Red Imported Fire Ants: Feeding on Radiolabeled Citrus Trees[1]

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ABSTRACT Navel orange and ruby red grapefruit trees made radioactive by soaking the roots in a ^{32}P solution were fed upon by workers of the red imported fire ant (RIFA), *Solenopsis invicta* Buren. Workers obtained radioactivity from all radiolabeled citrus trees; after 24 h, 53% of ants sampled were radioactive and ca. 80% were radioactive from day 2 through day 10. New leaves, blossoms, and small fruit were consumed by the ants. The availability of supplemental food (house fly pupae) did not reduce feeding on the citrus trees.

KEY WORDS Insecta, radioisotope, *Solenopsis invicta*, economic damage

RECENT REPORTS have shown that red imported fire ants (RIFA), *Solenopsis invicta* Buren, feed directly on a number of crop plants. The feeding has been shown to damage and reduce the yield of soybeans (Lofgren & Adams 1981, Adams et al. 1983, Apperson & Powell 1983), Irish potatoes (Adams et al. 1988), and eggplant (Adams 1983). Eger (1985), in a study to evaluate insecticides for control of RIFA in citrus groves, found that the ants damaged young citrus trees. W.A.B. et al. (unpublished data) found that RIFA foraged on the sap of young citrus trees and caused death of 12.5% and 17.2% of the young citrus trees in groves in Volusia County and Indian River County, Fla., respectively. Smittle et al. (1983) reported that RIFA fed extensively on corn, okra, and soybean plants labeled with radioisotopes. This study reports the results of tests that demonstrate feeding by RIFA on radiolabeled citrus trees.

Materials and Methods

The test trees, navel orange and ruby red grapefruit, were ca. 1 yr post-graft on sour orange rootstock and about 1 m in height. The trees were removed from their potting containers, washed thoroughly to remove any soil or other residue, and examined to ensure that no insects were present. The roots of the trees were immersed in a well-water solution containing radioactive phosphorus (^{32}P). The solution was aerated during treatment with an air stone connected to an aquarium air pump. After treatment, the roots were thoroughly washed with water and detergent to remove external ^{32}P, and the trees were potted in 20-liter buckets with potting soil. Each bucket containing a tree was placed in a plastic tray (15 by 40 by 80 cm) containing water to a depth of ca. 10 cm. A similar bucket containing a queenright RIFA colony consisting of ca. 50,000 workers and 20 ml or more of immatures was placed in each tray. The buckets were connected with a plastic hose (2.5 cm diameter). The inner walls of the trays and the buckets and the entire outside of the hose were coated with Fluon to prevent escape of the ants. The water moat and the Fluon coating forced the ants to traverse the inside of the tube to travel from one bucket to the other. Water was added to both buckets of soil as required to maintain proper moisture conditions.

At the end of the ^{32}P treatment, leaves on the trees were monitored with an end-window G-M radiation survey meter to select representative leaves for assay of changes in radioactivity from start to end of the tests. To do this, 1-cm circles were cut from one side of the primary vein of each leaf and assayed for radioactivity in a liquid scintillation counter. At the end of the tests, 1-cm circles were cut from the opposite side of the leaf and assayed for comparison with assays at the start of the test.

Ants were collected daily from the soil surface in both buckets or from the tree for 8–10 d. Some colonies were very inactive on some days and disturbance of the soil was necessary to obtain 10 to 20 ants for assay. Initially the ants were collected with a vacuum device (Smittle et al. 1983); however, featherweight forceps were used for subsequent collections. After collection, the ants were placed in a freezer for 1 h, then washed, placed individually in vials with scintillation cocktail, and assayed in a liquid scintillation counter. The washes were discontinued after assays showed that the ants had no external radiotracer contamination; thereafter, the collected ants were placed immediately in the scintillation cocktail. Because disturbance

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Table 1. Radioactivity of ^{32}P treatment solution and leaf samples of citrus trees (avg of three to eight samples)

Sample	Radioactivity (count/min) of samples from indicated test		
	1	2	3
1 μl treatment solution	40,820 ± 748	91,456 ± 2,511	28,400 ± 560
1 cm circle of leaf			
Start of test	30,883 ± 22,235	6,188 ± 3,565	178,625 ± 95,476
End of test[a]	33,165 ± 19,686	6,490 ± 4,860	105,707 ± 43,541

[a] Test 1, 8 d; test 2, 9 d; test 3, 28 d.

frequently caused the ants to drop from the trees, a paper collar was placed around the base of each tree to catch the ants and facilitate their collection.

The liquid scintillation counter used for these tests had an efficiency of >95% for ^{32}P. All ants with 10 or more counts/min above background were considered radioactive. After assay, the ants were removed from the vials and the vials were reassayed to ensure that radioactivity was caused by ants. In a few instances, vials were radioactive because of the presence of small bits of plant tissue. In these cases, the ants were placed in new vials and the cocktail was then reassayed.

The first test was conducted in an outdoor screen room under ambient conditions with average daily minimum and maximum temperatures of 17.6 and 30.2°C. In this test the roots of one navel orange tree were immersed for 76 h in 464 ml of well water containing 11.6 mCi (429.2 M Becquerel [MBq]) of ^{32}P.

The second test was conducted inside the laboratory in January when ambient temperatures were unsuitable for ant foraging. Light was provided by Gro-lux bulbs on a photoperiod of 12:12 (L:D). Average temperature in the laboratory was maintained at 22.8 ± 1°C with an average relative humidity of 38%. In this test, the roots of one navel orange and three ruby red grapefruit trees were immersed for 92 h in 800 ml of well water containing 25 mCi (925 MBq) of ^{32}P.

The third test was conducted in the outdoor screen room under average daily minimum and maximum temperatures of 14.2 and 33.9°C. In this test, the roots of four navel orange and four ruby red grapefruit trees were immersed for 72 h in 4 liters of well water containing 53 mCi (1,961 MBq) of ^{32}P. Two navel orange and two ruby red grapefruit trees were connected to the ant colony buckets as in previous tests; however, the tubing in this test was only 1.2 cm in diameter. The tubing connecting the other two buckets of each tree variety to the ant colonies had a Y-tube inserted to allow the ants access to a supplemental food container of house fly pupae as well as to the citrus trees.

Table 2. Radioactivity of ants with access to ^{32}P-labeled citrus trees

Test tree	No. ants		Radioactivity (counts/min) of ants	
	Collected	Radioactive	Mean ± SD	Maximum
		Test 1		
NO[a]	170	132	207.6 ± 529.5	4,825
		Test 2		
RGF 1[b]	100	84	268.9 ± 739.9	5,961
RGF 2	100	53	82.5 ± 153.8	997
RGF 3	100	85	63.4 ± 75.6	360
NO 1	100	88	157.5 ± 270.6	1,875
		Test 3		
RGF 1[c]	100	97	156.4 ± 449.3	4,192
RGF 2	100	61	20.1 ± 17.4	104
RGF 3 + SF[d]	100	31	176.6 ± 454.7	1,895
RGF 4 + SF	100	88	28.1 ± 34.5	204
NO 1	100	81	36.7 ± 49.0	321
NO 2[c]	100	100	1,322.6 ± 1,021.4	9,243
NO 3 + SF	100	71	83.8 ± 152.7	850
NO 4 + SF	100	92	136.0 ± 323.8	2,018

[a] Navel orange.
[b] Ruby Red grapefruit.
[c] Plant with blossoms.
[d] Supplemental food provided.

Results and Discussion

The citrus trees readily took up the ^{32}P from the treatment solution, and the entire plant became radioactive. The radioactivity of the treatment solution and leaf samples are shown in Table 1. The trees in test 2 did not take up as much radioactivity as those in the other tests, possibly because this test was conducted in the laboratory under Gro-lux fluorescent bulbs, whereas the other tests were conducted in direct sunlight. There was considerable variation in radioactivity on different parts of the tree; the large mature leaves had more radioactivity than developing terminal leaves. However, blossoms and fruit became highly radioactive. One petal from a blossom had 344,730 counts/min on day 1. One small orange that was chewed off at the stem by ants on day 4 had 1,108,994 counts/min, while the stigma and style from a blossom damaged by the ants had 113,741 counts/min. As indicated in Table 1, the radioactivity in the leaves increased after 8 and 9 d in tests 1 and 2. After correction for decay, these leaves had 1.6 times as much radioactivity as on day 1. In test 3, the leaf samples after 28 d had 2.3 times as much radioactivity as on day 1 after correction for decay. This increase in radioactivity was not expected, because Smittle et al. (1983) reported a decrease in corn and okra plants injected with ^{32}P. Apparently ^{32}P in the roots and stems moved into the leaves.

The radioactivity of ants collected is shown in Table 2. Assays of washes of ants collected on the trees indicated the ants did not become radioactive by crawling on the trees. An average of 53% of the ants became radioactive in the first 24 h in all three tests. This suggests that the ants readily found their way through the tubes into the buckets containing the citrus trees and then began to feed on the trees. From day 2 through day 10, the percentages of ants radioactive were 85.8, 81.7, and 79.6% for tests 1, 2, and 3, respectively.

There was considerable variation in activity of workers of the various colonies. In test 2, one colony was very inactive, so on day 3 we dug into the colony bucket and found the ants clumped near the bottom. By day 9, activity in this colony had increased greatly and 90% of the ants were radioactive. This variability in worker activity in test 3 limited our ability to compare feeding by the ants on the radioactive trees with and without supplemental food.

Blossoms on the trees may influence feeding, because in test 3, one orange and one grapefruit tree were blooming at the start of the test and these trees had the highest number of radioactive ants. The ants were observed feeding on the blossoms and visual damage was evident. Approximately 10 fruit were set on each tree. On the grapefruit, most damage was observed at the base of the fruit, causing the fruit to fall off the tree. Minimal damage was observed on the apical end of the grapefruit. The ants readily fed at the base and apical ends of the orange fruit. Some oranges were totally consumed by the ants, whereas only the inside portion of other fruit was eaten. Feeding at the base of the fruit ultimately caused it to fall from the tree. After the oranges were eaten, the ants continued to feed on the sap, stems, and leaves. All of the oranges were eaten or aborted and about one-half of the grapefruit aborted. After the 10-d test period, one bloom formed on the grapefruit tree (RGF 3) that had the lowest number of radioactive ants. The ants completely consumed this bloom before it opened, even though supplemental food was available. New growth on all trees was readily attacked by the ants. Several colonies moved from the colony bucket into the bucket with the citrus tree and piled the tumulus at the base of the tree.

The results of our radioactivity tests confirm our visual observations of feeding on young citrus trees by workers of RIFA. Damage to blossoms and young fruit was similar to that reported by Smittle et al. (1983) on okra blossoms and pods. The sap of citrus trees is fed upon readily by foraging workers, probably to obtain sugars. Sorenson et al. (1985) reported heavy utilization of sugars by foraging workers of RIFA. The bark removal and girdling of trees by ants reported by Eger (1983) was not observed during this short test. Considering the losses of young citrus from RIFA reported by W.A.B. et al. (unpublished data), the replacement costs could approach $750.00 for each ha of citrus <4 yr old. The severe freezes of December 1983 and January 1985 have forced the replacement of a high percentage of the citrus trees in the northern portion of the citrus region of Florida. Therefore, the impact from RIFA could become more severe in the near future.

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