Using the DAS-ELISA Test to Establish an Effective Distance Between Bait Stations for Control of *Linepithema humile* (Hymenoptera: Formicidae) in Natural Areas

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**ABSTRACT** *Linepithema humile* (Mayr), the Argentine ant, is an invasive pest that has spread throughout the United States and is a problem in natural and managed habitats in South Carolina. Foraging patterns and the effectiveness of liquid baits for control of this pest have been studied in urban areas. However, similar studies have not been conducted in natural areas such as parks, picnic grounds, or campsites. *L. humile* populations can be large and widespread, making them a major nuisance pest for visitors to these natural areas. The primary objective of this study was to determine an effective distance between bait stations for control of *L. humile* in a natural area. A double antibody-sandwich enzyme-linked immunosorbent assay (DAS-ELISA) procedure was used to detect individual ants that consumed rabbit immunoglobin (IgG) protein for marking and tracking. In both lab and field conditions, there was a significant difference in the detection of IgG in ants fed protein marker mixed with sugar water compared with ants only fed sugar water. Additional field studies revealed that an individual ant could retain detectable levels of protein marker for 3 d and that an ant feeding on IgG containing bait could be detected over 15 m from the original bait source. Overall, we found that using liquid ant baits, with a placement of 20 m between stations, was effective in reducing *L. humile* numbers between April to October, 2012 in a natural park area of Lake Greenwood State Park, SC.

**KEY WORDS** foraging pattern, protein marker, ELISA, Argentine ant, liquid bait

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*Linepithema humile* (Mayr), the Argentine ant, is an invasive pest introduced to the United States in 1891 (Newell and Barber 1913). It has spread throughout the southern and western United States. Even though *L. humile* is an urban pest, it causes ecological problems in natural habitats by displacing native ants and other arthropods (Markin 1970, Holway et al. 2002). Many ants, including *L. humile*, take part in central foraging. The foragers collect food around the nest and bring it back to the colony (Holldobler and Wilson 1990). *L. humile* does not show intraspecific aggression (Suarez et al. 1999, Holway et al. 2002); thus, Argentine ants form large, overlapping colonies housing multiple queens (Aron et al. 1990). Additionally, *L. humile* takes part in dispersed central-placed foraging to reduce foraging time and energy expenditure (Holway and Case 2000). The ants transport workers and brood to resources rather than bringing food back to the nest (Holway and Case 2000). *L. humile* foraging patterns and distances have mostly been studied in urban areas (Cooper et al. 2008), but few foraging studies have been conducted in recreational areas within natural habitats such as parks, picnic grounds, and campsites (Ellis et al. 2010). To make the most effective use of baiting for the control of Argentine ants in natural habitats, an understanding of the foraging range of *L. humile* in these habitats is essential.

The enzyme-linked immunosorbent assay (ELISA) is a valuable test method for detecting and quantifying a specific protein in a complex mixture. Engvall and Perlmann (1971) demonstrated quantitative measurement of immunoglobin (IgG) in rabbit serum when it was linked to the enzyme, alkaline phosphatase. Buczkowski and Bennett (2006) used rabbit IgG protein (Sigma Chemical Co., St. Louis, MO) in 30% sucrose–water solution to mark the odorous house ant, *Tapinoma sessile* Say, to evaluate central-place foraging. Previous research findings (Hagler and Naranjo1997) suggest that because rabbit IgG degrades less quickly than chicken IgG, rabbit IgG is retained by ants for a longer period of time post feeding than chicken IgG. Furthermore, hornworm caterpillars, *Manduca sexta* (L.) (Lepidoptera: Sphingidae), fed rabbit IgG solution had greater retention time than those marked with chicken IgG (Kelly et al. 2012). Using double antibody-sandwich enzyme-linked immunosorbent assay (DAS-ELISA; Hagler and Naranjo 1997), Buczkowski and Bennet (2006) tracked foragers and reported that *T. sessile* exhibited high foraging site fidelity, traveled...
Materials and Methods

Much of the preparatory work for these experiments was completed using L. humile individuals collected from, or colonies located on, the Clemson University campus. Voucher specimens for all phases of this research were placed in the Clemson University Arthropod Collection.

ELISA Test. A DAS-ELISA procedure was used for detecting the presence of technical grade rabbit IgG protein marker in ants in all experiments. Each well of a 96-microtiter plate was coated with 100 μl goat anti-rabbit IgG (Sigma Chemical Co.) diluted 1:500 with carbonate coating buffer (0.05 M sodium carbonate + 0.02% sodium azide, pH 9.6) and incubated at room temperature for 1 h. The goat anti-rabbit IgG was discarded and the microtiter plate washed three times with phosphate buffered saline (PBS), pH 7.4 (+0.5 ml/liter Tween-20 (PBS-Tween) for 3 min per wash. Individual frozen ants were ground in 150 μl PBS buffer and added into a single well. The plates were incubated overnight at 4°C after which the samples were discarded and washed two times with PBS-Tween and one time with tris-buffered saline (TBS; 10 mM Tris, 150 mM NaCl, pH 7.5) + 0.5 ml/liter Tween-20 (TBS-Tween) for 3 min per wash. Blocking solution (1% dried milk [Carnation Brand] + 0.5% bovine serum albumen in TBS) was added to each well for 1 h at room temperature to block nonspecific binding sites. Each well was washed three times for 3 min per wash with PBS-Tween. Then, 100 μl of anti-rabbit IgG alkaline phosphatase conjugate (Sigma Chemical Co.) diluted 1:5,000 in blocking solution (diluted 1:10 with TBS-Tween) was added into each well and incubated for 4 h at room temperature. Each well was washed three times for 4 min per wash with TBS-Tween. One hundred microliters of p-nitrophenol phosphate substrate (1 mg/ml) in 10% diethanolamine (pH 9.8) was added to each well. After 1 h of incubation, the absorbance at 405 nm was determined by using an E(max) microplate reader (Molecular devices, Sunnyvale, CA), and readings were subjected to analysis of variance and comparison with a control was analyzed by using a Dunnett's test unless otherwise stated.

Optimal IgG Protein Marker Concentration. To determine optimal concentration of technical grade rabbit IgG protein required to mark individual ants, specimens of L. humile were collected and fed rabbit IgG (IS140, Sigma Chemical Co.). According to Buzckowski and Bennett (2006), 0.5 mg/ml in 30% (weight/volume) sucrose water was a minimal concentration to mark odorous house ants. However, a preliminary test showed that 1 mg/ml of IgG protein marker would be easily detected in L. humile. To reduce potential costs we selected three concentrations of IgG protein marker (0.1, 0.01, and 0.001 mg/ml) below 1 mg/ml. The ants were placed into plastic boxes and fed with 30% sugar water. After 1 wk, a group of at least 10 ants was placed into a Petri dish and fed with one of the three concentrations of IgG serum protein, 0.1, 0.01, or 0.001 mg/ml, diluted into a 30% sugar–water solution. After 3 d the ants were collected at random and frozen at −20°C. A DAS-ELISA test was performed on individual ants and the mean absorbance was recorded.

Optimal Retention Time of IgG Protein Marker Under Laboratory and Field Conditions. To test retention time of the protein marker in the laboratory, L. humile were collected and placed into Petri dishes and fed with the protein marker (0.1 mg/ml) in 30% sugar water. After 3, 5, and 7 d of continuous feeding, ants were recollected and frozen at −20°C. For field tests of the retention time of the IgG protein marker, a 30% sugar water solution was prepared and placed in bait stations. The stations used were KM AntPro liquid bait stations (KM AntPro, Nokomis, FL). KM AntPro bait stations are designed to slowly release bait, hold bait up to 500 ml to feed many ants, and protect the bait from degradation. In other research, this bait station has been successfully used for control of L. humile in a citrus orchard (Greenberg et al. 2006). Control stations with only 300 ml sugar water were compared to stations containing 300 ml sugar water and 0.01 mg/ml of the IgG protein marker. Control stations were placed over 100 m from stations containing the IgG protein marker to avoid ants in the control area foraging to the stations containing IgG. Ants were collected within 5 m of each station at 2, 5, and 10 h, and 1, 2, and 3 d after continuous bait station feeding and stored in −20°C. A DAS-ELISA test was performed and the mean absorbance of individual ants and a control was recorded.

Field Foraging Distance for Which the IgG Protein Marker is Detectable in L. humile. This test was performed in natural areas of the Clemson University campus to determine the distance at which the IgG protein marker could be detected in field-collected L. humile. KM AntPro bait stations were used in these field trials. A control station had only a 30% sugar water solution and was compared with a station containing 30% sugar water (300 ml) and 300 μl of protein
The bait station used as the control was placed at least 100 m away from the station containing the IgG protein marker to avoid having ants in the control areas reach and feed on the station containing IgG. Three days post bait station placement in natural areas on the Clemson campus, several foraging trails were identified at the bait station. Each foraging trail distance was measured from a feeding source to their nest. The longest trail out of several foraging trails was selected and tested to determine foraging distance. Ten ants from each distance were collected at 5, 10, 15, 20, 25, and 30 m away from feeding station. The ant samples were stored in a C02/C14C freezer. The DAS-ELISA test was performed and the mean absorbance of individual L. humile fed with 0.1 and 0.01 mg/ml of IgG.

Effective Distance Between Bait Stations in the Field. A study was conducted at Lake Greenwood State Park (LGSP) (Ninety Six, SC; 81° 58' 0.8868'' W, 34° 11'58.7904 N) to determine the distance between bait stations that effectively manage foraging ants. LGSP had previously been shown to have large L. humile infestations and was ideal for a bait placement study (Ellis et al. 2010). It was difficult to find any other ant species in the test area. Based on earlier results, we hypothesized that significant differences of mean absorbance in bait station placement would occur between 10 and 20 m. Nine bait stations were placed in three rows of three at 10 and 20 m apart. The distance between each experimental area was at least 100 m to

![Fig. 1. Optimal IgG protein marker concentration. Mean absorbance detected for individual L. humile (10 ants per treatment) fed with protein marker at concentrations of 0.1, 0.01, 0.001 mg/ml ± SD. Data were analyzed by the Dunnett test. There were significant differences in mean absorbance of individual L. humile fed with 0.1 and 0.01 mg/ml of IgG.](https://academic.oup.com/jee/article-abstract/108/4/1961/2380257)
avoid collecting ants from neighboring stations. Each station contained 300 ml of sugar water with 0.1 mg/ml protein markers. After 3 d, 90 *L. humile* specimens were collected at mid-way points between stations in each experimental area. Detection of the protein marker was determined using DAS-ELISA. This test was replicated three times. Mean absorbance for each distance was compared using a mixed-model analysis of variance with distance as a fixed effect and replicate as a random effect.

**Evaluation of Bait Station Placement for the Control of *L. humile***. A study was conducted to evaluate bait station placements 20 m apart for the control of *L. humile* in a natural area (picnic area) of LGSP. The KM AntPro stations were used and placed from 21 March to 13 June 2012. Before treatment areas were assigned, ant numbers were evaluated once a week, from January to the middle of March. Ant numbers were counted on 30 trees where the individual number of *L. humile* crossed an arbitrary line (in both up and down directions) for 30 s (Moreno et al. 1987). If a tree had more than one trail, the ant trail with the greatest number of individuals was counted and recorded. When the mean number of ants counted was ~50 on at 30 trees, day-time temperatures were consistently 15°C or higher. On 21 March, three monitoring areas (an area with no stations [natural control (NC)], a control area with stations containing only sugar water [bait...
control (BC)], and a treatment area with stations containing sugar water and an insecticide [bait treatment (BT)] were established. Each area was ~1,600 m² in size. The peripheries of all three areas were at least 72 m apart to avoid the interaction of L. humile foraging from one area to another area (Cooper et al. 2008). Nine bait stations were placed in three rows at 20 m apart, based on a previous test in the BC and BT areas. In the BC area, each station contained 200 ml of 25% sugar water. The BT area had the same station placement, but stations were filled with 180 ml of 25% sugar water mixed with 20 ml Maxforce Quantum ant bait (0.03% imidacloprid, Bayer Environmental Science, Kansas City, MO) which resulted in a 0.003% imidacloprid bait solution. Maxforce Quantum ant bait was chosen for this study because of the active ingredient, imidacloprid. According to Daane et al. (2006), liquid bait containing imidacloprid had been successfully used to control L. humile in a grape vineyard.

Each area, NC, BC, and BT, contained 10 of the original 30 trees used for monitoring ant activity. All ants counted on foraging trails in each area were summed weekly and then averaged to provide a mean number of ants. For reporting and statistical evaluation, ant numbers were combined to obtain the mean number of L. humile for each area from 21 March through 15 October 2012. Bait stations were available to foraging L. humile in the BC and BT areas from 21 March through 13 June 2012. Each week, remaining liquid bait in each station in the BC and BT areas was

Fig. 3. Optimal retention time of 0.01 mg/ml IgG protein marker under field conditions. Mean absorbance number of L. humile (10 ants per treatment) fed with IgG protein marker collected under field conditions. Mean absorbance ± SD are given (n = 10). Data were analyzed by the Dunnett test.
measured and replaced with fresh liquid bait. After 13 June, bait replacement ceased but the weekly monitoring of ant activity continued. Data were analyzed at each sampling time by using analysis of variance. Mean separation was done using Fisher’s LSD.

**Results**

**Optimal IgG Protein Marker Concentration.** Preliminary evaluation with a 1 mg/ml IgG protein marker produced a significant absorbance (>0.2). To reduce protein marker expense, *L. humile* were fed with 0.1, 0.01, and 0.001 mg/ml IgG protein marker. The mean absorbance recorded for individual ants fed each concentration was 0.218, 0.108, and 0.082, respectively (Fig. 1). The average absorbance for ants fed 0.1 mg/ml IgG marker was almost three times greater than the absorbance of the control (buffer solution 0.081). The absorbance recorded for an individual ant fed with either 0.1 or 0.01 mg/ml IgG protein marker was significantly different from the control ($F = 127.87; df = 3, 8; P < 0.001$). The mean absorbance of ants fed with 0.001 mg/ml IgG protein marker did not differ from the mean absorbance for the control. Even though the 0.1 mg/ml absorbance value was two times higher than the 0.01 mg/ml absorbance, the 0.01 mg/ml

![Fig. 4.](https://academic.oup.com/jee/article-abstract/108/4/1961/2380257) Field foraging distance for which the IgG protein marker is detectable in *L. humile*. Mean absorbance of *L. humile* (10 ants per treatment) fed with IgG protein marker collected 5, 10, 15, and 20 m away from a food resource. Mean absorbance ± SD are given ($n = 10$). Data were analyzed by the Dunnett test. There was significant difference of mean absorbance of individual *L. humile* fed with 0.01 mg/ml IgG protein marker collected 5, 10, and 15 m away from a food resource.
Optimal Retention Time of IgG Protein Marker Under Laboratory and Field Conditions. In a laboratory test completed on ants fed 3 d previously with 0.01 mg/ml of IgG in sugar–water, the mean absorbance of an individual ant (0.204) was significantly different from the control (0.09; \( F = 9.32; \) df = 3, 36; \( P < 0.05; \) Fig. 2). However, there was no significant difference for the mean absorbance recorded for ant samples tested 5 d post feeding (0.0125) compared to the control. At 7 d post feeding, the mean absorbance value was almost the same as the control value, indicating the protein marker retained in a single ant was not distinguishable.

For the field test, ants were collected 3 d after setting up bait stations, based on the laboratory test results. There were significant differences in mean absorbance for individual ants collected 2 h, 5 h, 1 d, 2 d, and 3 d after exposure to a food resource compared with the control (\( F = 6.89; \) df = 6, 63; \( P < 0.05; \) Fig. 3). The mean absorbance of IgG protein marker detected in ants increased until 2 d. At 2 h, 5 h, 1 d, and 2 d the mean absorbance values were 1.346, 1.855, 2.119, and 2.77, respectively. After 2 d the amount of protein dropped quickly. At 3 d, the mean absorbance was less, but still detectable at 1.467.

Field Foraging Distance for Which Protein Marker is Detectable in L. humile. There were significant differences in mean absorbance for individual ants collected 5, 10, and 15 m away from sugar water bait containing 0.01 mg/ml of IgG compared to the control (\( F = 13.89; \) df = 6, 63; \( P < 0.05; \) Fig. 4). The mean absorbance of the samples was 2.280, 1.955, and 1.064, respectively. The mean background absorbance in the sugar–water control was 0.082. The mean absorbance of samples collected 20, 25, and 30 m away from a food source was 0.902, 0.638, and 0.238, respectively. However, these values were not significantly different from the control. Thus, the highest absorbance for an ant’s foraging trail was detected at 5 m from the food feeding source. In contrast, the lowest absorbance number was detected at 30 m from the feeding source where a nest was located. IgG levels were also still clearly detectable at distances of 10 and 15 m from bait stations.

Effective Distance Between Bait Stations in the Field. The mean absorbance of ants fed IgG at LGSP was significantly different between bait stations placed at 10 and 20 m as shown in Figure 5 (\( F = 4.23; \) df = 1, 50; \( P < 0.05; \)). The mean absorbance of each treatment was 0.363 and 0.192, respectively. The mean absorbance of the 10-m bait station placement sample was almost two times higher than the 20-m bait station placement. While this indicated that the closer bait stations had more L. humile feeding, marked ants could still be detected at 20 m. Because it was clear that ants were foraging up to 20 m, stations were set at this distance for evaluation of control of L. humile in a natural area to reduce material costs of the bait, the bait stations, and time for labor.

Evaluation of Bait Station Placement for the Control of L. humile. Before bait stations were placed in both the BC and BT areas at LGSP, the mean number of L. humile foraging on 10 trees per area was 65.0 in the NC area, 51.8 in the BC area, and 50.5 in the BT area (Fig. 6). The mean number of L. humile among the three areas on 21 March was not significantly different (\( F = 0.86; \) df = 2, 27; \( P = 0.4345; \)).

By April, the NC mean ant counts (98.0) were significantly greater than the mean ant counts in the BC (58.4) and BT (43.1) areas, respectively (\( F = 7.21; \) df = 2, 27; \( P < 0.05; \) Fig. 6). However, from May until the end of the experiment on 15 October, the mean number of ants in the BT area was significantly lower than the number of ants in either the BC or NC areas. The lowest mean ant count was 12.6 in September for the BT area. The highest mean ant count was 147.7 in June in the BC area. The highest mean ant count was 153.4 in July for the NC area. The mean ant counts for June and July in the BT area were 42.1 and 45.2, respectively.

Discussion

Optimal IgG Protein Marker Concentration. According to Buczkowski and Bennett (2006), a 0.5 mg IgG/ml concentration was selected for a feeding study of T. sessile because it allowed detection of ants that had been fed IgG. However, our preliminary test detected a significant difference between the absorbance recorded for controls (ants not fed IgG) and those fed only 0.1 mg/ml of IgG. This may reflect a difference between the species involved. Three different IgG concentrations (0.1, 0.01, and 0.001 mg/ml) were selected based on our preliminary test to determine the optimal IgG concentration for feeding L. humile workers. Workers fed 0.01 mg/ml IgG had the lowest optimal IgG concentration. Hagler (2004) applied 5 mg/ml rabbit IgG protein to Hippodamia convergens Gue\'rin-Me\'nville for a mark–recapture test. In our study we were able to reduce costs because IgG protein marker concentration was 50 times less than the selected protein concentration of Buczkowski and Bennett (2006).

Optimal Retention Time of IgG Protein Marker Under Laboratory and Field Conditions. Optimal retention time of 0.01 mg/ml IgG under our laboratory condition as indicated by mean absorbance was significantly different from the control at 3 d (\( F = 9.32; \) df = 3, 36; \( P < 0.05; \)) at 0.2039 and 0.0895, respectively. Data showed that at 3 d the mean absorbance was highest but dropped quickly by 5 d. These results were similar to the pattern reported by Buczkowski and Bennett (2006) where the average absorbance dropped sharply by 4 d using a higher protein marker concentration. In our laboratory study, the optimal retention time was 3 d, after which the absorbance dropped.

In field studies, the level of IgG protein detected in the ants increased over a 2-d period and then dropped sharply. The absorbance at 3 d was 1.467. However, this value was high enough to detect IgG protein and...
was significantly different from the control. Buczkowski and Bennett (2006) also mentioned that at 72 h the retention time of protein marker in a field study was shorter than in a lab study. Our results also showed that retention time of protein marker in a field test could be relatively shorter than under laboratory conditions. The results in both lab and field studies showed no significant difference in absorbance at 5 d post feeding. This suggests that the optimal sample collection time of *L. humile* fed with IgG protein was up to 3 d.

**Field Foraging Distance for Which IgG Protein Marker is Detectable in *L. humile***. The mean absorbance number at 5 m from a feeding station was highest and the mean absorbance at 30 m was lowest. We assumed that the foragers digested some IgG protein marker while travelling. We also assumed that *L. humile* shared food with nestmates on the trail by trophallaxis (Flanagan et al. 2013). Results of these data also indicated there was a significant difference from the control in mean absorbance at distances up to 15 m from the feeding source ($F=13.89; \text{df}=6, 63; P<0.05$), suggesting that the majority of *L. humile* populations stayed within 15 m. However, the mean absorbance at 20 m was almost 10 times higher than control. Furthermore, Cooper et al. (2008) speculated that most ants stayed within 20 m of a food source and that carrying bait beyond 20 m from a food source occurred in <10% of the ants. Even though there was not a significantly different absorbance value at 20, 25, and 30 m, the percentage of ants carrying IgG was 90, 100, and 50% positive. Cooper et al. (2008) focused on the positive percentage of IgG detection, but this study determined the significant difference of mean absorbance between individual ant sample and control. If our results were applied to the same data analysis method as the Cooper et al. (2008) study, the percentage of
L. humile carrying IgG protein marker was 100, 100, 100, 90, 100, and 50% at 5, 10, 15, 20, 25, and 30 m from a food resource, respectively. In spite of the high percentage of L. humile carrying in the IgG protein marker at 20, 25, and 30 m, there was no significant difference in the absorbance between each individual samples and the control samples, indicating that 15 m is the optimal foraging distance. However, up to 20 m appeared to be a suitable foraging distance due to higher mean absorbance (0.902) compared with control (0.082). Buczkowski and Bennett (2006) also suggested that foraging range and pattern studies were required in order to obtain better results when using bait for control.

**Effective Distance Between Bait Stations in the Field.** To determine an adequate distance for bait placement within the selected natural areas at LGSP, three rows of bait stations were placed at either 10 or 20 m apart. The results of these DAS-ELISA data indicated there was a significant difference in mean absorbance between individual ants from bait stations placed at 10 and 20 m (P < 0.05) apart. The mean absorbance was 0.363 and 0.192 at 10 and 20 m, respectively. Samples of 270 ants were collected from each plot. After conducting the DAS-ELISA test, the absorbance number was almost the same as the control value in 60 of 270 ants at the 10-m bait stations. At the 20-m stations, 62 of 270 at 20 m were close to the

Fig. 6. Evaluation of control of L. humile, from 21 March to 15 October 2012 using bait stations placed 20 m apart in a natural area at LGSP, SC. Three monitoring areas were established—an area with no stations (natural control [NC]), a control area with stations containing only 200 ml sugar water (bait control [BC]), and a treatment area with stations containing 200 ml sugar water and 0.03% imidacloprid insecticide (bait treatment [BT]). The mean (± SE) number of L. humile present on 10 foraging trails per site per month are represented. Data were analyzed at each sampling time by using analysis of variance. Mean separation was by Fisher’s LSD.
control number. The mean absorbance at 10 m was higher than at 20 m. This suggested that ants feeding at the 10-m bait stations ingested more IgG protein marker than those at 20 m. Because workers share food with colony members via trophallaxis (Knight and Rust 1991), *L. humile* could distribute the liquid bait faster with a 10-m bait station placement than a 20-m placement. Therefore, this study suggested that bait station placement at around 10 m apart would be a better distance. However, the previous study showed that the mean absorbance (0.902) at 20 m apart was 10 times greater than the control (0.092). The mean absorbance number (0.902) at 20 m was almost equal to the mean number (1.064) at 15 m. It suggested that the majority of foraging workers of *L. humile* could look for food at a distance of up to 20 m. For this reason, 20 m was selected to minimize the cost of labor and equipment, as well as insecticide use in the natural area at LGSP.

**Evaluation of Control of *L. humile* at LGSP.** In early spring, carbohydrate resources are scarce for foraging *L. humile*. According to Nelson et al. (2007), the springtime bait deployment date is important because ant colonies become active and there is increasing amount of brood. Thus, bait stations containing sugar water can become a focal site for foraging ants. This increases the ability to transfer toxic baits to *L. humile* workers and larvae early in the foraging season. This in turn can decrease *L. humile* numbers later in the year.

At the start of our field evaluations on 21 March 2012, the mean numbers of foraging *L. humile* among the three areas were not significantly different. After baits were placed in the BC and BT areas, the number of *L. humile* were summed and averaged by month. In April, the mean ant counts in the NC area were significantly greater compared to the BC and BT areas (Fig. 6). By May, the mean ant counts in the BT area were significantly lower than the NC and BC areas and remained this way for the duration of the field study. In June the mean ant counts peaked in the BC area. In July the mean ant counts peaked in the NC area. During June and July, the mean ant counts in the NC and BC areas were over three times greater than the mean ant counts in the BT area. By the end of the study on 15 October, the mean ant counts in the NC and BC areas were over four times greater than the mean ant counts in the BT area. While there was some fluctuation, mean ant counts in the NC and BC areas tended to decline from August to October. The reason for this decreasing trend in mean ant counts was not clear, but it may have been *L. humile* populations reducing ant numbers and combining colonies. Lower *L. humile* activity has been reported in late summer or early fall in other studies (Daane et al. 2006).

In conclusion, we were able to use IgG protein markers and DAS-ELISA to determine foraging distances for *L. humile* in natural areas on the Clemson University campus, Clemson, SC, and at LGSP, Greenwood, SC. With this information, we were able to determine adequate placement of sugar–water bait stations containing imidacloprid. Stations were placed at 20-m intervals for foraging ants starting in March, and this treatment was effective in reducing *L. humile* numbers between April and October in a natural area of LGSP. For this study, only one insecticide product was examined. In the future, a study that investigates other active ingredients for ant baits such as thiamethoxam and boric acid (Cooper et al. 2008) versus imidacloprid would be beneficial.

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