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# Controlling yellow jackets with fipronil-based protein baits in urban recreational areas

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#### **ABSTRACT**

The western yellow jacket, *Vespula penyslvanica* (Saussare), is a serious seasonal pest of outdoor venues in the western United States. In the spring, queens and low numbers of workers were captured in heptyl-butyrate-baited traps until early July, when the number of foragers dramatically increased. Microsatellite data suggest that 18 colonies were actively foraging within the park in 2012. Foragers from 11 different colonies were collected at one trap site. In 2012 and 2013, sufficient numbers of foragers were not captured until early August when baiting trials were initiated. Baits were prepared with canned chicken mixed with fipronil for a final concentration of 0.025%. In 2012, a single baiting provided >96% reduction of foragers for at least two months. A second baiting late in the season provided >80% reductions. In 2013, one baiting trial resulted in a 74% and a 93% reduction. A bait acceptance study was conducted in 2014 to test a bait consisting of the juices from canned chicken and 0.025% fipronil incorporated into a hydrogel, and provided a much longer lasting bait in the field.

#### ARTICLE HISTORY

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#### **KEYWORDS**

Vespula pensylvanica; hydrogels; microsatellites; bait; fipronil

#### 1. Introduction

The western yellow jacket, Vespula pensylvanica (Saussure), is a native North American species that exists throughout most of the United States, west of the Rocky Mountains from Mexico to Canada. V. pensylvanica is the most common pestiferous native yellow jacket in California (Wagner & Reierson 1971; Ebeling 1975). It belongs to the Vespula alascensis subgroup (formerly Vespula vulgaris) and scavenges for human foods (unlike beneficial species of yellow jackets, which are solely insectivorous), and hence, it is a major seasonal pest species, detrimentally interacting with humans wherever they co-exist (Ebeling 1975; Akre et al. 1984; Akre & Macdonald 1986; Rust & Su 2012). Yellow jackets are attracted to places where proteins (in the form of meats) and carbohydrates (typically in the form of sugared beverages) are served outdoors. They typically make their nests underground in abandoned rodent burrows but may occasionally make their colonies in cavities in buildings (Akre & Macdonald 1986). V. pensylvanica frequently choose cavities for their colonies that are in close association with humans in parks, near schools, and settings where stinging threats are potentially dangerous. In warmer climates, such as in California, nests overwinter and become polygynous and persist for more than one season (Visscher & Vetter 2003).

V. pensylvanica is a frequent pest around amusement parks, campgrounds, recreational sites, and zoos,

increasing the likelihood of human encounters and stinging events. In Illinois, 75% of the reported Hymenoptera stings were from urban areas (Friedman et al. 2010). Incidents increased during summer holidays, particularly Labor Day. Stings from Hymenoptera, including honeybees, hornets, and yellow jackets, result in numerous visits to emergency departments (EDs) each summer. From 2001 to 2010, approximately 10.1 million individuals visited EDs, and 67% of these were caused by insect bites and stings (Langley et al. 2014). Of those visits placed in the insect category, 32% were categorized as stings from Hymenoptera. From 2006 to 2008, 161,791 (15%) of ED visits were caused by hornets (genus Vespa), bees, and other wasps. Males older than 18 years were the most likely individuals stung (Langley 2012).

Life-threatening systemic sting reactions (SSRs) to Hymenoptera venom such as severe anaphylaxis are experienced by 1.2% to 3.5% of the population (Przybilla & Rueff 2012; Sturm et al. 2013). From 1991 to 2001, there were 533 venomous fatalities due to bees/wasps/hornets (Langley 2005). Langley (2005) reported that "Males are more likely to die from venomous and nonvenomous animal-related injuries than are females. This probably reflects the greater exposure of males to outdoor activities such as farming and hunting. Some studies suggest that males may be more likely to develop a severe reaction after a bee sting. Males may also have more underlying diseases, such as coronary atherosclerosis, which may be a significant risk factor

for a major adverse event after an insect sting." Even though the number of individuals with SSRs is low, the likelihood of an event increases because of the thousands of people often attending outdoor recreational facilities in the summer.

Severe local reactions greater than 10 cm in diameter that last for several days may occur in as much as 25% of the population. Sensitization to Hymenoptera venom without SSRs is commonly observed in the general population (Sturm et al. 2013). About 43.6% of patients showed large local reactions.

In addition to the annoyance factor and potential medical concerns, foraging yellow jackets disrupt recreational activities. In San Mateo County, CA, V. pensylvanica and V. vulgaris caused an estimated 90% reduction in park attendance and outdoor entertainment (Grant et al. 1968). In New Zealand, where beech forests were near recreational areas, loss in recreational opportunities caused by Vespula spp. cost \$2 million (MacIntyre & Hellstrom 2015). Yellow jacket attacks frequently make the newspapers and newscasts (Grisak 2015; Olenyn 2015; Taylor 2015), reminding us of the need to stay alert in outdoor recreational settings.

Intensive trapping with heptyl butyrate attractant can reduce the numbers of foraging yellow jackets, but trapping alone will not provide area-wide control (Rust et al. 2010). In addition to the destruction of yellow jacket nests with pesticide injection, baiting has been recommended as an effective alternative method for suppressing yellow jacket populations without the need for locating yellow jacket nests. Another approach is to repel foraging yellow jackets from areas of human activity. Seventeen essential oils, such as clove oil, lemongrass oil, ylang ylang oil, spearmint oil, wintergreen oil, sage oil, rosemary oil, geranium oil, and lavender oil, released at 30-45 mg/day were repellent to V. pensylvanica (Zhang et al. 2013). These repellents have been incorporated into commercial products for homeowner use.

In the search for effective baits for yellow jacket control, several kinds of processed meats have been tested for their attractiveness and acceptance by the foraging wasps when mixed with small amounts of insecticides. For example, pet foods, canned chicken or fish, freeze-dried chicken, fish, or kangaroo have been tested by numerous studies (Chang 1988; Spurr 1991; Spurr 1995; Sackmann et al. 2001; Wood et al. 2006; Rust et al. 2010; Hanna et al. 2012). Overall findings of the previous studies are (1) when proteinaceous baits were provided, foraging wasps typically cut them into small pieces and carried them back to the nest, and (2) wasps preferred certain meats, such as chicken and fish, over the others. Consequentially, the likelihood of effective suppression of yellow jackets would be increased when the most preferred meats are used as the bait matrices.

The objective of the study was to reduce the number of yellow jacket foragers in a large urban park with a non-obtrusive monitoring and baiting program. We also developed and field-tested a novel bait matrix for yellow jacket baiting by exploiting highly water-absorbent hydrogel polymer. The research was conducted over a three-year period.

#### 2. Methods and materials

#### 2.1. Site

Irvine Regional Park is located among a grove of oak and sycamore trees in the foothills and wilderness areas of the Santa Ana Mountains in southern California. The park consists of 193 ha, and offers many picnic activities, concession stands, shady turf areas, a zoo, and small lake (Figure 1). Nestled in the foothills, the park provides an excellent foraging setting for V. pensylvanica.

#### 2.2. Monitoring traps

Modified wet traps (Reierson & Wagner 1975) provisioned with an 8-ml vial containing about 7.2 ml heptyl butyrate were used to monitor V. pensylvanica activity. The vials lose about 3.2 mg heptyl butyrate per hour. The design of the trap allowed the collection of wasps in the jar containing propylene glycol (Sierra® Antifreeze/Coolant, Old World Industries, Inc., Northbrook, IL) diluted with water (1:1, vol:vol). Antifreeze was chosen instead of 70% ethanol for our trapping because it is less expensive than alcohol, evaporates much slower than alcohol, kills captured specimens quickly, is not repellent, and does not discolor specimens (Rust et al. 2010).

The monitoring traps were hung under trees about 100-150 cm off the ground and about 20-80 m apart. Their location was often dependent on the topography and availability of low-hanging trees. Traps were checked every 14 days and the heptyl butyrate vials and containers with coolant and yellow jackets were replaced.

#### 2.3. Bait stations and baits

The bait consisted of canned chicken meat (Swanson Premium Chunk Chicken Breast, Campbell Soup Co., Camden, NJ) and 0.025% (wt/wt) fipronil (Rust et al. 2010). The juices within the can of chicken were strained from the meat and saved. A 0.4 ml aliquot of fipronil concentrate (9.1% fipronil, Termidor SC, BASF, Research Triangle Park, NC) was mixed with 50 ml of the juices from 150 g of chicken meat. This preparation was thoroughly mixed with the chicken meat using a laboratory spatula to break the meat into small pieces. Thirty grams of bait were measured out

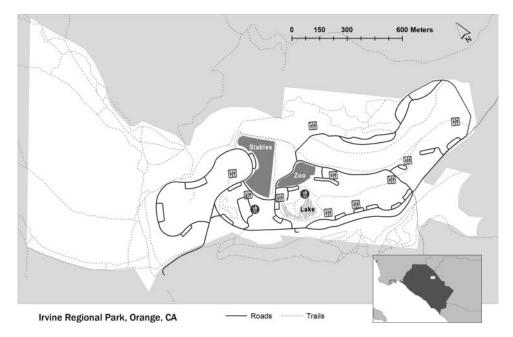


Figure 1. Irvine Regional Park in Orange County, CA.

into 59-ml plastic cups (Amerifoods Trading Co., Los Angeles, CA), and the cups were capped and stored in the refrigerator overnight until used.

Bait stations were constructed from two pieces of pine board about 30.5 cm<sup>2</sup> and 2.54 cm hardware cloth. The hardware cloth was stapled to the edges of the boards to construct the cage  $(18 \times 18 \times 14.5 \text{ cm}^3)$ . Each bait station had three bait cups containing 30 g of bait each (Figure 2). To estimate the potential weight loss due to evaporation of moisture from the bait, three bait cups were placed in a special bait station covered with fine screen (i.e. evaporative checks) to prevent yellow jackets from foraging in them. The bait stations were hung on trees within several meters of the monitoring traps. After the baiting period, the bait cups were capped, returned to laboratory, and weighed to determine the amount of bait consumed. To adjust data for evaporative loss of moisture, the average weight loss from the evaporative checks was subtracted



Figure 2. Bait station hanging underneath a small tree near a picnic area.

from the final weights of the bait cups that were foraged by wasps.

#### 2.4. Bait acceptance

The acceptance of an alternative bait consisting of hydrogel water-storing crystals (Miracle-Gro Lawn Products, Inc., Marysville, OH), chicken juice, and fipronil was tested. Each can of chicken meat yielded about 150 ml of chicken juice. The chicken juice was filtered through a filter paper to remove large particles and was diluted with 450 ml deionized water. The diluted juice (600 ml) was mixed with 1.6 ml of fipronil (Termidor SC) and 30 g of granular polyacrylamide hydrogel (total 630 g), resulting in 0.025% (wt/vol) fipronil liquid bait absorbed in the hydrogel. This preparation was thoroughly mixed with a spatula and left in an ambient condition overnight to ensure complete absorption of the liquid into the hydrogel. Thirty grams of bait hydrogels were measured out into 59-ml plastic cups, covered, and stored in the refrigerator until used.

#### 2.5. Baiting 2012

A total of 53 monitoring traps was installed in the park on 16 April 2012. A baiting program was initiated once the trap counts increased to 10 yellow jackets/ trap/day at a monitoring site.

Five different areas were baited during two separate baiting periods using a total 20 bait stations. On August 1, 10 bait stations were deployed for five days in two locations: the areas around trap #2 (traps #1-5) and around trap #31 (traps 29-31, and two additional locations nearby). On August 31, 10 bait stations were deployed for four days in three locations: the areas around trap #22 (traps #21-23), traps #41 and 42 (traps #40-43), and trap #46 (traps #45 and 46, and one additional location nearby). The bait stations were hung about every 50-80 m near the monitoring traps. Wagner and Reierson (1969) reported that increasing the number of bait stations decreased the time to control yellow jackets, the maximum distance between baits being 200 m. To prevent potential competition between monitoring traps and bait stations, the heptyl butyrate lure vials and collection jars from all monitoring traps were removed during the baiting period.

For a subset of traps (#2, 31, 41, and 42), we used microsatellite analysis of workers collected (1 August 2012) to estimate the number of different colonies in the area. DNA was extracted from V. pensylvanica workers using DNEasy kit (Qiagen). We obtained multilocus genotypes for 42 wasp foragers using eight microsatellite loci: Aa4, Aa9, LIST2001, LIST2014, RUFA5, RUFA19, VMA6, and VMA8 (Daly et al. 2002; Hasegawa & Takahashi 2002; Bushrow & Cowan 2005). Genotypes were examined using GENEMAP-PER 5 (Applied Biosystems). Relatedness was determined using COLONY 2.0.6 (J. Wang, Institute of Zoology) (Jones & Wang 2009). From these data, we estimated the minimum number of different colonies from each trap.

#### 2.6. Baiting 2013

In 2013, three additional monitoring traps were installed in the center of the park for a total of 56 (Figure 3). The new traps were located near picnic areas and concession stands. The first monitoring traps were hung on 22 April 2013.

On August 1, 10 bait stations were deployed at the areas around monitoring traps #34 and 35 and trap #53 for 24 hours.

#### 2.7. Baiting 2014

Fifty-seven monitoring traps were placed in the park on 24 April 2014. The location of the monitoring traps was identical to the 2013 study, except one additional trap was installed near the picnic area in the center of the park. The traps were checked about every two weeks.

A bait acceptance study was initiated at monitoring traps #27 and 57 on August 25 for 24 hours.

#### 3. Statistics

The number of yellow jackets trapped was analyzed with a Wilcoxon's-signed rank test. The traps located a distance of about 400 m from the bait station were analyzed. This distance was selected because yellow jackets forage near their nests. About 80% of V. pensylvanica were trapped within 335 m of the nest site and none detected beyond 942 m (Akre et al. 1975).

#### 4. Results

#### 4.1. Baiting 2012

The number of workers captured in the traps was initially low in April and May with a total of 383 workers captured at 53 monitoring stations between April 16 and June 2. During this time, 43 queens were captured. The last queen was captured on July 2. The numbers of

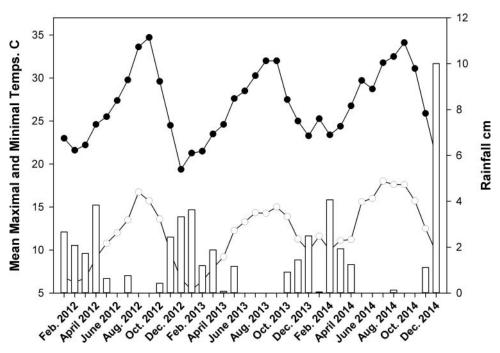


Figure 3. Map for 2013 bait treatments. Numbers indicate the locations of the monitoring traps. The white outlined boxes represent the bait sites.

Table 1. The efficacy of 0.025% fipronil bait applied on August 1 and August 31 in Irvine Park in 2012.

|                    | Initial avg. no.          | Avg. no. of yellow jackets/trap at week (%reduction) |                     |               |               |               |
|--------------------|---------------------------|--|---------------------|---------------|---------------|---------------|
| Sites <sup>a</sup> | Workers/trap <sup>b</sup> | 2  | 4                   | 6             | 8             | 10            |
| 1–5                | 67.4 (6–233)              | 2.6<br>(96.6)  | 4.8<br>(93.7)       | 9.4<br>(87.7) | 6.8<br>(91.1) | 2.8<br>(96.3) |
| 28–<br>32          | 183.0 (42–640)            | 6.8<br>(96.3)<br>0.6                                 | 11.4<br>(93.8)<br>2 | 5.6<br>(96.9) | 1.0<br>(99.5) | 4.4<br>(97.6) |
| 20–<br>24          | 70.8 (3–242)              | 25.8<br>(63.6)                                       | 14.0<br>(80.2)      |               |               |               |
| 38–<br>47          | 80.5 (2–193)              | 25.6<br>(68.2)                                       | 9.0<br>(88.8)       |               |               |               |

<sup>&</sup>lt;sup>a</sup> Sites 1–5 and 28–32 baited on August 1. Sites 20 and 38-47 baited on August 31.

workers trapped dramatically increased in July, and by August 1, a total of 3740 workers were trapped. There were at least six traps with more than 10 yellow jackets/trap/day.

Yellow jackets took 1.5  $\pm$  0.4 g (mean  $\pm$  SEM, n =30) of bait per cup (three cups per bait station) during treatments on August 1 at sites 1-5 and 28-32. After the bait treatment, there was a significant 96.6% and 96.3% reduction in trap counts of yellow jackets at week 2 at monitoring sites 1-5 and 28-32, respectively (Table 1, W = 15, P < 0.001). At week 10, there was a significant 96.3% and 97.6% reduction in the number of yellow jackets trapped at sites 1-5 and 28-32, respectively (W = 15, P < 0.001). Initially, there was also a 56.2% reduction in the number of workers trapped at the other 43 monitoring stations in the park at week 2. By week 4, this reduction had declined to 25.4%.

On August 31, a second baiting was conducted. Yellow jackets took 1.3  $\pm$  0.2 g (mean  $\pm$  SEM, n = 30) of bait per cup (three cups per bait station). There was a significant 80.2% and 88.8% reduction in the number of workers trapped at monitoring sites 20-24 and 38-47 at week 2, respectively (Table 1, H = 15, P <0.001). There was a 39.6% reduction at the other 38 monitoring stations in the park 10 days after the August 31 baiting.

From the wasp samples collected before the baiting trials, we estimated that at least 18 different colonies (Colonies A-R) were foraging within Irvine Regional Park. Microsatellite data suggest that 11 colonies were detected at trap #2, seven colonies at trap #31, and nine colonies at traps #41 and 42 (Table 2). We pooled data from traps #41 and 42 because all wasps at trap #42 had full siblings visiting #41. Workers from five colonies (Colonies A, C, D, H, K) were only detected at trap #2, whereas workers from colonies L & M were only detected at trap #31, and workers from Colonies O-R were only detected at traps #41 and 42.

#### 4.2. Baiting 2013

A total of 64 queens were trapped from April 29 until June 11. The total number of workers was initially low

Table 2. The survey data of colonies foraging in Irvine Park using molecular markers in 2013.

| Monitoring trap # | Minimum # of colonies detected | Minimum # of<br>unique colonies<br>detected | Putative<br>volony ID |
|-------------------|--------------------------------|---|-----------------------|
| 2                 | 11                             | 5   | A, C, D, H, K         |
| 31                | 7                              | 2   | L, M                  |
| 41/42             | 9                              | 4   | O, P, Q, R            |

in April (106) and gradually increased through June. By August 1, the total had increased to 3372 (61.3/trap).

After the baiting on August 1, a significant decrease in the total number of wasps in all traps per day was observed. Five bait stations were placed at each of two sites on August 1. Yellow jackets took 5.2  $\pm$  0.7 g (mean  $\pm$  SEM, n = 27) of bait per cup (three cups per bait station). Three bait cups from one bait station were filled with water from a sprinkler and excluded from the consumption data. In the center of the picnic area (sites 52-54), there was about a 74% reduction in the number of workers trapped over a 10-week period (Table 3). At the more peripheral baiting site (34–38), there was a significant 91.3% reduction in the number of workers at week 10 (W = 28, P < 0.05)

#### 4.3. Baiting 2014

By June 16, 79 queens were trapped. However, the numbers of yellow jackets remained very low and only 733 workers (2.6/trap) had been collected by July 14. None of the monitors exceeded 10 yellow jackets/trap/ day throughout the entire season and baiting trials were not conducted.

A bait acceptance test was conducted on 25 August 2014. Seven monitoring traps (#24, 25, 26, 28, 29, 55, and 56) in the test area collected 2.4 yellow jackets/ trap/day. Even with this low number of foragers, the workers took 2.0  $\pm$  0.4 g (mean  $\pm$  SEM, n=12) of bait per cup. The gel baits were cut into pieces small enough for yellow jackets to easily carry away.

#### 5. Discussion

Yellow jacket foraging activity and density are seasonal and may be affected by weather conditions. However, very little information exists concerning weather and its effect on yellow jacket abundance. In the Pacific

Table 3. Efficacy of 0.025% fipronil bait at two sites in Irvine Park in 2013.

|                    | Initial avg. no. | Avg. no. yellow jackets/trap at week (% reduction) |        |        |        |        |  |
|--------------------|------------------|--|--------|--------|--------|--------|--|
| Sites <sup>a</sup> | Workers/trapb    | 2  | 4      | 6      | 8      | 10     |  |
| 1                  | 76.3 (1–179)     | 11.1   | 5.7    | 5.0    | 4.4    | 6.6    |  |
|                    |                  | (85.4)   | (92.5) | (92.5) | (93.4) | (91.3) |  |
| 2                  | 153.7 (76-       | 50.0   | 43.0   | 52.3   | 42.0   | 32.3   |  |
|                    | 201)             | (67.5)   | (72.0) | (65.9) | (72.7) | (74.0) |  |

<sup>&</sup>lt;sup>a</sup> Site 1 consists of traps 34–38 and 50–51. Site 2 consists of traps 52-54.

<sup>&</sup>lt;sup>b</sup> Range in parentheses.

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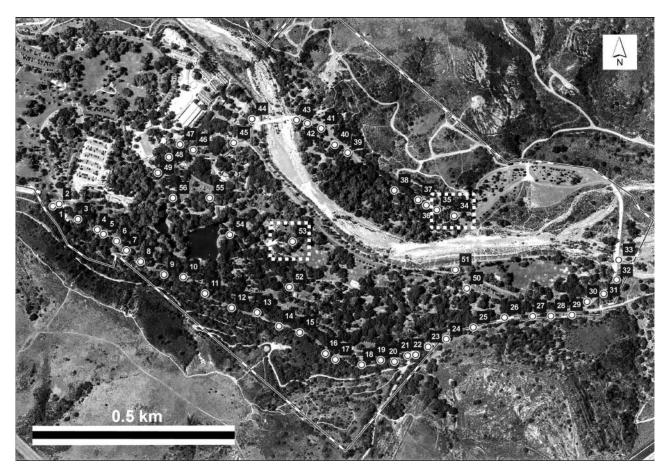


Figure 4. Monthly maximal average temperatures (solid circles) and minimal average temperatures (open circles) and rainfall (bars) at Irvine Ranch, CA, located about 0.3 km east of the park.

Northwest of the United States, warm, dry springs result in outbreak populations (Akre & MacDonald 1986). Even though more yellow jacket queens (79) were trapped in 2014 than in 2012 (43) or 2013 (64), the number of foraging yellow jackets that summer was very low. The general weather patterns for 2012-2014 are summarized in Figure 4. Weather conditions between 2007 and 2009 were examined because these were also years in which yellow jackets were numerous (Rust et al. 2010). The temperature and moisture profiles from 2007 to 2009 are similar to those of 2012-2014, except that the average monthly minimum temperatures were elevated from December 2013 to April 2014, and it was extremely dry during January 2014. The lack of rainfall and warmer temperatures potentially reduced the abundance of herbivorous insects that spring when queens were founding nests.

Various levels of yellow jacket foraging activity have been proposed as action thresholds for potential control. Grant et al. (1968) cited 50 yellow jackets/trap/ week as an "annoyance threshold" when traps were placed at no less than 183-m intervals. Likewise, Rogers (1972) established 50 yellow jackets/trap/week for V. vulgaris as an annoyance threshold. Considering many protein-based baits remains palatable only for a limited amount of time when placed in warm field conditions, the timing for bait deployment is critical to

maximize the bait take by the foraging wasps. Previous studies have shown that 10 V. pensylvanica /trap/day are necessary if baiting is to be successful (Rust et al. 2010). In addition, because V. pensylvanica only appears to exhibit primitive forms of cue-based recruitment strategy, unlike the sophisticated recruitment systems of ants and eusocial bees (Wilson-Rankin 2014), the accurate placement of the baits would also be critical.

Baiting to control yellow jackets is preferred over nest treatments because nests are difficult to locate and may be located off the property (Rust & Su 2012). Baits containing 0.025% fipronil quickly reduced yellow jacket foraging and provided significant reductions in their numbers for at least two months. Control at eight weeks ranged from 69% to 99% corroborating the findings of Rust et al. (2010). In the current studies, it was not necessary to bait again after 6-8 weeks because populations were already declining in the park by October. In 2013, baits placed in the main picnic area around site 52 provided slightly lower percent reductions of workers. It is likely that this occurred because of its central location. Clearly, a much larger area must be baited to obtain similar reductions to baiting along peripheral sites.

Akre et al. (1975) retrieved yellow jackets belonging to more than one colony at a given monitoring station,

indicating that the foraging ranges of colonies overlap. About 10%-20% of the foragers trapped were not unique to one particular monitoring station. Our microsatellite data support these findings and further suggest that the poison baits at the three selected stations could have affected at least 18 different yellow jacket colonies. Trap visits and bait take by multiple colonies may also suggest why a single baiting event could lower yellow jacket counts throughout the park, even at untreated sites, yet was not entirely effective in eliminating all yellow jacket activities in the baited area. If only a few workers from a particular colony visited a bait station, then an insufficient amount of bait would be returned to the nest. Thus, the colony could survive a single baiting episode and workers from this colony may continue to be trapped 10 weeks later. This may also explain why it was important to observe 10 yellow jackets/trap/day before initiating baiting (Rust et al. 2010). A sufficient level of foraging activity is needed to introduce an effective amount of bait to the nest. Lastly, the detection of workers from the same colony at multiple traps may provide insight into the location of those nest sites. For example, workers from two colonies (I and J) were detected in all three trap locations, suggesting that their colony locations may be central in the park. The use of molecular markers will provide an important new tool in evaluating future baiting and monitoring programs.

The use of food-grade proteinaceous food sources in yellow jacket baits has been problematic from several standpoints. The meat-based baits tend to dry quickly during hot and dry summer weather when baiting needs to be conducted, preventing further consumption by the foraging wasps even within a few hours after initial deployment of the baits. Second, many of these foods are expensive. Third, it has been difficult to formulate them with insecticides. Thus, the incorporation of meat flavors into the hydrogels allowed for longer bait acceptance and greater convenience. Additional field trials with the hydrogels are warranted.

The only product in the United States that is registered as a bait for yellow jacket control is Onslaught® (microencapsulated esfenvalerate), but in field tests, it was not effective (Rust et al. 2010). Unfortunately, fipronil has not been registered as a bait for yellow jackets in the United States. Consequentially, either a special registration for fipronil is needed or new toxicants will need to be tested.

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