which neither hatched were recorded.

3. Protocols for Estimating Damage and Beetle Population Densities.

Two pairs of field sites were chosen in southern California to monitor beetle defoliation damage and beetle density, with one site of each pair scheduled for subsequent parasitoid releases. Two additional sites were added in spring of 2000. Thirty trees at each site were checked at monthly intervals, rating each tree visually on a scale of 0-5 for damage using the criteria listed in Table 1. The damage rating scale was later revised to allow for better discrimination of severe damage levels (Table 1), including the resprouting which occurs after continued heavy defoliation.

For more detailed assessments, subsamples of six trees were chosen randomly to be sampled for leaf damage, presence of beetles under the bark, and presence of adult and immature beetles in leaf litter under trees (see below).

Leaf Damage. At each sampling date, two small randomly selected branches from the north and south sides of each of six trees per site were clipped using a pole pruner. The total number and the number of leaves damaged by adult or larval beetle feeding per branch were counted, and the percentage of damaged leaves calculated. Because feeding damage is very characteristic, and because there is only one other species of eucalyptus defoliator in California (the eucalyptus snout beetle, *Gonipterus scutellatus*, which is under good biological control, and which produces distinctly different patterns of damage), we are certain that all damage tabulated resulted from *T. sloanei* feeding.

Sampling beetle density on trees. Each tree was searched for 60 seconds by looking both on top of and under the bark surface for all stages of the beetle both live and dead, from ground level up to ~7 feet (i.e., the height that an observer on the ground could reach). The numbers of eggs, live and dead larvae, live and dead

pupae, and live and dead adults were recorded.

Sampling beetle density on the ground. Mature larvae pupate within the top couple of inches of leaf litter around the bases of trees, and we had observed that pupating beetles tended to congregate under objects on the ground. In an effort to take advantage of this behavior for sampling purposes, four one foot square clay tiles were placed on the ground at each of the cardinal directions at the base of the six test trees/site. Each tile was placed flush with respect to the ground and then covered with vegetative debris. At the next visit each tile was removed and the upper and lower surface was checked for all stages of the beetle, live or dead. The soil immediately below and around the edge of the tile was also checked. The numbers of live and dead larvae, live and dead pupae, and live and dead adults were recorded.

4. Releases of E. reticulata parasitoids.

Lab-reared parasitoids were released at two sites in Perris and Ontario CA. Initial releases were made by caging mixed sex wasps with several host egg masses on a Eucalyptus log for two days before release. Subsequent releases were made by releasing mated parasitoids directly onto host trees that were heavily infested with tortoise beetles.

RESULTS AND DISCUSSION

Laboratory rearing of parasitoids.

In the laboratory colonies, wasps took between 10 and 12 days to develop, and equal numbers of males and females were produced. The number of parasitoids emerging as a percentage of available host eggs varied from 0-20%, hatching beetle larvae varied from 0-45%, and the percentage of host eggs producing neither a parastoid nor a beetle larva varied from 66-100%. Hatch rates of beetle eggs not exposed to parasitoids were consistently >95%. Thus, even though the percentage of host eggs producing parasitoids was relatively small, the parasitoid females appeared to be killing the majority of the host eggs, presumably by probing with the ovipositor, host feeding, or superparasit-

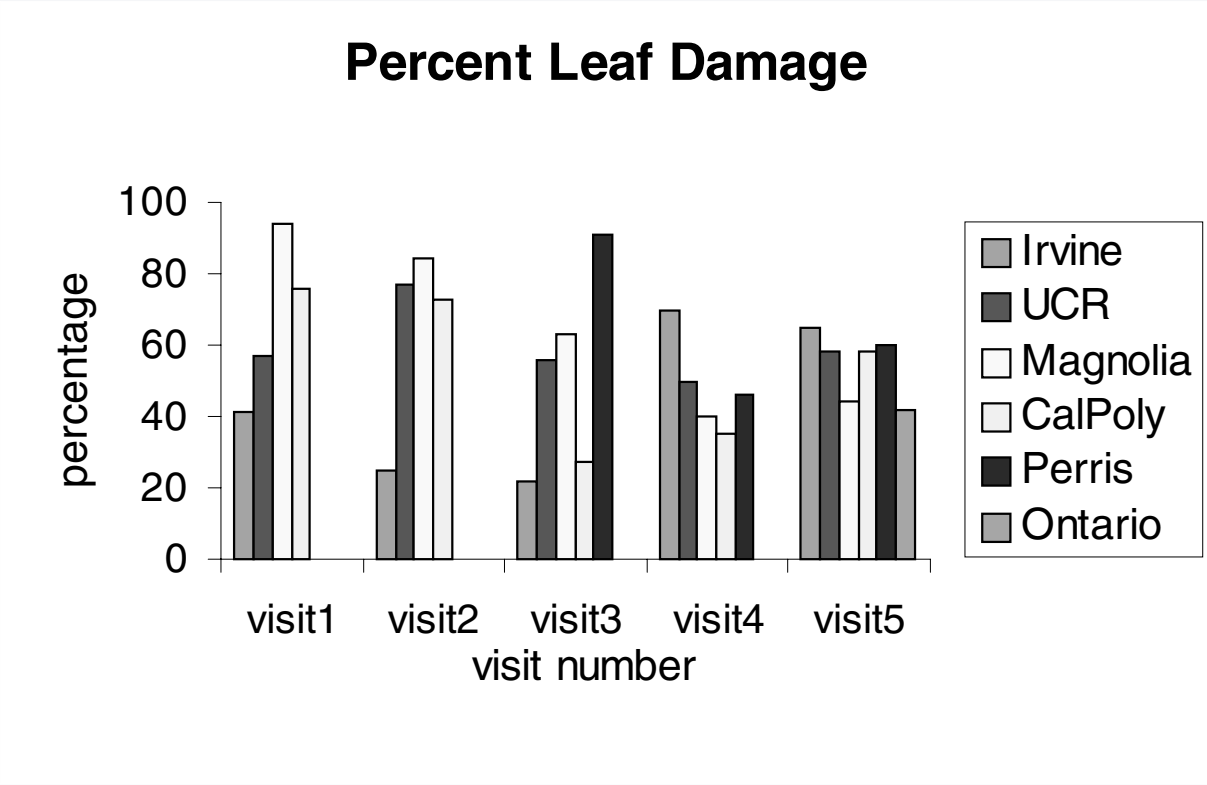

Fig. 4. Percent defoliation of eucalyptus trees at six monitoring sites during the first half of 2000.

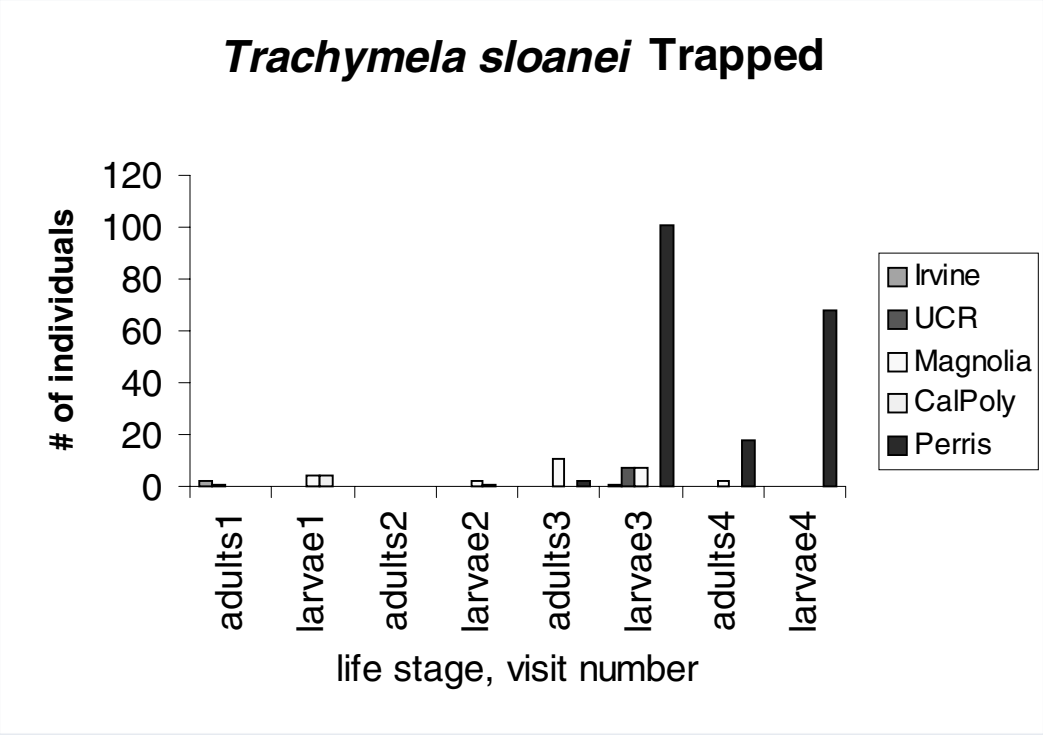

Fig. 5. Results from visual survey sampling of tortoise beetle larvae and adults at six monitoring sites.

ism (laying more than one egg per host). However, superparasitism is not considered a likely explanation because similar results were obtained with parasitoids given >100 host eggs per female per week.

The weekly production of parasitoids from the laboratory colony for the first 6 months of 2000 is shown in Fig. 1. Despite all efforts to increase production of parasitoids, including manipulation of light, temperature, humidity, and number and age of available hosts, we have not been able to produce more than several hundred parasitoids per week. However, the laboratory colony is relatively stable, with approximately 40 generations having been reared. We are maintaining contact with Dr. Geoff Tribe in South Africa should it be necessary to make further importations to improve the vigor or genetic base of the lab colony. We have also consulted extensively with Dr. Tribe to optimize our rearing conditions to the extent possible.

Monitoring and Quantifying Tortoise Beetle Damage.

For the latter half of 1999, defoliation damage was heavy at all 4 monitoring sites (Fig. 2). Damage increased during the course of the year, except for the Irvine site, where it seems likely a pesticide application was made, based on the sharp drop in damage, and the simultaneous sharp drop in insect fauna of all types at that site.

Damage levels at the four original plus two additional monitoring sites for the first half of 2000 are shown in Fig. 3, with percent defoliation shown in Fig. 4. Foliage at all six sites suffered extensive damage, with damage increasing from winter through early summer. Trees at the sites were also heavily infested with red gum lerp psyllid, and damage and leaf drop from the psyllid to some extent obscured the feeding damage to leaves caused by tortoise beetle.

Monitoring Tortoise Beetles.

Sites were monitored for the presence and number of tortoise beetle adults, larvae, and egg masses. Egg masses were difficult to find, and were relatively rare on the trunk segments sampled. Fig. 5 shows a

representative graph of adults and larvae counted during trapping of the bottom seven feet of trees at one site. Overall, this sampling method provided a qualitative estimate of beetle populations, but larger sample sizes may be needed in order to obtain more quantitative estimates, because of the highly clumped distribution of beetles under loose bark. Trees with a lot of adhering loose bark generally harbored much larger numbers of beetles than trees with smooth bark, or trees from which the exfoliated bark had fallen or been stripped off.

The second sampling method, counting the numbers of adults and larvae under tiles placed on the ground also proved to be too variable for general use. In particular, despite being marked as part of an experiment, tiles were frequently moved, broken, or stolen. Furthermore, leaf litter and soil characteristics strongly influenced the results. Tiles on loose, well-drained soil were good beetle pupation sites, whereas the ground under tiles at irrigated sites tended to become saturated. The ground under tiles also was frequently colonized by ants. Conversely, at sites with deep leaf litter, the beetles preferred to pupate in the leaf litter rather than under the tiles. Because of the large variability in this sampling method, both within and between sites, this sampling method was abandoned. This method was replaced with trunk banding. This involved using pieces of roofing flashing stapled to the trunks of trees, similar to a method developed for sampling the congeneric species, *T. tincticollis*, in South Africa (Tribe and Cillie 1985).

Releases of Enoggera reticulata parasitoids.

Four batches of mixed sex parasitoids were released at one study site in Perris CA on May 26, June 6, July 27, and August 4, 2000, and two further batches of parasitoids were released at the Ontario CA study site on July 18 and 24, 2000. Releases will be continued through the summer and fall of 2000, and the spring of 2001 as parasitoids become available from the laboratory colony. At the beginning of September, efforts to collect and monitor tortoise beetle egg masses will be increased to monitor for establishment of breeding populations of *E. reticulata*, percentage of egg masses parasitized, and percent parasitism within individual egg masses. The hatch rate of tortoise beetle eggs will also be monitored, to check whether host feeding or other damage to the eggs by *E. reticulata* causes extensive mortality, as was seen in the laboratory colony.

Conclusions

A biological control program for the eucalyptus tortoise beetle is in progress. Methods of sampling and quantifying damage by the tortoise beetle, and of monitoring beetle populations, have been developed and are being used in monthly surveys of six sites. A laboratory colony of the parasitoid *Enoggera reticulata* was started with individuals obtained from South African colleagues, and has been maintained for approximately 40 generations at UC Riverside. A limited number of releases of the parasitoids have been made at two sites, and releases will continue over the coming year, with additional support from the UCIPM Pest Management Project.

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