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# Management of subterranean termites, *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in a citrus orchard with hexaflumuron bait

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**Abstract**

The eastern subterranean termite, *Reticulitermes flavipes* (Kollar) is a pest of young citrus in Florida, killing trees by girdling at or just below the soil line. *Reticulitermes virginicus* (Banks), another inhabitant of citrus groves, may cause similar damage. Chemical and cultural methods developed previously to create barriers to termites around young citrus trees provided temporary or incomplete control. However, baiting systems may provide longer lasting control by eliminating or at least reducing termite activity. Triple mark–recapture with Nile blue dye was used to delineate foraging ranges and to estimate populations sizes of two *R. flavipes* and one *R. virginicus* colony located in two different blocks of a south Florida citrus grove. This dye has been observed to be detectable from 6 to 9 months after ingestion by subterranean termites, and not to be transferred between individuals by tropholaxis (Su, unpublished data). Termite activity was monitored by counting workers and estimating wood consumption at buried monitoring stations. Termites were recruited to bait stations initially containing spruce wood which was then replaced with 0.1 or 0.5% hexaflumuron in either a particulate (wood flour) or non-particulate (paper) cellulose matrix and consumption was monitored. After a 2–3 month baiting period, no new termite activity was detected within the areas of baited *R. flavipes*. Similar amounts of toxicant were consumed per termite in all cases. No subsequent tree injury was observed within the areas of the baited colonies. Baiting with hexaflumuron appears to be a viable alternative for managing subterranean termites in citrus and possibly other agricultural systems. © 2001 Published by Elsevier Science Ltd.

*Keywords:* Florida; Mark–recapture

Termites have been cited as agricultural pests from most of the world's tropics and subtropics, including Asia, Africa, South America, India, Australia, and Hawaii (Harris, 1969; Sands, 1973; Li et al., 1979). Attacks of agronomic crops such as sugarcane, forage, cotton, peanuts, tubers, and numerous tree crops have been reported. Representatives of the major termite families have been cited as crop pests including fungus cultivating and non-fungus cultivating members of the Termitidae, various genera of Rhinotermitidae and *Neotermes* in the Kalotermididae. Termite damage to crops is often grossly underestimated (Harris, 1969).

The eastern subterranean termite (*Reticulitermes flavipes* (Kollar)) has long been recognized as a potentially serious pest of citrus in Florida (Watson, 1926), especially in new groves established on cleared pine woodland where remnant populations may cause serious loss of young trees (Watson, 1926; Stansly et al., 1991). Termites remove bark and cambium from the tree crown between the soil line and scaffold roots, often girdling the tree (Stansly et al., 1991, 1992). Feeding on sapwood during this initial phase was not observed, although termites did sometimes return and feed on seasoned wood months or years after abandoning the dying tree (Stansly et al., 1992). Partially girdled trees abandoned by the termites often recovered. Other termites reported to attack citrus include *R. lucifugis* (Rossi) in Israel, *Paraneotermes simplicicornis* (Banks) in Texas, *Coptotermes* spp. in Malaya, Australia, and Surinam, *Microcerotermes diversis* Silvestri in Arabia, *Macrotermes* spp. in Nigeria and *Nasutitermes costalis* (Holmgren) in Surinam (Harris, 1969). Control measures in Florida have included removal of remnant wood from the orchard soil and protection of the vulnerable crown by soil removal and applications of insecticide to the base of the tree (Stansly

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et al., 1991, 1992). However, these latter measures are costly, temporary, and only partially effective in that they do not control the source of the problem, the termite colony.

Insecticidal baits for controlling subterranean termites have been under investigation for some time (Findlay, 1971) and recently put into practical use to control large colonies of subterranean termites. Successful baiting has been reported using the chitin inhibitor hexaflumuron (Su, 1994; Su et al., 1995). These authors believed that a critical characteristic of hexaflumuron was dose-independent killing time that allowed for sufficient ingestion of active ingredient to assure cessation of termite activity. Baits using hexaflumuron and other toxicants are now used for structural termite control but none are currently labeled for agricultural use in the US. The objectives of the present study were to evaluate the applicability of a baiting technique utilizing hexaflumuron to reduce or eliminate populations of *Reticulitermes* spp. in an agricultural setting, specifically a young citrus grove in southwest Florida.

## 1. Materials and methods

### 1.1. Field sites

Two sites with a history of tree loss due to termites were chosen within a large grove of citrus in Collier county, southwest Florida. Soil type was Immokalee fine sand. Each site was located within a block of 'Valencia' orange scion budded to 'Carrizo' citrange (*C. sinensis* × *Poncirus trifoliata* Rafinesque) rootstock planted 4 years previously in the spring of 1990. Spacing was 7.6 m between rows and 3.1 m between trees within rows (Figs. 1 and 2). Termite damage to trees had been monitored intermittently since 1990 (Stansly et al., 1991, 1992). Soil moisture was maintained by a microsprinkler under each tree. Site I consisted of a 0.75 ha area, 18 rows wide and 15 trees deep. The area encompassed all trees (18) in the block killed by termites plus a buffer area of 40 m on all but the north side bordered by a road and a canal (Fig. 1). Termite damage was identified by characteristic signs (Stansly et al., 1991, 1992): bark removal from the crown area, and the presence of termite galleries on recently killed trees. Site II was located near to but not adjacent to Site I, and consisted of another block of 3.2 ha containing some 1200 trees (Fig. 2). Losses due to termite damage had caused over 200 trees to be replaced, at some sites on multiple occasions, especially along the eastern and northern perimeters (Stansly et al., 1991, 1992). However, activity had moved away from the eastern perimeter by the time these experiments were initiated and two locations toward the center of the block were subsequently chosen for baiting (Fig. 2).

### 1.2. Termite monitoring

All trees within the two sites were surveyed for termite activity in August 1994 by examining the crown for damage (bark removal), galleries and/or presence of termites. Trees were again monitored for damage on 11

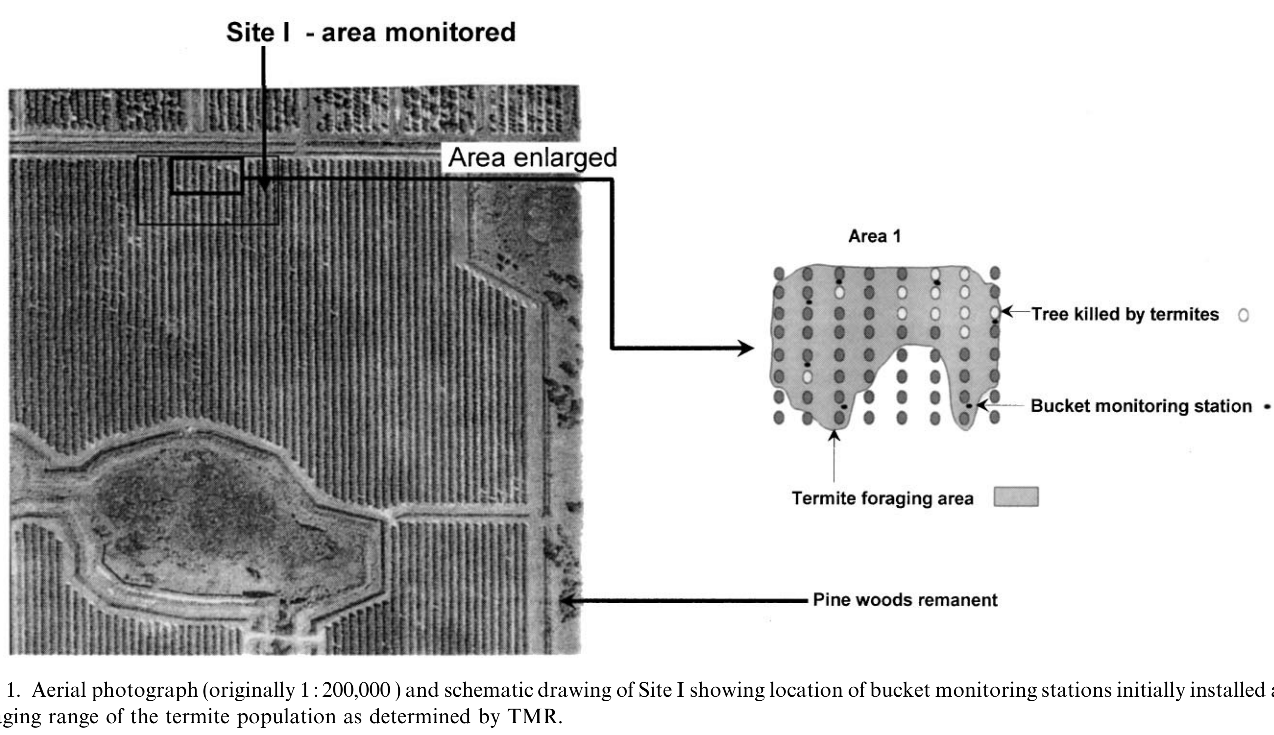


Fig. 1. Aerial photograph (originally 1 : 200,000 ) and schematic drawing of Site I showing location of bucket monitoring stations initially installed and foraging range of the termite population as determined by TMR.

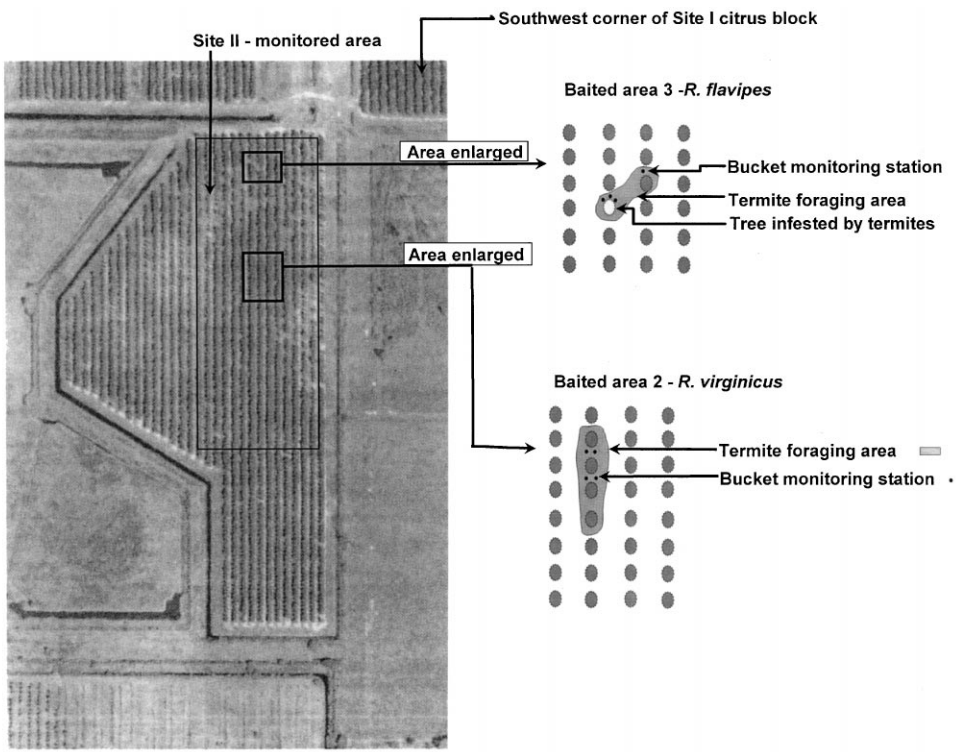


Fig. 2. Aerial photograph (originally 1 : 200,000 ) and schematic drawing of Site II showing location of bucket monitoring stations initially installed and foraging ranges of two baited termite populations.

May 1995, 22 July 1996 and 5 January 1999 and around each baiting location prior to and periodically following placement of bait. Spruce (*Picea* sp.) stakes $45 \times 3.5 \times 1$ cm driven 3/4 of the way into the ground were used to monitor for termite activity. The stakes were placed between every tree in each row including the 40 m buffer zone of Site I on 6 June 1994, and between every 5th tree on 2 June 1994 at Site II. Stakes were checked monthly and augmented around both sides of every tree at Site II wherever termite activity was detected. Consumed stakes were replaced as needed at both sites and ranges of termite activity were delineated (Figs. 1 and 2). Species determinations were made morphometrically based on Scheffrahn and Su (1994) and Hostettler et al. (1995) and verified by R. Scheffrahn (University of Florida, Fort. Lauderdale).

Monitoring stations similar to those described by Su and Scheffrahn (1986) were placed in the soil under tree canopies in the most active areas at each site; initially 7 at Site I and 4 in each of two areas at Site II. Monitoring stations consisted of plastic buckets 19 cm high and 15.5 cm wide at the bottom with snap-on lids. Bucket bottoms were removed so that a wooden block placed inside came in direct contact with the soil. The rectangular blocks were made by nailing together six spruce boards $2 \times 13 \times 10$ cm in size, separated by small wooden dowels 2 mm in diameter provided as spacers to facilitate termite access to the boards. Blocks were oven-dried for 48 h at 70°C, cooled in a desiccator, weighed and remoisturized before being placed in bucket stations. Bucket stations were buried under the tree canopy at a depth of approximately 2.5 cm below the lid. Following the baiting procedure described below, trees, stakes and bucket stations were monitored for termite activity at approximately 30-d intervals. Monitoring commenced on 24 August 1994 and 24 June 1995 and terminated on 16 January 1997 and 16 April 1997 at Sites I and II, respectively. Since baiting was only conducted around monitoring stations where dyed termites were observed, counts from stations where no dyed termites were observed were considered as controls. These were all located at Site II.

### *1.3. Consumption rates, population estimation and foraging ranges*

Infested blocks were brought to the laboratory and carefully disassembled. Termites were removed by gently tapping the pieces over a $25 \times 38$ cm Teflon-coated cookie sheet. All wood was oven-dried and weighed to compute consumption. Termites were separated from the remaining debris by allowing access to a stack of three pine boards 25 cm long that had been soaked in water. Termites aggregated in the stacks of wood within 3–4 h and were removed and weighed (Tamashiro et al., 1973). Recovered termites were weighed on an analytical balance and their number estimated based on the weight of 100 workers from the same wood block.

One active bait station was chosen to initiate mark–recapture. Termites captured at that station were fed on filter paper disks (Whatman No. 1) stained with a 0.05% wt/wt solution of Nile Blue A (Su and Scheffrahn, 1988). Marked termites were counted and weighed again before being released back into the same monitoring bucket 3 d after collection. Nine additional bucket stations at Site II were monitored for foraging activity and to catch marked termites to delineate foraging ranges and estimate the colony population. At 2 week intervals, blocks and termites were collected from all monitoring buckets at the site and the process repeated with all blocks containing marked termites until three iterations had been completed (Begon, 1979; Su et al., 1993). Triple mark–recapture was initiated on 25 August 1994 at Site I and on 24 July 1995 and 29 April 1996 in the two areas of Site II.

Colony population was estimated using a weighted mean model (Begon, 1979; Su et al., 1993):

$$N = (\sum M_i n_i)/[(\sum m_i) + 1],$$

$$\mathrm{SE} = [1/(\sum m_i + 1) + [2/(\sum m_i + 1)^2] + 6/(\sum m_i + 1)^3]^{1/2}$$

where $N$ is the mean foraging population and SE the associated standard error for the $i$th cycle, $M_i$ the total number of marked individuals up to the $i$th cycle, $m_i$ the number of marked individuals among the captured termites, and $n_i$ the number captured. Foraging ranges were delineated by observation of marked termites in monitoring stations or on stakes.

### *1.4. Baiting procedures*

The baiting station (Dow AgroSciences, Indianapolis, IN) used consisted of a slotted plastic tube, 5.5 cm in diameter and 24 cm in length closed with a screw cap. The tube was initially provided with two rectangular pieces of pine wood (*Pinus* sp.) $18 \times 2.5 \times 1$ cm in size held in place by a polyethylene harness to facilitate removal. Baiting stations were installed in the ground flush with the soil line under trees where marked termites had been observed and nowhere else. Infested pine wood was replaced within 3–4 weeks by a perforated polyethylene tube, 2.9 cm in diameter and 16.5 cm in length, filled to within 2 cm of the top with weighed wood flour or a paper matrix impregnated respectively with 0.1% or 0.5% (AI) wt/wt hexaflumuron. Ten to 20 termite workers recovered from the pine wood were carefully placed into the free space between the matrix and the cap, or recruiting chamber (Su, 1994) of each tube. Tubes containing the 0.1% wood flour formulation were placed in 32 of 55 stations on 6 October 1994 in Site I. A possible effect of additional moisture was evaluated by adding 10 ml of water to the top of 8 randomly chosen tubes. At Site II, 8 of 14 stations (4 with the 0.1% and 4 with the 0.5% wood flour formulation) were baited on 19 October 1995 in the first area and 5 of 8 stations, all with 0.5% active ingredient in a paper matrix were set out on 23 July 1996.

Baiting stations were monitored at weekly intervals and tubes were replaced when about 2/3 depleted. When no more termite activity was observed, tubes were brought to the laboratory where debris was removed from the matrix. The cleaned matrix was then air-dried and reweighed to estimate bait consumption.

### *1.5. Analysis*

*t*-Tests (SAS institute, 1988) were used to compare consumption rates between moistened and unmoistened baits and between 0.1 and 0.5% baits. *t*-Tests were also used to compare consumption rates from baited and unbaited areas.

## 2. Results

### *2.1. Site I*

Termite activity was limited to an area of about 3400 $m^2$ delineated by seven monitoring buckets, all of which eventually captured marked termites. An estimated 129,387 workers were captured in the monitoring buckets during the triple mark–recapture cycle of which 990 were dyed. The number of foraging workers in the area was estimated at $4.7 \times 10^6$ ($\mathrm{SE} = 1.5 \times 10^5$) (Table 1).

Consumption of the 0.1% bait matrix from the 32 bait stations totaled 1260 g from October through February, with 1120 g of the total consumed by 29 November 1994. The 1260 mg of active ingredient consumed would correspond to 0.27 μg/termite estimated per capita consumption. Approximately the same proportion of dry bait tube devices was fed upon (23 of 24) as moistened tubes (8 of 8). Estimated consumption rates of dry and moistened bait for the first 11 d were similar (7.4 g, SE = 1.8 and 9.2 g, SE = 3.0) or 23 and 29%, respectively, with no significant differences ($t = 0.53$, $P < 0.61$).

Total workers captured in the seven active bucket monitoring stations peaked on 18 October 1995, 2 weeks after baiting had commenced, and decreased thereafter to 0 by 9 March 1996 (Fig. 3). No more *R. flavipes* were taken at Site I through 16 January 1997, either from monitoring buckets or wooden stakes, including those in the buffer zone. In comparison, a mean of 1347 termites (SE = 128) were collected from an average 14 bucket stations over 18 sampling dates in unbaited areas of nearby Site II during the same period. A small number of *R. virginicus* was found at Site I in one of the previously active monitoring buckets approximately 7 months after the last *R. flavipes* was trapped. Additional termites collected from monitoring buckets and

Table 1
Initial estimate of foraging populations, consumption of bait matrix and hexaflumuron toxicant, and duration of termite activity at three citrus orchard locations in southwest Florida

| Species | Site | Population ($\times 10^3$) | Consumption: Bait matrix[a] (g) | Consumption: Hexaflumuron (mg) | Duration (d) |
|---|---|---|---|---|---|
| *R. flavipes* | I | 4700 | 1260 | 1260.0 | 155 |
| *R. virginicus* | II | 290 | 28 | 120.3 | 136 |
| *R. flavipes* | II | 120 | 17 | 85.4 | 210 |

[a]Wood flour matrix for first *R. flavipes* and *R. virginicus*; paper matrix for second *R. flavipes*.

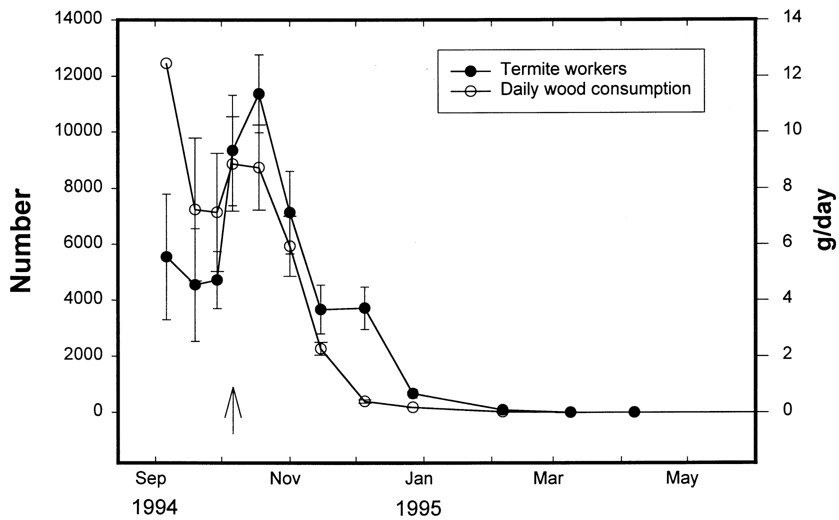


Fig. 3. Mean number of *R. flavipes* workers recovered and average daily wood loss (g) from seven bucket monitoring stations at Site I during 1994 and 1995. Bars indicate SEM. Arrow indicates initiation of baiting.

stakes set out around the perimeter of the baited area were also identified as *R. virginicus*.

The pattern of wood consumption from bucket stations followed closely that of termite abundance (Fig. 3). Consumption peaked on 6 October, coinciding with the initiation of baiting, then declined rapidly. There was no measurable consumption observed from 6 February 1995 through January 1997. In comparison, consumption in unbaited areas of Site II averaged 0.47 g/d (SE = 0.043) during the same period.

Of 15 trees observed with actively foraging termites at the beginning of the baiting phase, only 2 showed activity by January 1995 and no activity was observed 3 months later and thenceforward. No damaged trees were seen within the baited area, the buffer zone or the rest of the block through 5 January 1999 at Site I, over 4 years after commencement of baiting.

### 2.2. Site II

Patterns of termite numbers and wood consumption in unbaited areas followed each other closely, peaking in summer and decreasing in winter (Fig. 4). In addition, an overall downward tendency was observed over the 19-month monitoring period.

Marked termites in the second baited area were all *R. virginicus*. Marked termites were not observed beyond the original four, closely placed monitoring stations used for marking. Termites (*R. virginicus*) were also observed in a monitoring station located in an adjacent row across a temporarily flooded swale, but none were marked. A total of 16,950 termites were captured during the mark–recapture cycle, of which 457 were marked. Colony size was estimated at $2.9 \times 10^5$, SE = $8.7 \times 10^3$ (Table 1).

Approximately 4 times more matrix containing the 0.5% formulation of hexaflumuron (22.9 g, SE = 2.0) was consumed per station, compared with the 0.1% formulation (5.7 g, SE = 0.80). Although the difference was not significant ($t = 1.9$, $P < 0.13$), 20 times more active ingredient was delivered by the 0.5% formulation because of its higher concentration. Estimated consumption of active ingredient was 120.3 mg, or 0.41 μg/termite, approximately equal to that estimated for the *R. flavipes* colony described below. None of the eight baiting devices

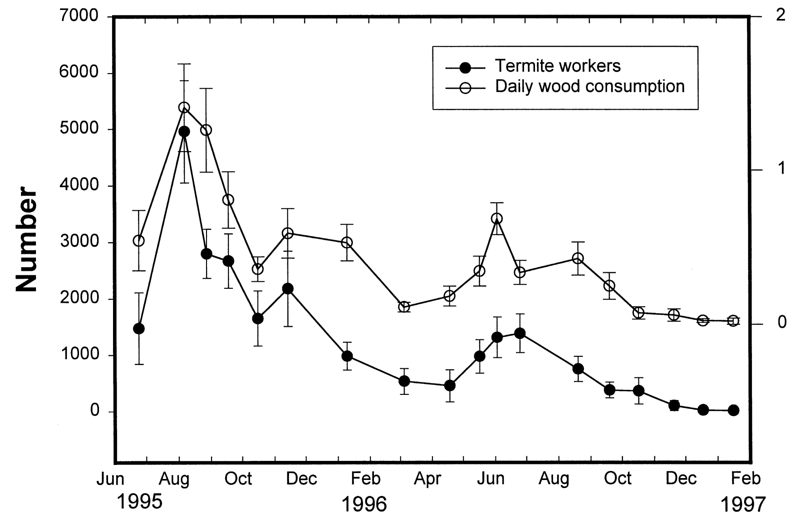


Fig. 4. Mean number of *Reticulitermes* spp. workers recovered and average daily wood loss (g) from 25 unbaited bucket monitoring stations at Site II. Bars indicate SEM.

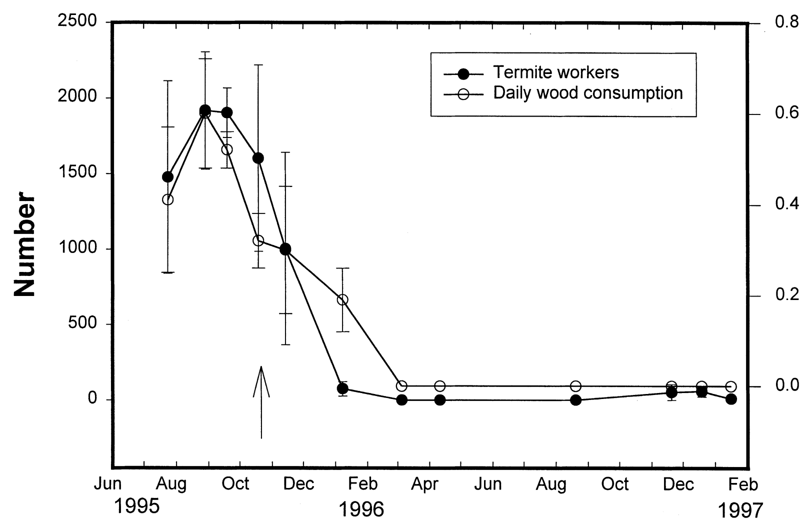


Fig. 5. Mean number of *R. virginicus* workers recovered and average daily wood loss (g) from four bucket monitoring stations in the second baited area during 1995 and 1996. Bars indicate SEM. Arrow indicates initiation of baiting.

required replacement during the 5-month baiting cycle.

Termite numbers in monitoring buckets decreased following the commencement of baiting on 19 October 1995 to 0 by 5 March 1996 (Fig. 5). Mean number of termites on 5 March in 19 control buckets was 532 (SE = 228). No termites were captured within the baited foraging range through October 1996, during which time the mean number of termites in control buckets was 734 ($N = 123$, SE = 97.7). Subsequently, 208, 230 and 37 *R. virginicus* unmarked workers were captured in 2 of the 4 original bucket stations on 20 November and 19 December 1996 and 16 January 1997. Nevertheless, over the period 5 March 1996 to 16 January 1997, mean worker numbers in the baited area (19.1, SE = 10.7) were significantly less ($P < 0.0001$) than in the unbaited area (595, SE = 81, $N = 155$) (Fig. 4). Wood consumption during the same period averaged 0.22 g/d (SE = 0.026) in the unbaited area but was undetectable in the baited area.

In the third baited area, marked *R. flavipes* were observed in four bucket stations, three located closely together near the original damaged tree and the fourth across a swale in an adjacent row (Fig. 6). Colony size was estimated at $1.2 \times 10^5$, SE = $2.6 \times 10^3$ *R. flavipes* based on the capture of 9754 workers of which 2096 were marked (Table 1).

Consumption of active ingredient was estimated at 85.4 mg during the baiting phase of the trial or

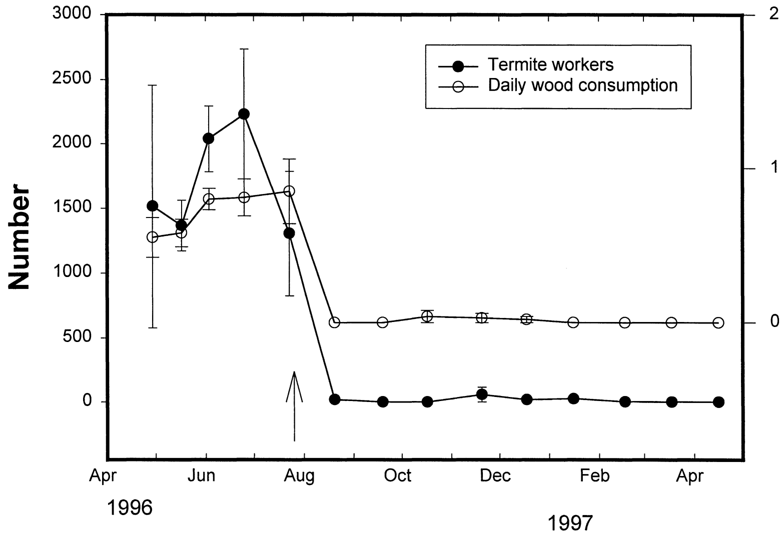


Fig. 6. Mean number of *R. flavipes* workers recovered and average daily wood loss (g) from four bucket monitoring stations in the third baited area during 1996. Bars indicate SEM. Arrow indicates initiation of baiting.

0.71 μg/termite, similar to that observed previously. Most (91%) of bait consumption had occurred by 20 August within the first month of baiting. The paper matrix baits tended to become mushy and had to be replaced on several occasions. Termite counts went from a mean 1308 ($N = 4$, SE $= 958$) on 23 July to 0 by 19 September, 2 months after baiting commenced (Fig. 6). Mean number of termites in control buckets during the period was 532 ($N = 35$, SE $= 132$). A few termites were observed in the fourth bucket station in the late fall and persisted through the winter (228 on 20 November, 72 on 18 December, 101 on 16 January and 7 on February 1997), but none were seen after 7 February through 16 April when monitoring was discontinued. Over the post-baiting period (20 August 1996–16 January 1997), a mean of 13.4 (SE $= 7.3$) termites per bucket station were found in the baited area compared to 330 (SE 77) in the unbaited area ($P < 0.0001$). No further tree damage was observed at the site through January 1999.

## 3. Discussion

Colonies of *Reticulitermes* spp. in Florida citrus are thought to represent vestige populations that inhabited the pine/palmetto habitats prior to orchard development (Stansly et al., 1991, 1992). The primary food source in orchards is probably buried remnants of pine trees with citrus contributing little to colony growth and maintenance. Stansly et al. (1991, 1992) predicted that termite damage to new groves planted on recently cleared land would probably abate as pine remnants were degraded or consumed and maturation of citrus caused bark to thicken and better resist attack. This abatement was observed at unbaited locations in Site II where termite captures fell off in control monitoring buckets toward the end of the study and no new damage to trees was observed since 1996.

Nevertheless, the toxic baiting procedure caused natural abatement in Site II to proceed at a more rapid rate. Where *R. virginicus* reappeared in monitoring stations following an 8 month absence, termites were unmarked and probably not from the baited colony. The apparent demise of the colony at Site I was even more dramatic, although there was no other colony at the location for comparison. Neither was there evidence for displacement of the foraging range across the buffer zone, nor was any subsequent damage to trees observed elsewhere in the block. Therefore, it is unlikely that anything but ingestion of toxic bait caused this vigorous colony of 5 million workers to disappear within 2 months and without a trace for over 2 years.

Under fall conditions in Florida, moistening the wood flour matrix did not appear to enhance acceptance by the termites. The 0.5% formulation was accepted at least as well as the 0.1% formulation and resulted in more rapid acquisition of active ingredient by the colony during the baiting period, increasing likelihood that a lethal dose was acquired. The cardboard matrix tended to disintegrate during the wet summer months, requiring frequent changes making it a less than ideal formulation for wet conditions. The large bucket stations were convenient for monitoring termites, and if adapted for baiting, might be more efficient than the smaller system used.

Our results support the use of a baiting system based on hexaflumuron for management of subterranean termites in Florida citrus. It was perhaps unfortunate for the grower that baiting in these blocks began 4–6 years after the trees were planted. Had baiting begun at planting or even before, replacement of more than 200 trees in the test blocks might have been avoided. Based on our experience, prospects should be good for using this system in citrus against *Reticulitermes* spp. and possibly adapting the system to other crops and termite species.

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