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Food Safety and Rodent Control in Leafy Green Crops

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ABSTRACT: In 2006, spinach contaminated with *Escherichia coli* resulted in the death of 3 people and over 200 illnesses. The contaminated spinach was traced to a field in central California. In 2007, produce industry representatives developed safety guidelines (known as the Metrics) for lettuce and other leafy green crops. The Metrics addressed encroachment and crop contamination by wildlife and livestock. Rodents are treated by growers and buyers as suspects in the food-borne illness matrix. This has resulted in many unnecessary, ineffective, and expensive rodent control practices in leafy green crops. The Metrics require periodic monitoring of animal activity in and around the crop fields, but there are no specific guidelines on how to monitor for rodents. In 2009/2010, we conducted field work in the Salinas Valley region of Monterey County. This included surveying iceberg lettuce and spinach fields to identify the rodents present and test rodent monitoring techniques. As a result of this fieldwork, a specific index of abundance calculation was developed to enable growers to accurately assess the rodent population in their fields. We reviewed the Metrics and identified all portions related to rodents and rodent control. Monitoring techniques were evaluated for their practicality and ability to detect rodents. Control strategies from the California Vertebrate Pest Control Handbook (CDFA 2008) were combined with the results of the monitoring program to create a pocket-sized field guide containing relevant monitoring and control information regarding rodents and leafy green production.

KEY WORDS: food safety, index of abundance, leafy greens, lettuce, rodent monitoring strategies, snap traps, spinach, wax block, WaxTag

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INTRODUCTION

The safety of the food supply is a primary consideration of farmers, wholesale and retail establishments, and ultimately consumers. While past food safety efforts related to rodents and rodent control have primarily focused at the food processing level, recent food safety outbreaks have attracted more attention to farm production activities. Since 1995, there have been 20 outbreaks of foodborne illness in the U.S. from *Escherichia coli* O157:H7 on leafy greens such as spinach (RCDMC 2007). In 2006, a major outbreak of food-borne illness caused by *E. coli*contaminated bagged spinach resulted in 3 deaths and over 200 illnesses (Beretti and Stuart 2008). The contaminated product was traced to bagged leafy green spinach from Central California.

As a result, in 2007 produce industry representatives, with oversight from CDFA, developed the *Commodity Specific Food Safety Guidelines for the Lettuce and Leafy Greens Supply Chain*, known as the Metrics. The guidelines focus on the entire production operation from farm to packaging and distribution with the goal of minimizing microbial contamination. One issue specifically addressed in the guidelines is encroachment by animals (e.g., livestock, deer, wild pigs). Growers are advised to determine whether or not to harvest portions of a field with signs of wildlife pest activity (e.g., damaged crop, wildlife feces). In addition, buyers and shippers can and do impose additional requirements on growers as a condition of buying their crop.

Rodents are not mentioned as species of concern in the Metrics, nor has research established a strong link between rodents and contamination of leafy green crops in the field. Until the actual link, or lack thereof, is identified, growers, shippers/buyers, and others are treating rodents as suspects in the food-borne illness complex. Growers, in some cases, have been told by buyers to eliminate rodents without any proof that rodents are actually present.

DESCRIPTION AND EXTENT OF THE PROBLEM

Growers must comply with the Metrics and the various interpretations of the Metrics imposed by buyers to ensure their crop is safe for consumption. The response by growers to these requirements has been significant. A recent survey of growers in the Central Coast region of California indicated that of the 140,000 acres managed by the respondents, management activities to deter or eliminate wildlife has occurred on 133,000 acres (RCDMC 2007). Activities targeting rodents include the use of bait stations and poison baiting on 108,283 acres and bare ground buffers on 91,890 acres. There are significant efficacy and environmental risks with this large number of treated acres:

- 1. The target rodent pest species is largely unknown to the growers (D. Huss, pers. commun.). Generally, growers do not identify the species or know the specific habits of the animals being targeted. It is difficult to select a suitable control strategy when the target species is unknown.
- 2. Inappropriate or unnecessary control techniques are possibly being used by growers. For example, traps are often used as a direct control technique. It is hard to imagine trapping as an effective approach to prevent rodents from entering leafy green crops.

Some processors require a bait station every 50 ft. It is a common sight in the central coast growing region to see field borders lined with PVC bait stations. Bait stations designed for ground squirrels placed at 50-ft intervals represent an excessive use of bait that can result in increased hazards to other wildlife (Salmon 2007). A ground squirrel bait station is not necessarily the best design for smaller rodents. The costs for the bait and bait stations at 50-ft intervals are unnecessarily large.

3. Buffer strips (bare ground strips) are being used as a means to prevent rodent movement into crops. However, little is known about the size of the strip needed and in many cases the buffer strips are maintained around the crops without knowing the target species. Without knowing the target species and the strip size needed to provide separation, this approach may be creating significant vegetation-free areas that have little impact on potential crop contamination.

4. According to the Metrics, growers must conduct periodic monitoring (pre-season, pre-harvest, and harvest assessments) of animal activity in and around the crop fields. Presently, there are no specific guidelines on how to monitor for rodents.

The overall goal of this project was to develop a systematic approach for monitoring and controlling rodents in and around leafy green crops and to develop training materials for growers on managing rodents in and around leafy green crops.

Field work was conducted in the Salinas Valley region of Monterey County from May 2009 through June 2010. Ten iceberg lettuce and 4 spinach fields were surveyed for rodent presence and to test rodent monitoring techniques. All of the fields bordered habitat that could harbor rodents. We referred to this habitat as the "wild edge".

METHODS

We contacted major buyers of leafy green crops to obtain information on what they require from growers regarding rodent control. We conducted site visits to meet with growers to identify rodent issues related to leafy green cops. We reviewed the Metrics to identify portions that related to rodents and rodent control.

Rodents were identified in leafy green crops and adjacent lands during 2 site inspections at each field: during the periods of 7 to 14 days prior to harvest, and 1 to 6 days prior to harvest. These time periods roughly correspond to the pre-harvest and harvest inspections suggested in the Metrics. The pre-planting and post-harvest inspections suggested in the Metrics were not undertaken, as we considered such inspections non-productive. Harvested fields were typically plowed under soon after harvest and reworked several times in preparation for replanting, leaving only bare soil, no rodent presence, and no reason for rodents to invade.

Direct observation of pest animals or signs (e.g., burrows, mounds, trails, damage) was the technique used to detect the presence of California ground squirrels (*Spermophilus beecheyi*) and pocket gophers (*Thomomys* spp.). As ground squirrels are diurnal and active above ground during the day, we scanned for squirrels as we drove our vehicle on or off site. Other visual signs of squirrels and other rodents were obtained by walking the perimeter of the fields and by walking across the fields through the crop on at least 2 occasions during each inspection period. We noted the occurrence of any animals, burrows, tracks, mounds, trails, or droppings.

We used mouse-sized snap traps to reveal the presence and identity of small rodents such as voles (*Microtus* spp.), house mice (*Mus musculus*), and deer mice (*Peromyscus* spp.). The snap traps were also evaluated as a monitoring technique.

We evaluated 4 techniques for monitoring rodent populations. Two of the techniques, direct observation and snap trapping, as noted above, also served to identify the

species of rodents present in the field. The 2 remaining techniques, wax blocks and WaxTags, served to indicate the presence and relative abundance of rodents in the field. The wax blocks, known as Detex Blox, were commercially available from Bell Labs (Madison, WI). The wax block is non-toxic and made from 16 human foodgrade ingredients. It is intended to detect the presence of rats or mice and is typically put out at intervals inside or around the outside of a building. Feeding (consumption and toothmarks) on the wax block indicated the presence of a rodent.

The WaxTag is commercially available from Pest Control Research (Christchurch, New Zealand; www. pestcontrolresearch.co.nz). It was designed to monitor the presence of the brushtail possum (*Trichosurus vulpecula*), which is a reservoir of bovine tuberculosis. It is also used to detect the presence of rats or mice on tropical islands where seabirds nest. An animal that chews on the wax will leave tooth marks on the WaxTag, which in some cases can be identified to species. The plastic tag is intended to enhance visibility (for the possum) and also permits the tag to be nailed to a tree, which puts it out of reach of nontarget species. These features are not considered important for rodent use of the wax block. However, the wax is impregnated with a peanut butter scent, which is highly attractive to rodents. Unlike the snap trap, both the WaxTag and the wax block pose no danger to non-target species.

Monitoring Procedures

We used snap traps, wax blocks, and WaxTag to determine the presence or absence and the relative abundance of rodents in leafy green crops and adjacent lands during 2 periods at each field, the period 7 to 14 days prior to harvest, and the period 1 to 6 days prior to harvest. At each site, we established 2 transects, one along the edge or up to 5 ft (1.5 m) into the crop, and the second along the wild edge or up to 5 ft into the wild edge adjacent to the transect in the crop. The 2 transects were in all cases separated by a dirt road used by farm vehicles.

We established monitoring stations at approximately 20-foot (6.1 m) intervals along each transect. The stations were set up in an alternating fashion, with one station consisting of a wax block and a WaxTag, then the next station consisting of a snap trap. We set out a total of 20 wax blocks, 20 WaxTags, and 20 snap traps along each transect. We used a wire stake with a vinyl flag to pin each wax block and WaxTag to the ground. The WaxTags and wax blocks were located a minimum of 20 inches (51 cm) apart. We stuck a wire stake with a flag next to each snap trap. We used peanut butter to bait the snap traps. If any rodent sign was observed (e.g., a runway or burrow), then the trap or wax item was placed appropriately to enhance the likelihood of capture or use.

The stations were set up during the morning or early afternoon of day 1, then were checked for any activity in the morning on days 2 and 3. The stations were removed after the day 3 check. For the snap traps, we recorded the species of rodent caught (if any), whether the bait had been taken without springing the trap, or if the trap was sprung with no catch. We rebaited and reset traps as needed for the next day's check. We examined the wax blocks and WaxTags for any sign of chewing or feeding by rodents,

Table 1. Potential index of abundance (IA) values base on 0 to 40 trapped mice or chewed wax blocks or Wax-Tags over a 2-day sampling period with 20 traps or wax items deployed each day. The calculated values assume no sprung traps, and no missing wax blocks or WaxTags.

| No. of captures or chewed items | IA | No. of captures or chewed items | IA |
|---------------------------------|------|---------------------------------|------|
| 0 | 0 | 21 | 52.5 |
| 1 | 2.5 | 22 | 55 |
| 2 | 5 | 23 | 57.5 |
| 3 | 7.5 | 24 | 60 |
| 4 | 10 | 25 | 62.5 |
| 5 | 12.5 | 26 | 65 |
| 6 | 15 | 27 | 67.5 |
| 7 | 17.5 | 28 | 70 |
| 8 | 20 | 29 | 72.5 |
| 9 | 22.5 | 30 | 75 |
| 10 | 25 | 31 | 77.5 |
| 11 | 27.5 | 32 | 80 |
| 12 | 30 | 33 | 82.5 |
| 13 | 32.5 | 34 | 85 |
| 14 | 35 | 35 | 87.5 |
| 15 | 37.5 | 36 | 90 |
| 16 | 40 | 37 | 92.5 |
| 17 | 42.5 | 38 | 95 |
| 18 | 45 | 39 | 97.5 |
| 19 | 47.5 | 40 | 100 |
| 20 | 50 | | |

as evidenced by marks from the rodents' incisors. On day 2, we lightly marked the chewed areas of the wax blocks with a black Sharpie pen. This allowed us to identify any new chewing when the blocks were checked on day 3. We used our fingers to smooth out any signs of chewing on the WaxTags, as the wax was pliable and easily molded to cover over the incisor marks.

We calculated an index to rodent abundance (IA) following Caut et al. (2008). This calculation took into account the number of corrected trap nights (Nelson and Clark 1973). The calculation was:

$$IA = \frac{100 \times captures}{(TN - S/2)}$$

where $TN = P \times T$. P is the number of trapping intervals, T is the number of traps, S is the total number of traps sprung (but without any catch), TN is the number of trap nights, and TN - S/2 is the number of corrected trap nights. With regard to the wax blocks and WaxTags, "captures" in the above calculation would refer to the number of blocks or tags that had been chewed on. Also, a missing wax block or WaxTag (e.g., taken away by a coyote or racoon) would be analogous to a sprung trap. For comparative purposes, we calculated an IA value for each item (snap trap, wax block, WaxTag) for each transect (in crop, wild edge) for

Table 2. Changes in the numbers of mice trapped in-crop and along the wild edge from the 1st sampling period to the 2nd sampling period at 14 sites in Monterey County.

| Location | Decrease | No Change | Increase |
|-----------|----------|-----------|----------|
| In-crop | 3 | 7 | 3 |
| Wild edge | 5 | 4 | 5 |

each sampling period (7 to 14 days before harvest, 1 to 6 days prior to harvest). If there were no sprung traps or missing wax blocks or WaxTags, the IA value could range from 0 to 100 (Table 1).

We combined control strategies from the California Vertebrate Pest Control Handbook (CDFA 2008) with the results of the monitoring programs to produce a field guide specific to rodent control in and around leafy green crops.

RESULTS

We conducted visual surveys and used snap traps on 14 sites. Visual surveys indicated no ground squirrels or ground squirrel burrows in or around any of the fields. With the exception of 2 fields, pockets gophers were not present in-crop. In the 2 fields where we observed gopher mounds, the infestation appeared light, consisting of limited areas with <5 mounds each. Gopher sign, however, was common in the wild edge adjacent to the crop sites. We found gopher mounds in the wild edge at 11 of 14 sites. We did not find any evidence (e.g., burrows, runways) of mice inhabiting any of the crop sites. Mouse-sized burrows or runways were found in the wild edge at 12 of 14 sites.

Mice were trapped both in-crop and in the wild edge. In 27 sampling periods, no mice were trapped in-crop during 14 sampling periods. Low numbers of mice, usually 1 or sometimes 2 mice, where trapped in-crop during 13 sampling periods. No mice were caught in the wild edge during 13 of 28 sampling periods. In comparison to the in-crop trapping, relatively large numbers of mice (e.g., 7 to 9 mice per sampling period) were caught along the wild edge during 4 sampling periods. Overall, 17 mice were caught in-crop, while 51 were caught along the wild edge. Deer mice and brush mice (Peromyscus spp.) were the most frequently caught mice, with 15 caught in-crop and 33 caught along the wild edge. House mice were the next most abundant species, with 15 mice caught along the wild edge at 4 sites but only 2 caught in-crop at 2 sites. Only 3 voles were trapped, all taken along the wild edge.

In terms of changes in the number of mice caught in snap traps from the 1st sampling period to the 2nd sampling period, in 10 out of 13 cases the catch in-crop remained the same or decreased (Table 2). For the wild edge, the catch remained the same or decreased in 9 out of 14 cases. Combining the trap catch data for in-crop and wild edge surveys, in the 8 cases where the catch increased from the 1st sampling period to the 2nd sampling period, the increase was only 1 mouse in 6 of the 8 cases. This illustrates that in most cases there was a decrease, no change, or only a small increase in mouse populations from the 1st to 2nd sampling period, which calls to question the value of the 2nd sampling period undertaken only 1 week after the 1st sampling period0

Table 3. Summary of data showing the number of wax blocks or WaxTags with no activity, chewed by rodents, or missing, and the number of snap traps either sprung, missing, or with rodent catch in spinach and iceberg lettuce fields surveyed during the period from May 2009 through June 2010 in Monterey County, California.

| Status | Wax Blocks | | WaxTags | | Snap Traps | |
|------------------------------------|------------|------|---------|------|------------|------|
| | In Crop | Edge | In Crop | Edge | In Crop | Edge |
| No activity | 964 | 696 | 1052 | 1024 | 997 | 890 |
| Chewed or with catch | 110 | 397 | 12 | 72 | 17 | 51 |
| Percent chewed or with catch | 10.2 | 36.3 | 1.1 | 6.6 | 1.7 | 5.4 |
| Sprung or missing | 5 | 7 | 6 | 4 | 64 | 158 |

Table 4. Changes in IA values from 1st to 2nd surveys for spinach and iceberg lettuce fields surveyed for rodents during the period from May 2009 through June 2010 in Monterey County, California.

| Item | Decrease | | No change | | Increase | |
|-----------|----------|------|-----------|------|----------|------|
| | In Crop | Edge | In Crop | Edge | In Crop | Edge |
| Wax block | 6 | 2 | 3 | 1 | 4 | 11 |
| WaxTag | 4 | 6 | 8 | 4 | 1 | 4 |
| Snap trap | 4 | 5 | 4 | 4 | 5 | 5 |

We evaluated visual surveys, wax blocks, WaxTags, and snap traps as techniques to monitor rodent populations in and around 14 sites. Data on the number of items chewed, sprung, or missing, and the rodent catch are summarized in Table 3. The wax blocks had the greatest amount of chewing activity both in-crop and along the wild edge at 10.2% and 36.3%, respectively (Table 3). In comparison, the WaxTags were less preferred by the rodents, with activity levels of 1.1% in-crop and 6.6% along the wild edge. The catch levels for snap traps at 1.7% in-crop and 5.4% along the wild edge were similar to the chewing activity for WaxTags.

Regarding IA values, in 15 of 27 (56%) surveys incrop and in 28 of 28 (100%) surveys along the wild edge, the wax blocks had higher values compared to the Wax-Tags or snap traps. As the average IA values are based on the number or items chewed or mice caught with a correction for trap nights, the calculated IA values are nearly identical to the percentage rankings above (see Table 3 to compare): wax blocks – 10.2 in-crop, 36.9 along the wild edge; WaxTags – 1.1 in-crop, 6.6 along the wild edge; snap traps – 1.6 in-crop, 5.4 along the wild edge. The highest individual IA values were: wax blocks – 55.0 in-crop, 80.0 along the wild edge; WaxTags – 7.5 in-crop, 32.5 along the wild edge; snap traps – 5.1 in-crop, 30.8 along the wild edge.

Regarding changes in IA values from the 1st sampling period to the 2nd sampling period, for all items both in-crop and along the wild edge, the IA values, with 1 exception, either decreased or stayed the same in the majority of cases (Table 4). For example, IA values for wax blocks decreased or stayed the same in-crop in 9 of 13 cases, or decreased or

stayed the same for snap traps in 9 of 14 cases along the wild edge. The exception was wax blocks along the wild edge, where the IA values decreased or stayed the same in only 3 of 14 cases.

The wax blocks and to a much lesser degree the WaxTags were fed upon by snails (species unknown) at several sites, particularly those that bordered the slough. The snail activity occurred along the wild edge, never in-crop. Snail feeding could be differentiated from rodent feeding. Snail feeding looked like smooth shallow scoops in the wax block, compared to the jagged incisor marks left by rodents. Snails typically left a slime trail on or around the wax blocks, which also indicated their presence. Rodent feeding usually, but not always, resulted in "crumbs" on the ground around the wax block. Snail feeding did not result in crumbs on the ground.

The WaxTags were impacted by weather conditions. Eight WaxTags either melted or partially melted during the 13 - 15 July 2009 sampling period at Field 6. The high temperature in Salinas reached 88° F (31° C) during that period.

DISCUSSION

Whisson and Engeman (2003) examined various indexing techniques to measure relative abundance of meadow voles (*Microtus*

californicus), a common pest in artichokes. They concluded that wax blocks provided the most sensitive index measurements and were logistically the easiest to use. This study attempted to develop a monitoring system based on the findings of Whisson and Engeman, but one that avoided the complicated computations used by Whisson and Engeman that were best suited to scientific research rather than "real world" situations. Thus, our goal was to develop a monitoring system that identified the species of rodents present, and provided an index to relative abundance that was practical, easy to apply and to interpret.

We evaluated 4 techniques, wax blocks, WaxTags, snap traps, and visual surveys. The wax blocks and Wax-Tags provided different information than the snap traps and visual surveys. The wax blocks and tags only indicated the presence or absence of small rodents (e.g., mice). The snap traps and visual surveys indicated the presence of rodents, but more importantly the species of rodent that were present. Visual surveys could effectively detect large rodents (e.g., ground squirrels, pocket gophers) both incrop and along the wild edge, but were not effective in detecting small rodents in-crop. We suspect that the mice detected in-crop by the wax items or the snap traps were living in the wild edge. Thus, there would be no sign (e.g., burrows, runways) of small rodents to be detected by visual surveys in-crop. The visual surveys and snap trapping complimented each other, with the snap traps essential to small rodent detection and identification.

In comparing the wax blocks to the WaxTags, it is apparent the wax blocks were preferred by small rodents and were superior at detecting small rodents. Other factors fa-

vor the wax block. The wax block at ~\$0.41 each is less expensive that the WaxTag at ~\$1.00 each (2008 prices). The wax block does not melt at high temperatures, unlike the WaxTags, several of which melted at temperatures in the mid- to high 80's (°F). In regions with higher temperatures, the number of melting failures would be greater.

We did not conduct surveys while the fields were being irrigated, because we thought the sprinkler irrigation might degrade the wax blocks, wash the peanut butter off the traps, or inhibit rodent foraging. On several occasions, we requested that irrigation be delayed until our surveys were completed. However, rain, heavy at times, fell on 27 May during the surveys of fields 12, 13, and 14. We did not observe any degradation of the wax blocks or of the peanut butter bait on the traps as a result of the rain. Nonetheless, we suggest that surveys should be conducted during dry periods (e.g., no sprinkler irrigation or rain).

We conducted the surveys during 2 periods at each field, the period 7 to 14 days prior to harvest and the period 1 to 6 days prior to harvest. We initially timed the surveys to follow the guidelines for wildlife surveys recommended in the Metrics. However, when comparing the number of mice caught or the IA values from the 1st to the 2nd survey, we found a decrease, no change, or only a small increase in the number of mice (n = 1) or IA values (IA < 2.5) in the majority of cases. This suggests there is little additional information to be gained from the 2nd survey and therefore it is unnecessary. In addition, from a control standpoint, if there was an significant increase in IA values from the 1st to the 2nd survey to the point where control actions were deemed necessary, there would be little time left before harvest to significantly reduce damage. We suggest that only 1 survey for small rodents using wax blocks and snap traps is necessary, and that it should be conducted 2 to 3 weeks before harvest. Visual surveys for ground squirrels and pocket gophers should be undertaken on a regular basis throughout the crop cycle and should begin no later than the time of germination.

It is apparent from the surveys that there are more rodents in the wild edge than in-crop. However, high numbers of small rodents along the wild edge did not translate into high numbers or damage in the crops. Based on incrop visual surveys, there were no small rodents living in any of the fields we surveyed. We suspect that any small rodent activity in the fields resulted from animals that lived in the wild edge and crossed over the service road, primarily during the night, to access the crop or the field. We did not observe any crop damage from small rodents in the fields we surveyed. Given that the animals may enter the crop at different locations every time (rather than via established runways) and due to the dense growth and leafy nature of lettuce and spinach, it would be difficult to identify any damage if it were present.

A pertinent question is what IA value warrants control action? First, the in-crop IA is the most important value to consider. As noted above, a high IA from the wild edge does not necessarily mean that small rodents are entering the crop or that there is damage. However, if the in-crop IA exceeds an established threshold, then control action is warranted. The threshold could be based on pest tolerance (e.g., no mice can be tolerated in the crop), economic factors (e.g., weighing the value of costs versus benefits), or

health and safety factor (potential disease transmission). Once the threshold has been reached, then control actions would be undertaken in the wild edge as it represents the source of small rodents entering the crop.

RECOMMENDATIONS FOR A RODENT MONITORING SYSTEM

The rodent monitoring system described below is based on results from the field surveys conducted in 2009 and 2010. There are 2 components to the monitoring system, the visual survey for larger rodents, and the wax block/snap trap survey for small rodents.

Visual Survey

Target species: Primarily California ground squirrels and pocket gophers; secondarily small rodents.

When: Every time when driving vehicle on or off site; when conducting wax block/snap trap survey 2 to 3 weeks before harvest.

Materials required: Ranch map or notebook for recording observations.

Survey procedure: Survey for small rodents (e.g., mice) while walking along the wild edge and the field perimeter. Small burrow openings, runways between burrows or in the vegetation, tracks in the mud, and droppings are signs of small rodents. Record the general location of any burrows, runways, or other small rodent sign. The presence of larger rodents (e.g., pocket gophers, ground squirrels, tree squirrels) should also be recorded.

Wax Block-Snap Trap Survey

Target species: Small rodents including deer mice, house mice, and voles.

When: 2 to 3 weeks before harvest.

Materials required: Ranch map or notebook for recording location of transects; data sheets for recording survey results; mouse-sized snap traps (40 plus spares to replace broken or missing traps); a bucket for carrying the traps and wax blocks; peanut butter for bait; knife to apply peanut butter to the trap; wax blocks (40 initially with reserve of at least 20 per survey to replace chewed or missing blocks); Sharpie pen; wire flags (80 plus spares); latex gloves, rubber gloves, disinfectant.

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