Elimination and Reinvasion Studies with *Coptotermes formosanus* (Isoptera: Rhinotermitidae) in Louisiana

MATTHEW T. MESSENGER,[1, 2] NAN-YAO SU,[1] CLAUDIA HUSSENEDER,[3] AND J. KENNETH GRACE[4]

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ABSTRACT Three Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), colonies located inside the 12.75-ha Louis Armstrong Park, New Orleans, were selected for elimination by using the chitin synthesis inhibitor hexaflumuron. Once eliminated, each vacated foraging territory was monitored for reinvasion by neighboring *C. formosanus* colonies, *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) colonies, or both. Each selected colony was eliminated in ≈3 mo by using baits containing hexaflumuron. Overall activity of each untreated colony in the park remained unchanged during the same period. New *C. formosanus* and *R. flavipes* activity was detected in two of the three vacated territories, and in both areas, within days of selected colony elimination. The third vacated territory was completely reoccupied by a new *C. formosanus* colony ≈7 mo later. Mark–recapture studies and DNA fingerprinting confirmed the distinctness of the reinvaders from eliminated and neighboring colonies.

KEY WORDS Formosan subterranean termite, *Reticulitermes flavipes*, succession ecology, termite baiting, DNA fingerprinting

THE FORMOSAN SUBTERRANEAN TERMITE, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), has become one of the most economically important species in the United States since its introduction to Hawaii in the early 1900s and the southeastern United States in the 1950s (Su and Tamashiro 1987, Su and Scheffrahn 1990). The inability of conventional soil barrier treatments to eliminate colonies of *C. formosanus* was probably the primary reason that allowed for the establishment and subsequent spread of this species throughout the southeastern Unites States in the last 30 yr (Su 2003a). New baiting technologies have lead to increased efficacy in controlling *C. formosanus* and other economically important subterranean termite species (Grace et al. 1996, Su and Scheffrahn 1996). Numerous studies have shown the benefits of incorporating the chitin synthesis inhibitor hexaflumuron into a baiting strategy to eliminate field populations of *C. formosanus* and *Reticulitermes* spp. (Isoptera: Rhinotermitidae) (Su 1994, 2003b; Su and Scheffrahn 1996; Getty et al. 2000). A technology now exists to eliminate individual colonies of *C. formosanus*, possibly leading to the implementation of an areawide program to eliminate all detectable colonies in a given area one by one. However, field studies have documented occasions where neighboring *C. formosanus* colonies reoccupy the vacant gallery systems, including bait stations, of eliminated colonies (Grace et al. 1996). Because simultaneous elimination of all *C. formosanus* populations from a large area is unlikely, there is a need to understand the reinvasion scenario by neighboring populations (Su 2003a). Therefore, it would be beneficial to determine how quickly reinvasion can occur in an area with exceptional termite pressure.

Beginning in 1998, mark–recapture studies were conducted on *C. formosanus* colonies in Louis Armstrong Park, New Orleans, LA, revealing the presence of >13 individual colonies (Messenger 2003). The distinctness of these colonies was confirmed by mark–recapture studies (Messenger and Su 2005) and genetic studies (Husseneder et al. 2003). All 13 colonies remained active from 1998 to 2001. Then, beginning in 2001, three well-established and characterized *C. formosanus* colonies were selected for elimination by using 0.5% (wt:wt) hexaflumuron, the active ingredient in the Sentricon Termite Colony Elimination System (Dow AgroSciences LLC, Indianapolis, IN). The main objective of this study was to monitor the response of adjacent *C. formosanus* colonies to the elimination of these three colonies and determine whether or when reinvasion would occur.

[1] Department of Entomology and Nematology, Fort Lauderdale Research and Education Center, University of Florida, 3205 College Ave., Fort Lauderdale, FL 33314.

[2] Current address: Dow AgroSciences LLC, 6650 Fleur de Lis #9, New Orleans, LA 70124 (e-mail: mmessenger@dow.com).

[3] Department of Entomology, Louisiana State University, 404 Life Sciences Bldg., Baton Rouge, LA 70803.

[4] Department of Plant and Environmental Protection Services, University of Hawaii at Manoa, 3050 Maile Way, Room 310, Honolulu, HI 96822.

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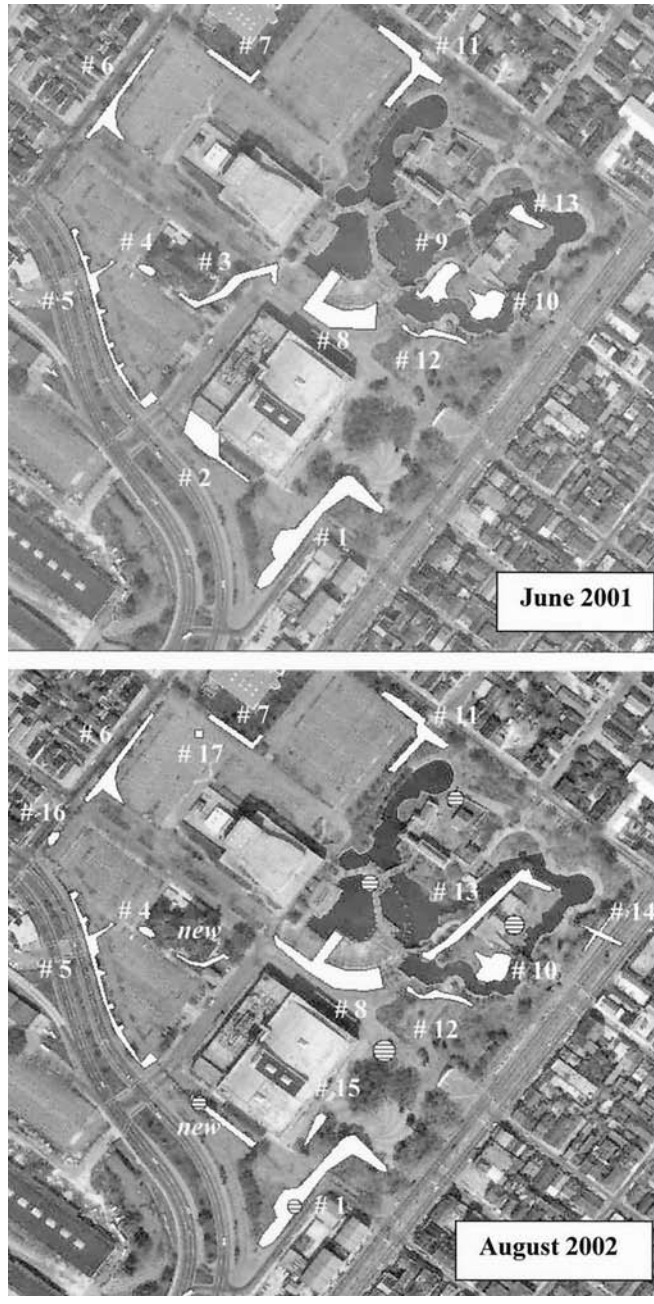

Fig. 1. Location of each *C. formosanus* colony in Louis Armstrong Park, New Orleans, in June 2001 (prebaiting) and in August 2002 (postbaiting). Lined circles represent *R. flavipes* colonies.

Materials and Methods

In August and September 2000, commercial Sentricon stations were installed in the foraging territories of *C. formosanus* colonies 2, 3, and 9 (Fig. 1). In total, 25 stations were installed in the territory of *C. formosanus* colony 2, 16 stations in the territory of *C. formosanus* colony 9, and 17 stations in the territory of *C. formosanus* colony 3. In several cases, pine stakes (*Pinus* sp.) harboring *C. formosanus* activity within each territory were replaced with Sentricon stations. On 21 June 2001, commercial Recruit II Baitubes (Dow AgroSciences LLC) containing 0.5% hexaflumuron were added to active Sentricon stations located in the foraging territories of colonies 2 (Fig. 2), 9 (Fig. 4), and 3 (Fig. 6). These three colonies were chosen for elimination because their foraging territories were near other characterized *C. formosanus* colonies. The foraging territory, wood consumption rate,

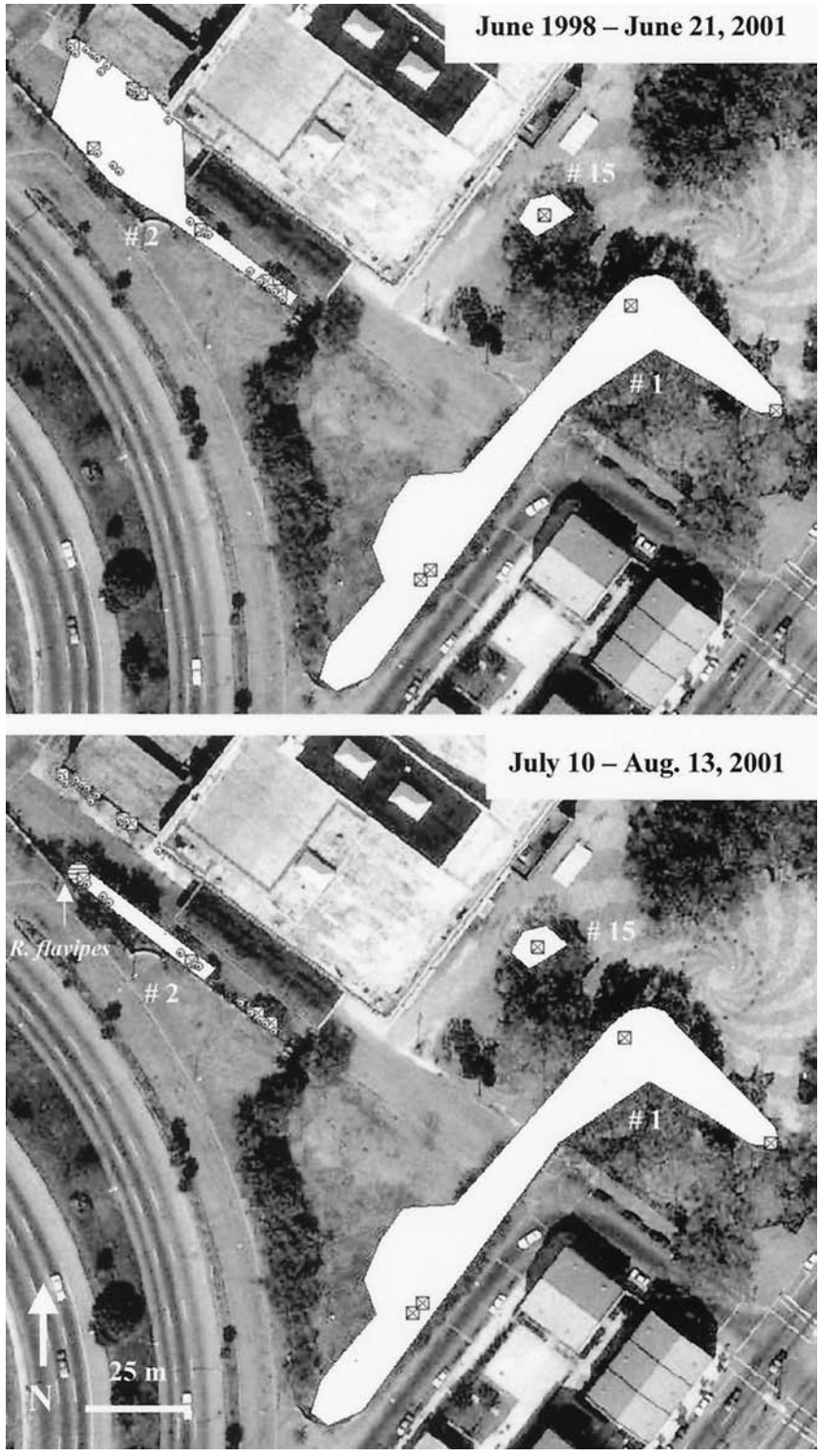

Fig. 2. Change in *C. formosanus* colony 2 foraging territory during baiting period and the movement of a previously undetected *R. flavipes* colony into the territory, in Louis Armstrong Park, New Orleans. Bait introduced on 21 June 2001. Squares represent UMS location, and small, dotted circles represent bait station location. Foraging territory area for each colony includes UMSs, bait stations, infested trees, and pine stakes connected via dyed individuals.

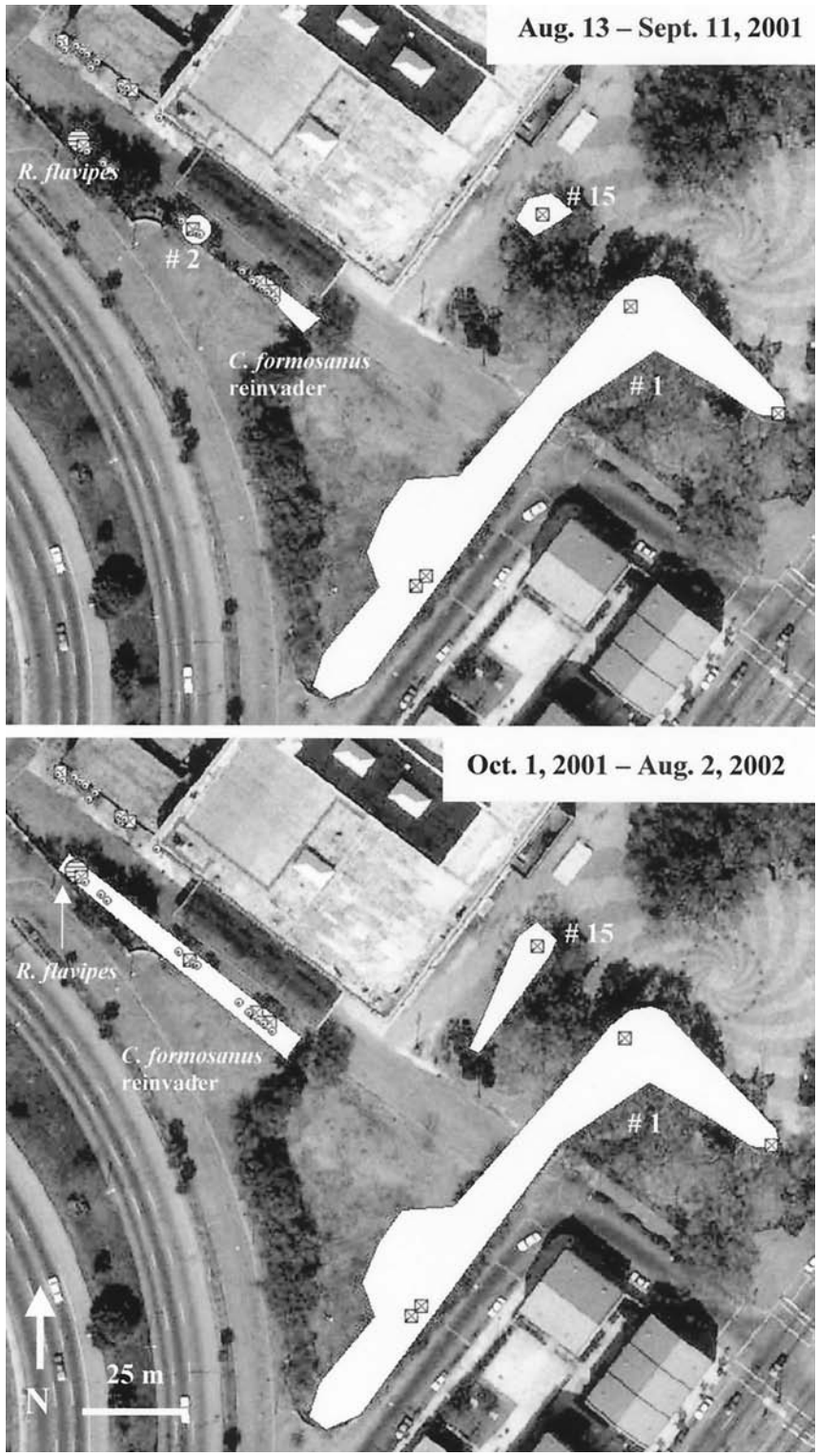

Fig. 3. Elimination of *C. formosanus* colony 2 and further movement of reinvading and previously undetected *C. formosanus* and *R. flavipes* colonies into vacated territory, in Louis Armstrong Park, New Orleans. Squares represent UMS location, and small, dotted circles represent bait station location. Foraging territory area for each colony includes UMSs, bait stations, infested trees, and pine stakes connected via dyed individuals.

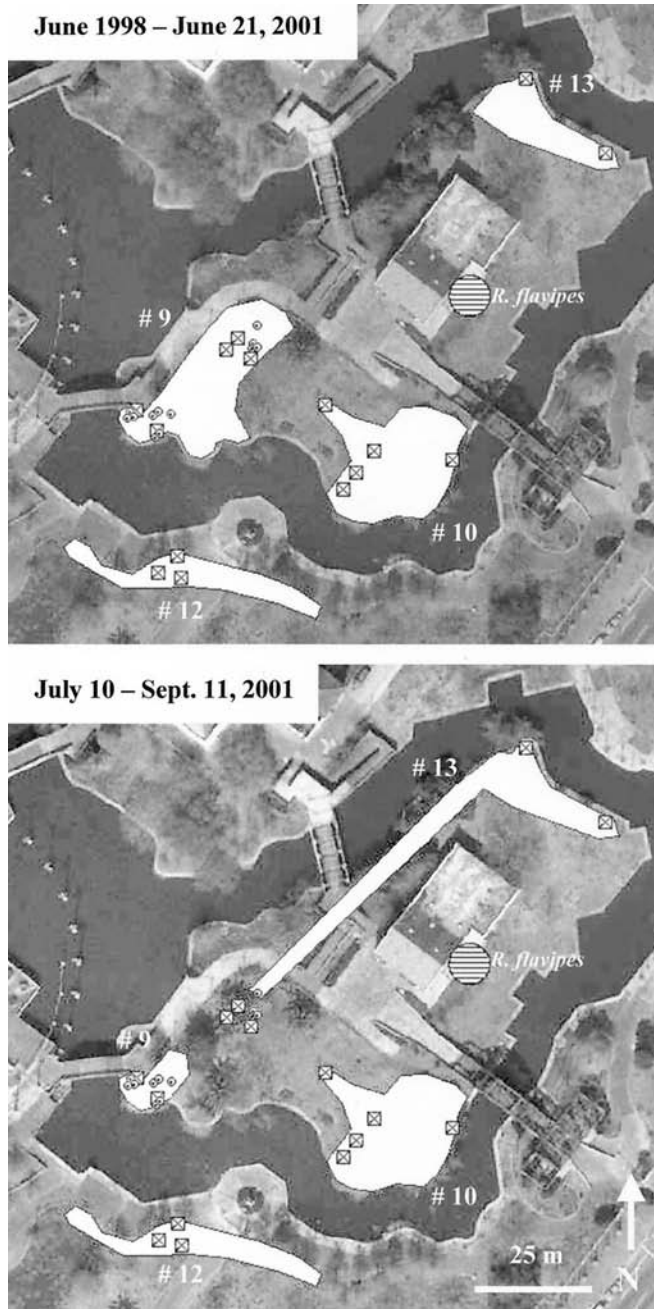

Fig. 4. Change in *C. formosanus* colony 9 foraging territory during baiting period, including eventual elimination and the movement of neighboring *C. formosanus* colony 13 into vacated territory, in Louis Armstrong Park, New Orleans. Bait introduced on 21 June 2001. Squares represent UMS location, and small, dotted circles represent bait station location. Foraging territory area for each colony includes UMSs, bait stations, infested trees, and pine stakes connected via dyed individuals.

and mean worker and soldier weights for each colony had been monitored since 1998 by using underground monitoring stations (UMSs). In addition, the total number of infested trees within each territory was visually monitored on a bimonthly schedule since 1998. Termites from each baited and neighboring colony were continuously collected, weighed, fed filter paper dyed with either Nile Blue A [0.1% (wt:wt)], Neutral Red [0.5% (wt:wt)], or Sudan Red 7B [0.5% (wt:wt)] (Fisher, Pittsburgh, PA), and released into the appropriate UMS to connect active stations with active UMSs. Foraging territories and UMS and Sentricon station location were mapped on a georeferenced, aerial orthophoto image (1 pixel = 0.8 ft^2) of

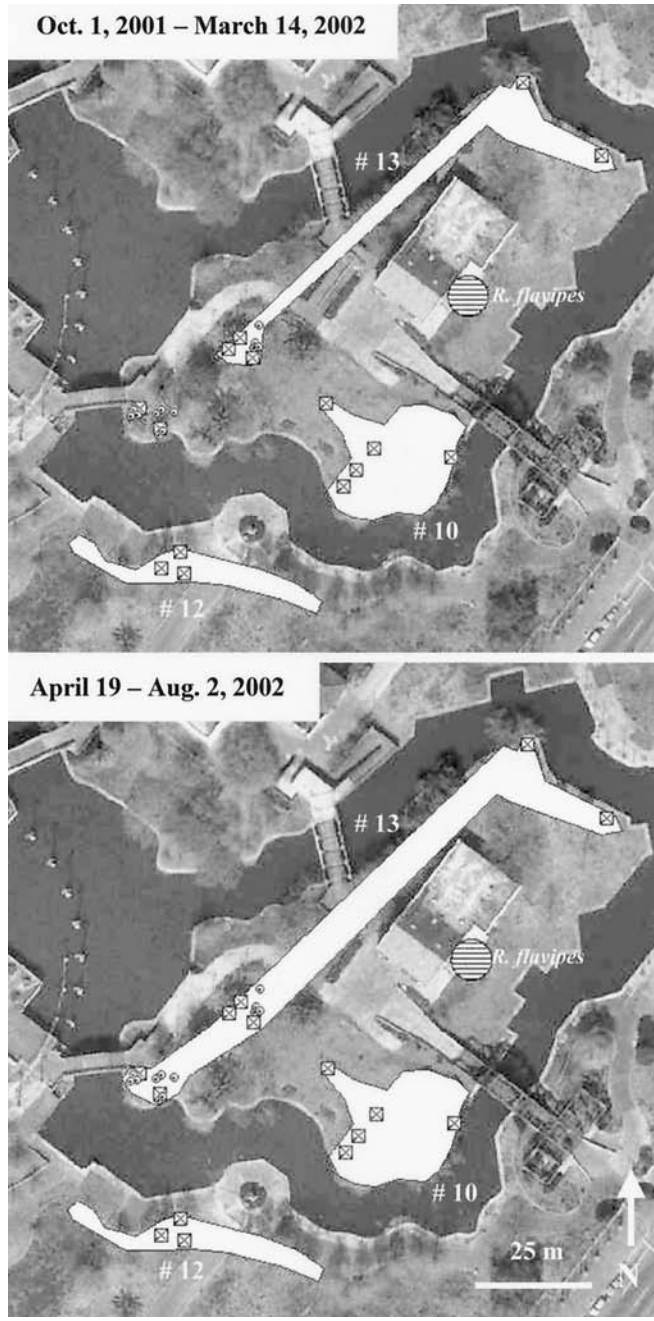

Fig. 5. Elimination of *C. formosanus* colony 3 and further movement of neighboring *C. formosanus* colony 13 into vacated territory, in Louis Armstrong Park, New Orleans. Squares represent UMS location, and small, dotted circles represent bait station location. Foraging territory area for each colony includes UMSs, bait stations, infested trees, and pine stakes connected via dyed individuals.

Louis Armstrong Park by using ArcView GIS version 3.1 software (Environmental Systems Research Institute, Inc., Redlands, CA).

Because most stations became active with each *C. formosanus* colony within a week after installation in fall 2000, the wood-monitoring devices in each Sentricon station were replaced periodically until actual baiting began in June 2001. At the same time, wood consumption rates were continuously calculated after replacing wood feeding blocks in each UMS for all 13 colonies in the park. Blocks collected in the field were first separated from the termites, and loose debris was removed. The wood consumption rate (pixel loss per UMS per day) was calculated using the technique of

Fig. 6. Change in *C. formosanus* colony 3 foraging territory during baiting period, including eventual elimination, in Louis Armstrong Park, New Orleans. Bait introduced on 21 June 2001. Squares represent UMS location, and small, doted circles represent bait station location. Foraging territory area for each colony includes UMSs, bait stations, infested trees, and pine stakes connected via dyed individuals.

Su and Messenger (2000) from 1998 to 2002. The wood consumption rate over time was estimated using videoimage analysis based on the amount of pixel loss (1,000 pixels is ≈0.9 g of wood) for each block.

Wood consumption rates and UMS activity for colonies 1, 10, and 4, which were adjacent to hexaflumuron-treated colonies (2, 9, and 3) were continuously recorded during the baiting period, and these three colonies were considered untreated controls, in addition to other colonies in the park (Fig. 1). Every Sentricon station and UMS was checked biweekly after baits containing hexaflumuron were applied to each colony.

Workers from existing colonies, including any new reinvading colonies, were preserved in 100% ethanol for establishing colony identity and distinctness through DNA fingerprinting. DNA extraction and multilocus DNA fingerprinting was conducted as de-

Fig. 7. Elimination of *C. formosanus* colony 3 and the movement of reinvading and previously undetected *C. formosanus* colony into vacated territory, in Louis Armstrong Park, New Orleans. Squares represent UMS location, and small, doted circles represent bait station location. Foraging territory area for each colony includes UMSs, bait stations, infested trees, and pine stakes connected via dyed individuals.

scribed in Husseneder and Grace (2001a, b) and Husseneder et al. (2002). Because the main focus of this study was to establish whether groups of termites belong to the same colony, we pooled 10 individuals from each sample to characterize the genetic profiles of groups of termites. The number of individuals necessary to achieve a representative genetic profile of a certain colony was determines based on our previous studies determining intra- and intercolony genetic similarity in Armstrong Park (Husseneder et al. 2003). Comparisons between genetic profiles derived from different pools of termites from the same colony run

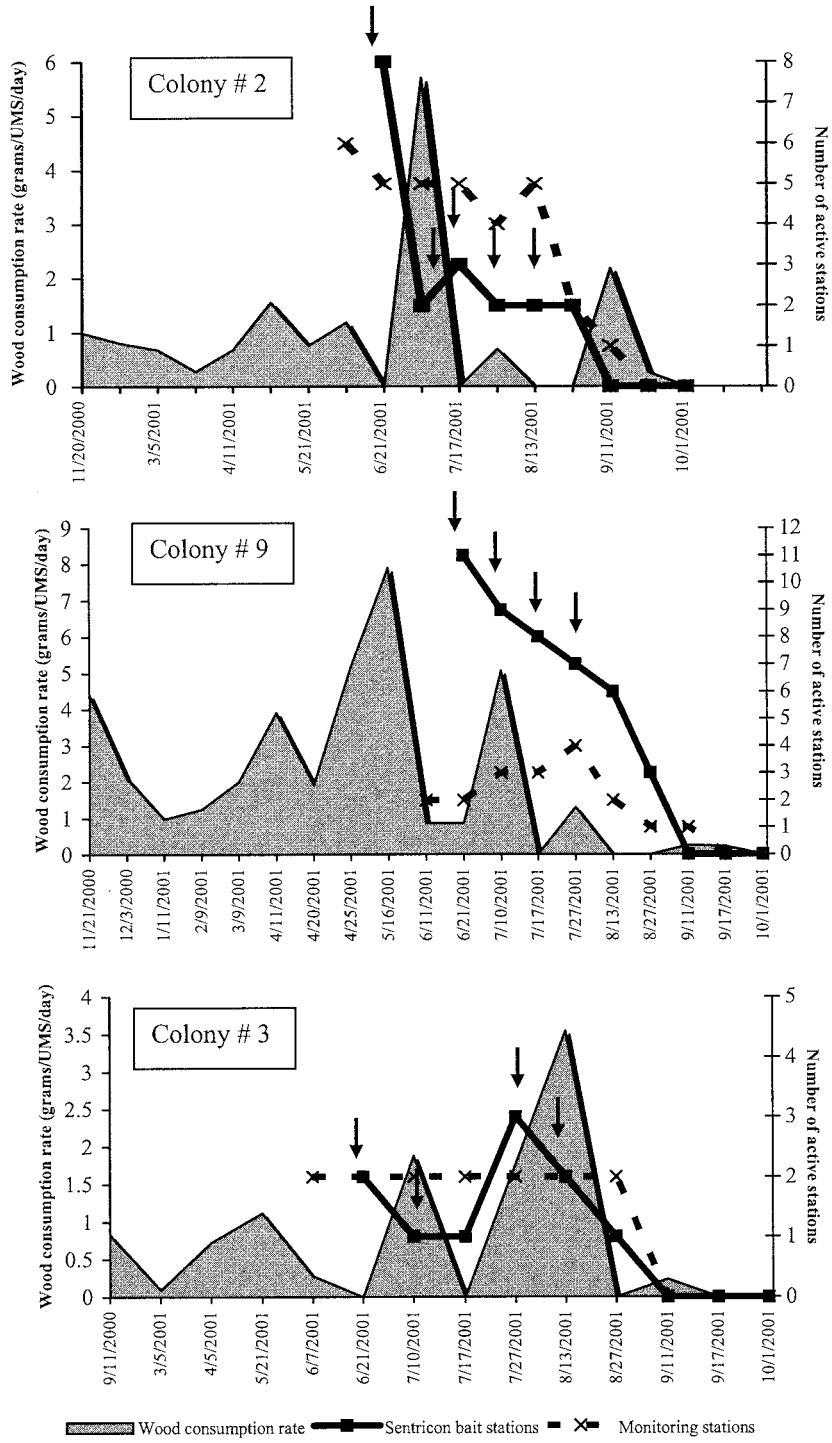

Fig. 8. Wood consumption rate (1,000 pixels is ≈0.9 g of wood), baiting history, and UMS and bait station activity of *C. formosanus* colonies 2, 9, and 3 in Louis Armstrong Park, New Orleans, from November 2000 to October 2001. Arrows indicate application of bait toxicant.

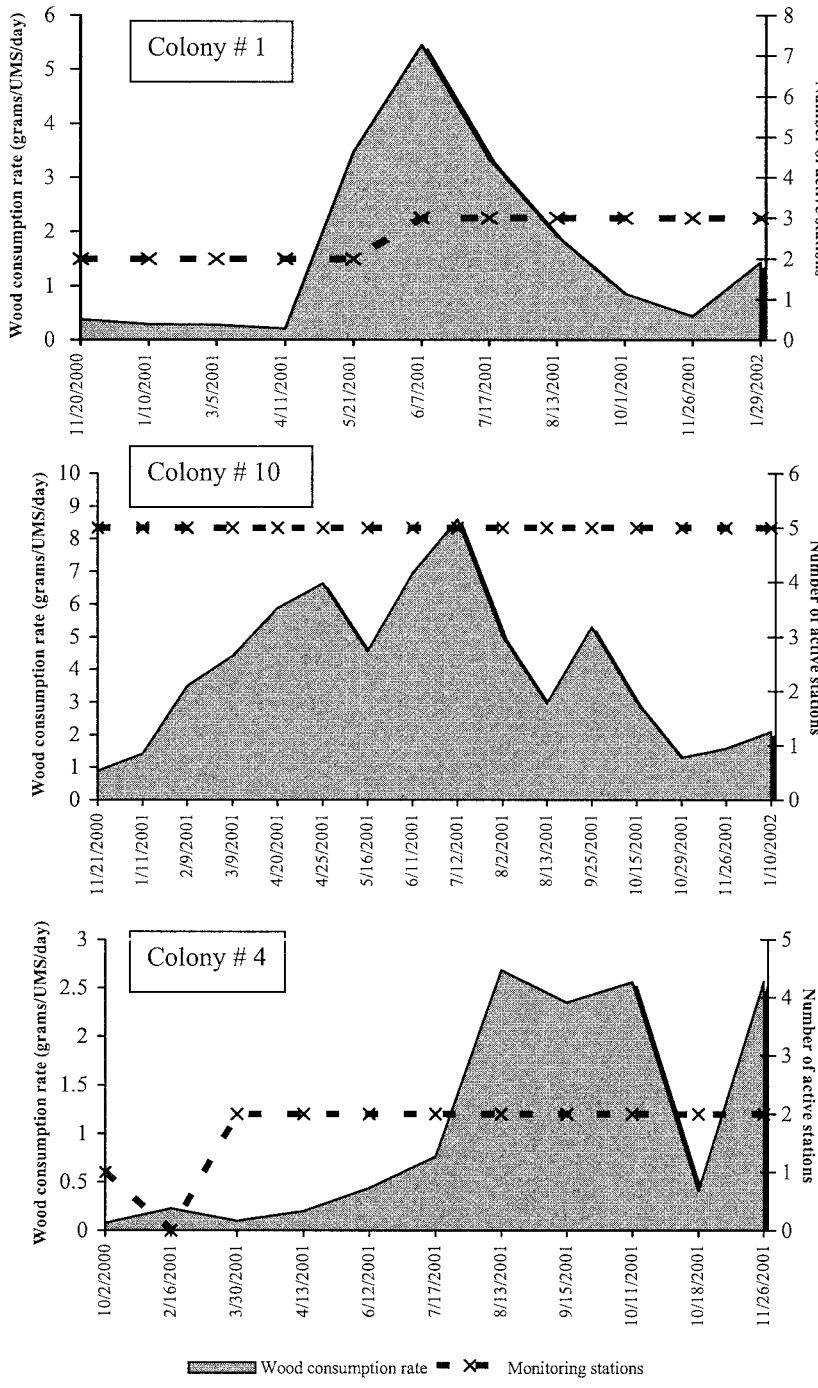

Fig. 9. Wood consumption rate and UMS activity of neighboring *C. formosanus* colonies 1, 10, and 4 (untreated controls) in Louis Armstrong Park, New Orleans, during treatment period.

on independent gels show genetic similarities >0.95 in all cases. Hence, we define groups of termites with >95% identical genetic profiles as identical/belonging to the same colony.

Results

Baiting Strategies. By late August, there was a general decline in Sentricon station and UMS activity,

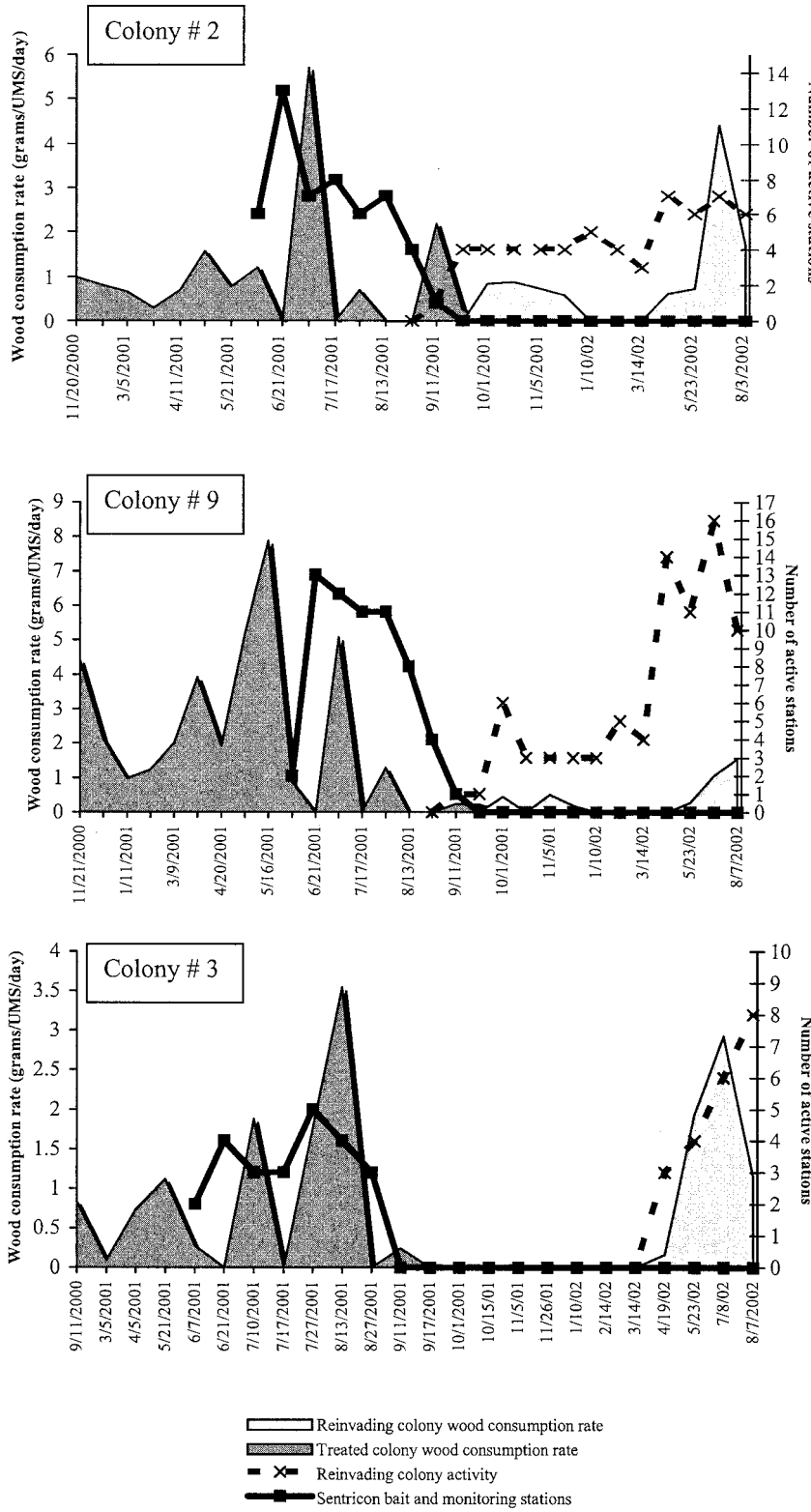

Fig. 10. Reinvading *C. formosanus* colony activity inside foraging territory and monitoring stations of eliminated *C. formosanus* colonies 2, 9, and 3 in Louis Armstrong Park, New Orleans, from September 2001 to August 2002.

including wood consumption rates, for all three treated colonies (Fig. 8). Field observations confirmed colony decline whereby activity within each territory contracted from previously active areas, especially in *C. formosanus* colony 2. Wood consumption rates and UMS activity for all three colonies reached zero and were considered eliminated by 17 September 2001, ≈3 mo after baiting began in late June. In comparison, wood consumption rates and UMS activity for the three neighboring, untreated colonies (1, 10, and 4) remained unchanged during the same period (Fig. 9). Final bait consumption revealed colony 2 consumed an equivalent to 12 Baitubes (240 g of matrix containing 1,200 mg of hexaflumuron), colony 3 consumed a total of 5.5 Baitubes (110 g of matrix containing 550 mg of hexaflumuron), and colony 9 consumed a total of nine Baitubes (180 g of matrix containing 900 mg of hexaflumuron). Toward the end of the 3-mo baiting period, UMS activity had decreased to the point where only one UMS was active for each colony, and usually nymphs and soldiers were the only castes present. The few workers present seemed very sluggish. After elimination, inactive UMSs contained decaying termite bodies and a large number of active collembolans (Insecta: Collembola). On 4 October 2001, trees, which had been actively and consistently harboring these three colonies for over 3 yr, were inspected internally for termite activity by using a video borescope (Everest VIT, Inc., Slidell, LA) after drilling a 9.525-mm hole into each tree. These trees included two sycamores, *Platanus occidentalis* L.; and one live oak, *Quercus virginiana* Mill. (colony 2); two American elms, *Ulmus americana* L.; and another live oak (colony 3); and three baldcypress, *Taxodium distichum* (L.) Rich.; and two red maple, *Acer rubrum* L. (colony 9). Each of these trees had interior cavities large enough to inspect using the video boroscope. As a result, every tree was devoid of termite activity and the interior cavities contained large numbers of collembolans.

Reinvasion Studies. Before the elimination of colonies 2 and 9 occurred, there was new *C. formosanus* activity in Sentricon stations and UMSs along the perimeter of each foraging territory (Figs. 3 and 4). Bait was not added to these previously inactive stations for two reasons. First, blue-dyed individuals from colony 13 were recovered in a single station in the territory of Neutral Red-dyed colony 9. In the territory of colony 2, there was a visual difference in mean worker size and weight between the unknown, reinvading *C. formosanus* colony (3.57 mg) and *C. formosanus* colony 2 (3.93 mg). Second, each baited colony was in decline and the ratio of soldiers and nymphs to workers was very high, and reinvaded stations contained very active workers. Interestingly, a Sentricon station became active with *Reticulitermes flavipes* (Kollar) during the decline of *C. formosanus* colony 2 in August 2002 (Fig. 2). When *C. formosanus* colony 2 was eventually eliminated, the reinvading *C. formosanus* colony slowly reoccupied Sentricon stations and UMSs over time. By August 2002, the new *C. formosanus* colony had reinvaded a majority of the vacated territory. The *R. flavipes* colony remained active in the Sentricon station, even when the new colony reoccupied an adjacent UMS. Wood consumption rate and overall station activity of the colony 2 reinvader steadily increased after September 2001 (Fig. 10). DNA fingerprinting analysis confirmed the new colony was genetically different from treated colony 2, and any other detectable *C. formosanus* colonies, including untreated colony 1.

A similar situation occurred in the foraging territory of colony 9. Colony 13 (*C. formosanus*) initially reinvaded a single Sentricon station and then foraged further into the foraging territory of eliminated colony 9, until colony 13 had reoccupied the entire foraging territory by August 2002 (Fig. 5). This new activity was reflective in station activity and wood consumption rate (Fig. 10).

The decline and eventual elimination of colony 3 occurred throughout the entire foraging territory at about the same time (Fig. 6), which differed from the other two treated colonies. The entire foraging territory of colony 3 remained inactive until 14 March 2002, when new *C. formosanus* activity was detected in Sentricon stations and UMSs. The workers from the new, reinvading *C. formosanus* colony were very small (2.71 ± 0.05 mg) in comparison with those from colony 3 (4.25 ± 0.3 mg). By August 2002, the new *C. formosanus* colony had reoccupied the entire vacated territory, including Sentricon stations and UMSs (Fig. 10).

Again, DNA fingerprinting analysis confirmed genetic differences in all baited Formosan subterranean termite colonies and reinvading *C. formosanus* colonies. As of August 2002, there were at least 15 individual *C. formosanus* colonies in Louis Armstrong Park, including at least six *R. flavipes* colonies (Fig. 1).

Discussion

A relatively small amount of hexaflumuron (≈550–1,200 mg for each colony) was needed to eliminate each colony in the park, which was similar to previous reports by Su (1994). After elimination, field observations of previously infested trees revealed each tree closed outer openings, which had been maintained by the invading colony for over 3 yr. However, a few trees were reinfested with individuals from the reinvading *C. formosanus* colonies and therefore were unable to heal open wounds. The effectiveness of using hexaflumuron to eliminate tree-infesting *C. formosanus* colonies was confirmed during this study.

The rate of reinvasion by neighboring *C. formosanus* and *R. flavipes* colonies was surprising considering the rates of reinvasion from previous studies. In Hawaii, three *C. formosanus* colonies were eliminated using hexaflumuron from three locations, and every monitoring station remained inactive for at least 10 mo (Grace et al. 1996). In California, *Reticulitermes* spp. colonies were eliminated from two sites, and monitoring stations remained inactive for at least 9 mo (Getty et al. 2000). Therefore, the current study con-

firms that, in a high-pressure area, termite colonies are able to reinvade vacated foraging territories and food sources in a relatively short time.

It is unclear why the reinvading *C. formosanus* colonies 2 and 3 were not detected before initiating the baiting/elimination program. These may have been young colonies with limited foraging territories and food resources and were outcompeted by established colonies, such as 2 and 3. Colony 2, 3, or both may have displayed aggression toward these neighboring colonies, thus preventing colony fusion or invasion (Messenger 2003). Only one case of *C. formosanus* colony fusion has ever been confirmed using mark–recapture studies (Su and Scheffrahn 1988a). Therefore, the decline in each *C. formosanus* colony may have reduced the ability of each colony to defend established food sources and tunneling systems, especially in the *R. flavipes* colony reinvading the territory of declining *C. formosanus* colony 2. In southeastern Florida, *R. flavipes* colonies are commonly displaced by the more aggressive *C. formosanus* in urban locations (Su and Scheffrahn 1988b). The ability of the soldier caste to defend the territory also may be affected because of a reduction in the total worker population after bait application, particularly when the soldier caste relies on workers for feeding and grooming.

New Orleans is considered a city with extremely high termite pressure because of the highly conducive environment, which allows the subtropical *C. formosanus* to thrive and use virtually any material containing cellulose. The result of eliminating one colony in a structure or area may lead to further reinvasions by neighboring and opportunistic *C. formosanus* colonies, *R. flavipes* colonies, or both. This study clearly documents that only a relatively short time (days before elimination) is needed for one colony to begin reoccupying a vacated territory and continue feeding on the same food sources, such as UMSs and bait stations. At the same time, DNA fingerprinting proved to be a valuable tool in confirming the distinctness of detectable and undetectable colonies in a high termite pressure environment, such as Louis Armstrong Park.

Some of the benefits of using a baiting system and establishing active monitoring stations within a foraging territory include treatment and future monitoring of new colonies, which may use the same tunneling system connecting stations. This study confirms and reflects the findings of Grace et al. (1996) and Su (2003b) in that the establishment of active monitoring stations and their connections using mark–recapture studies was essential in evaluating and developing short- and long-term control strategies for subterranean termites in a large area, such as Louis Armstrong Park. Further areawide studies involving continuous baiting and monitoring for alate establishment and young, undetectable colonies are needed to gain a better understanding of termite succession ecology in urban environments.

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