REPORT 2: Springtail
Project Title

Survey and management of springtails in lettuce

Project Investigator

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Abstract

A series of experiments were conducted to determine the impact of *Protaphorura fimata* feeding on seeds and germinating seedlings of lettuce. First, various densities of *P. fimata* were incubated with 25 lettuce seeds for 7-day and feeding injury was evaluated in three soilless arena experiments. As a second step, 100 *P. fimata* were incubated with 25 lettuce seeds in three arena experiments with soil media. In experiments without soil, the number of ungerminated seeds, feeding injury sites, and plants with injury were significantly greater in arenas with *P. fimata* than without. Similarly, the number of germinated seedlings, shoot fresh and dry weights, and length and width of fully opened-leaves was greater in arenas without than with *P. fimata* in assays with soil. The results clearly show that *P. fimata* is a pest of lettuce and can cause severe feeding injury to germinating seeds or seedlings, thereby reducing their growth rate. The potential implications of *P. fimata* feeding and feeding injury characteristics are discussed. In 2014 and 2015, potato slice, typically used to monitor Garden symphylan, *Scutigerella immaculata* were compared with beet and Berlese funnel for sampled soil in commercial lettuce fields. Results suggest that both potato and beet slices captured *P. fimata* when deployed in the soil than *P. fimata* extracted using Berlese funnel. Overall, beet slices capture significantly more number of *P. fimata* than potato slices in both years. Survey in the Salinas fields indicated that *P. fimata* is widespread; however, the factor contributing incidence, abundance and crop loss are under investigation.

Objective

1. Develop expertise in identification of springtails of economic importance in the Salinas Valley affecting lettuce production.
2. Survey - determine distribution of this new species of springtail in various lettuce fields in the Salinas Valley.
3. Compare and contrast monitoring methods – potato slices and Berlese funnel for detection of springtails.
4. Determine the effects of timing, and number of applications of various insecticide materials.

Procedures

Objective 1: Develop expertise in identification of springtails of economic importance in the Salinas Valley affecting lettuce production.

I visited Dr. Felipe N. Soto-Adames lab for two days and studied the key characters to identify springtails. Dr. Soto-Adames is an insect systematist and curator of entomology part of Illinois Natural History Survey, Prairie Research Institute at University of Illinois, Urbana-Champaign – IL. Dr. Soto-Adames is an expert of insect group (Collembola or springtail). I mounted springtails myself so that I could compare and contrast the springtail received in the samples.

To follow-up with the field work done in 2013 (as reported in 2014 annual report), I conducted laboratory assays to confirm that the pattern we observed in the field was indeed caused by springtail, *Protaphorura fimata*.

Lab soilless-arena study. Three soilless arena experiments were conducted in the laboratory. An experiment unit or assay consisted of plastic petri dish with a filter. The filter paper was soaked in 50 mL of distilled water for 5 seconds before being added to the petri dish. Twenty-five uncoated and untreated lettuce seeds were added onto the moistened filter paper before the springtails were introduced. In the first experiment, two *P. fimata* densities (treatments), 0 and 100 individuals were added and were replicated five times (five petri dishes) per treatment. Later, two more experiments were conducted with four *P. fimata* densities (treatments): 0, 20, 50 and 100 individuals. The treatments in both the experiments were replicated five times (five petri dishes per treatment) in a completely randomized design. All petri dishes were sealed using parafilm and secured using rubber bands around the petri dishes. The petri dish assays provided a value in understanding the feeding behavior of *P. fimata* when other environmental factors were controlled.

The parameters evaluated were ungerminated lettuce seeds due to feeding injury, total number of feeding injury sites, and number of germinated seedlings with distinct feeding injury. In the third experiment, the location (e.g. leaf, stem, plant crown or root) of the feeding injury on the plants was also recorded. This information was not recorded from two previous soilless experiments.

Lab soil-arena study. Three experiments were conducted with soil media in the laboratory. For each experiment, the soil was collected from a field in Salinas, CA where *P. fimata* was previously collected from the soil and later identified. The soil was oven dried twice. An experiment unit consisted of plastic petri dish with 25 g of oven-dried soil. To maintain uniform soil moisture, 3-mL of distilled water was added to each petri dish. For each experiment, there were two *P. fimata* densities (treatments), 0 and 100 individuals and were replicated five times (five petri dishes) per treatment. The petri dish assays were covered by inverting another 4.5-cm petri dish and sealed using parafilm around the edges. These assays were maintained at controlled conditions for 14 day. After 14 day, 25 uncoated and untreated lettuce seeds were added onto the soil surface of each petri dish assay. The petri dish assays were maintained at controlled for 7 more days then evaluated to determine the effects of *P. fimata* feeding. The parameters evaluated were number of lettuce seeds germinated, shoot fresh and dry weights, and length and width of fully-opened leaves of seedlings. To determine the length and width of seedling leaves, 10 leaves were randomly selected from each petri dish and measured.

Objective 2: Survey - determine distribution of this new species of springtail in various lettuce fields in the Salinas Valley.

Ten fields in the northern Salinas Valley were investigated with soil samples and seven fields had *P. fimata* infestation. *P. fimata* are detected when the populations are high in the field. When soil moisture decline in the upper soil profile, *P. fimata* detect declined.

Objective 3: Compare and contrast monitoring methods – potato slices and Berlese funnel for detection of springtails.

The experiments were conducted in lettuce fields in northern Salinas Valley of California from March to April 2014 and 2015. In both years, the treatments were arranged in randomized complete block design with 12 and 10 replications in 2014 and 2015, respectively. The three treatments were beet root slice and potato slice and soil core samples. The treatments were 15.2 m apart within a block and blocks were two meter apart. The beet root, and potato were purchased from the local produce stores in Salinas, CA. Thin slices (~1-cm thick) of beet root (~5.4 cm diameter) and potato were cut in the field before deployment. These bait slices were deployed in the sub-surface of the soil about ~5 cm deep (Fig. 4A and B) and were covered with disposable white, 8.5-cm diameter, 4-cm deep plastic-bowls (Fig. 4C). The *P. fimata* were collected from the underside of the bait slices that were in contact with the soil. The baits were serviced at 2-d interval up to three times within a field. At the end of each two day exposure period, bait slices were removed, placed into plastic bags and transported to the laboratory in Salinas, CA. The captured *P. fimata* on the bait slices were quantified within 24 h using a dissecting microscope. Soil samples (~ 310 g) were collected using a bulb planter from randomly assigned spots from ~10-cm deep sub-soil on the day when the bait slices were removed for evaluation. The collected soil samples were transported to the laboratory and were introduced into the modified small Berlese funnel traps for seven days. In the small Berlese funnel, 25 W incandescent bulb was used as light or heat source and springtails were collected in 70% ethyl alcohol in a 100-mL plastic cup (Fig. 4D). The small Berlese funnel was modified by cut and removal of 9 cm diameter section from the top container to allow heat and light transmission and prevent settlement of water vapors within the sealing of the top container. The baits or soil samples were collected on 28 Feb, and 4 and 12 Mar 2014, and 2, 4, and 5 Feb 2015.

In 2014, two experiments were conducted in two lettuce fields. The beet root slice was used for these experiments because beet performed better in the preliminary experiments. The beets were deployed as described in the previous section. The treatments were the discrete periods the slices were exposed to *P. fimata* and were 1, 2, 3, 4 and 5 day in the soil. The treatments were arranged in randomized complete block design with 10 replications. The captured *P. fimata* were quantified within 24 h by examining the slices using a dissecting microscope. The bait traps were removed on 4, 9, 11, 14, 16, and 18 Apr 2014.

Objective 4: Determine the effects of timing, and number of applications of various insecticide materials.

A. **Field timing study.** This study was conducted on 10 February 2015 in a lettuce field in Salinas where the *P. fimata* caused the stand loss. With permission from the grower, the trial was deployed

in the beds with maximum stand loss. The pre-trial survey showed high densities of *P. fimata* in the beds. Belay (12 fl oz/ acre) and Mustang (4 fl oz/acre) was chosen for the trial based on efficacy result in the laboratory bioassays. The treatments were insecticide: a) applied 2 days before planting (beds not shaped), b) at planting, and c) both. The lettuce seeds were planted on 12 February 2015. Four replicates of each treatment were assigned to a 25-feet long 80"beds according to a randomized complete block design. Spray application was done using four-nozzle sprayer at 30 PSI. The water volume used all applications was 40 gal/acre. No adjuvant was added.

B. **Insecticide efficacy trial.** This study was conducted on 14 February 2015 in a lettuce field in Salinas that was reported with garden symphylan incidence and plant damage. The insecticides used and theirs rates were Radiant at 10 fl oz/acre, Verimark at 13.5 fl oz/acre, Belay at 12 fl oz/acre, Warrior II at 1.92 fl oz/acre, and Mustang at 4 fl oz/acre. The field that was chosen for the study always gets *P. fimata* problem every other year. The lettuce seeds were planted on 13 February 2015. Four replicates of each treatment were assigned to a 25-feet long 80"beds according to a randomized completely block design. All insecticides were applied along the seed line (3-inch band). Spray application was done using one-nozzle sprayer at 30 PSI. The water volume used all applications was 40 gal/acre. No adjuvant was added.

Results

Objective 1: Develop expertise in identification of springtails of economic importance in the Salinas Valley affecting lettuce production.

Injury characteristics. Figure 1 provides a visual illustration of *P. fimata* feeding injury on germinating lettuce seeds in the presence and absence of *P. fimata*. *P. fimata* could feed on various parts of seed or young seedling (Fig. 2). *P. fimata* could directly feed and injure the seed with only the seed coat remaining (Figs. 2a and b) or completely sever the radicle (Fig. 2c) of germinated seed, and/or partially feed on the radicle (Fig. 2d). If the seedlings survive the *P. fimata* feeding, the plants demonstrate a reduced seedling development (Fig. 1b). A section of the plant tissue was removed and the injured living tissue surrounding the feeding site appeared stained with reddish-brown coloration (Fig. 2d). On developed seedlings, most of the feeding activity was noticed at the crown area of the seedling and not much on other plant structures such as root, leaves or stem (Fig. 2d and Fig. 3).

Lab soilless-arena. In experiment 1, the number of ungerminated lettuce seeds, total *P. fimata* feeding injury sites, and number of plants with feeding injury were greater when *P. fimata* were present in the assay than absent (Table 1). In experiment (Exp) 2 and 3, the number of ungerminated lettuce seeds, total *P. fimata* feeding injury sites, and number of plants with feeding injury were greater when *P. fimata* were present regardless of *P. fimata* densities than absent (Table 1).

Lab soil-arena. In experiment 1, the number of germinated seeds, fresh weight, and both the length and width of the leaves of seedlings were lower in arenas with *P. fimata* than without (Table 2). The only variable that was not different between treatments was dry weight. In the experiment 2, the number of germinated seeds, dry weight, and both the length and width of the leaves of seedlings were lower in arenas with *P. fimata* than without (Table 2). The fresh weight was not different between treatments. In experiment 3, although the number of germinated seeds, fresh and dry weight

was not different with and without *P. fimata*, the leaf length and width was smaller when *P. fimata* were present than absent.

Objective 2: Survey - determine distribution of this new species of springtail in various lettuce fields in the Salinas Valley

Of 10 fields surveyed, seven fields had *P. fimata* infestation. *P. fimata* are detected when the populations are high in the field. When soil moisture decline in the upper soil profile, *P. fimata* captures was reduced.

Objective 3: Compare and contrast monitoring methods – potato slices and Berlese funnel for detection of springtails.

A total of 1775 and 1564 *P. fimata* were captures in all the trap types in 2014 and 2015, respectively. In 2014, number of *P. fimata* was greater on beet bait than potato, and Berlese funnel during experiment one, two, three and in all the experiments combined (Fig. 5). In 2015, in experiment one, more *P. fimata* were collected on beet and potato than in Berlese funnel (Fig. 6). Similarly in experiment two, number of *P. fimata* was significantly greater in beet and potato than in Berlese funnel. In experiment three, *P. fimata* densities found on beet was greater than on potato and extracted using Berlese funnel. When all the *P. fimata* combined, higher number of *P. fimata* was captured on beet followed by potato than those collected using Berlese funnel.

There was no statistical difference on number of *P. fimata* captured when the length of exposure of beet slices in the soil (Fig. 7).

Objective 4: Determine the effects of timing, and number of applications of various insecticide materials.

A. Unfortunately, the seeds did not germinate and the trial was not successful. Possibly reason was the planted seeds were not consistent with maturity of other lettuce plants in the field. This might cause change in irrigation schedule in the trial. Also, I noticed that seeds were exposed and cracked open. It is likely that birds might have picked the seeds. Therefore, no data to report.

B. Unfortunately, the incidence of *P. fimata* was low and they did not cause the stand loss or any issues. Therefore, no data to report.

Conclusion

The results from this study clearly demonstrate that *P. fimata* is an important pest of lettuce and is capable of reducing the crop stand. Incidence of high populations of *P. fimata* could be detrimental to germination of seeds in the field. *P. fimata* could be effectively suppressed to a large extent with early applications of synthetic insecticides directed to the seed line. The beet and potato can attract *P. fimata* in the field setting and could be used for monitoring rather than sampling soil then extracting *P. fimata* using Berlese funnel. It is more likely that baits draw *P. fimata* around the soil, although the active radius of attraction is not yet established. Overall, beet (*B. vulgaris*) bait captured significantly more number of *P. fimata* than potato bait. Also, using beet for monitoring will help the scouts to easily detect the *P. fimata* on it and accurately

quantify because of the dark background of beet and white or off-white color of *P. fimata*. Although *P. fimata* move slowly on the bait surface, they curl-up when disturbed and are likely to fall out of the bait surface. Future studies will focus on research that will help determine threshold density for treatment decisions.

Table 1. Mean (± SE) of ungerminated lettuce seeds, total number of *P. fimata* feeding sites and plants with *P. fimata* feeding injury after exposing two densities of *P. fimata* for 7-day in a soilless assay.

Experiment	No. of *P. fimata*	Ungerminated seeds[1]	Injury sites[2]	Plants with injury[3]
1				
	0	0 ± 0b	0 ± 0b	0 ± 0b
	100	4.6 ± 1.3a	19.8 ± 2.1a	13.8 ± 2.9a
2				
	0	0 ± 0b	0 ± 0b	0 ± 0b
	20	4.2 ± 1.2a	22.4 ± 4.7a	12.8 ± 1.6a
	50	8.0 ± 3.9a	24.4 ± 3.0a	12.2 ± 2.3a
	100	14.2 ± 1.8a	22.8 ± 4.2a	9.8 ± 1.7a
3				
	0	0 ± 0b	0 ± 0b	0 ± 0b
	20	13.0 ± 3.1a	34.0 ± 5.5a	11.2 ± 2.8a
	50	7.8 ± 1.1a	40.2 ± 1.4a	16.6 ± 1.2a
	100	13.2 ± 3.3a	44.2 ± 4.9a	11.4 ± 3.1a

[1]Seeds not germinated to *P. fimata* feeding injury. [2]Includes total number of distinct *P. fimata* feeding sites. [3]At least one *P. fimata* feeding injury site detected. Symbols following means with similar case letters within the same column and experiment are not different.

Table 2. Mean (± SE) of lettuce seedling, fresh and dry weight, and leaf dimensions after exposing two densities of *P. fimata* for 7-day in the soil assay.

Experiment	No. of *P. fimata*	No. of seedlings	Fresh weight (g)	Dry weight (g)	Leaf length (cm)	Leaf width (cm)
1						
	0	21.0 ± 0.44a	0.298 ± 0.026a	0.023 ± 0.001a	1.63 ± 0.05a	0.48 ± 0.05a
	100	15.6 ± 1.94b	0.119 ± 0.014b	0.018 ± 0.003a	1.08 ± 0.07b	0.29 ± 0.01b
2						
	0	20.0 ± 0.6a	0.194 ±0.017a	0.021 ± 0.002a	0.41 ± 0.03a	0.74 ± 0.04a
	100	16.5 ± 0.9b	0.253 ± 0.047a	0.013 ± 0.000b	0.34 ± 0.13b	0.54 ± .03b
3						
	0	19.6 ± 0.7a	0.192 ±0.014a	0.023 ± 0.002a	0.37 ± 0.04a	0.52 ± 0.02a
	100	20.2 ± 0.4a	0.144 ±0.017a	0.016 ± 0.003a	0.26 ± 0.01b	0.42 ± .02b

Symbols following means with similar case letters within the same column and experiment are not different. Not transformed data are presented.

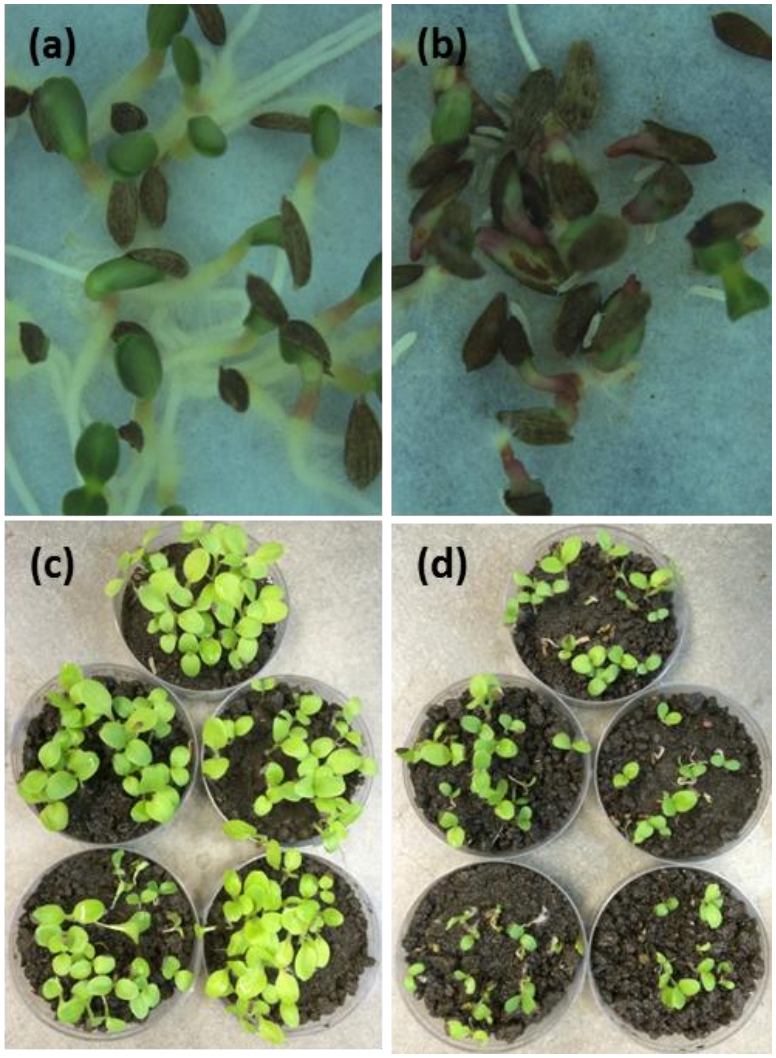

Fig. 1. Effects of *P. fimata* on germinating lettuce seeds exposed after 7-day in assays (a) without soil and *P. fimata*, (b) without soil but with *P. fimata*, (c) with soil but without *P. fimata* and (d) with soil and *P. fimata*.

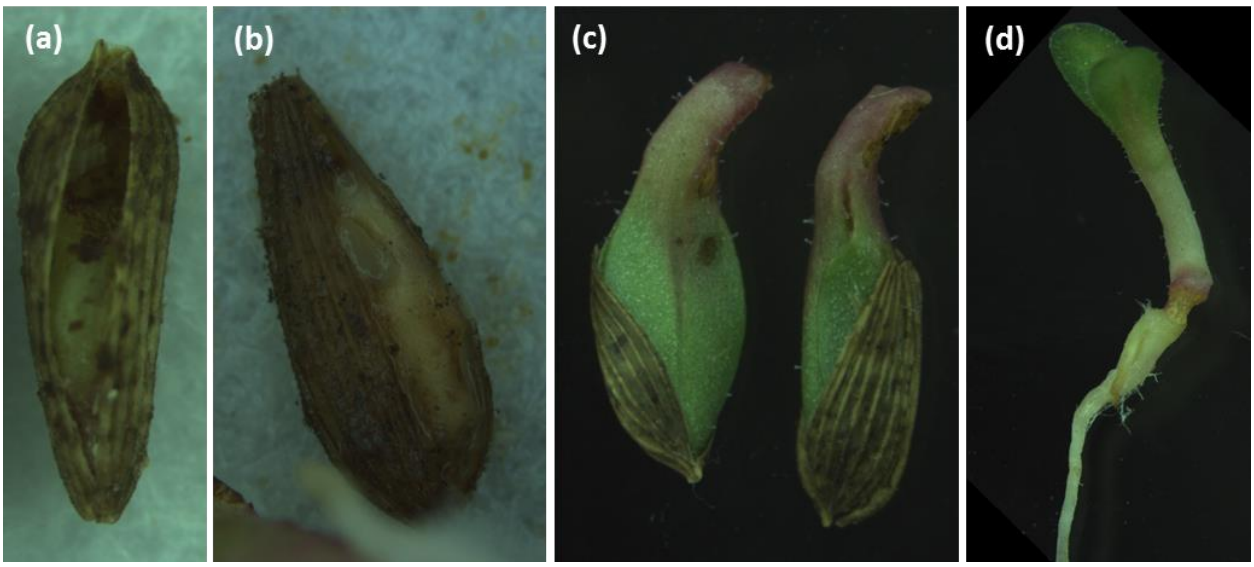

Fig. 2. Feeding injury of *P. fimata* on (a) seed – completely injured, (b) seed – incompletely injured, (c) radicle – completely severed, and (d) radicle –partially severed after 7-day of exposure.

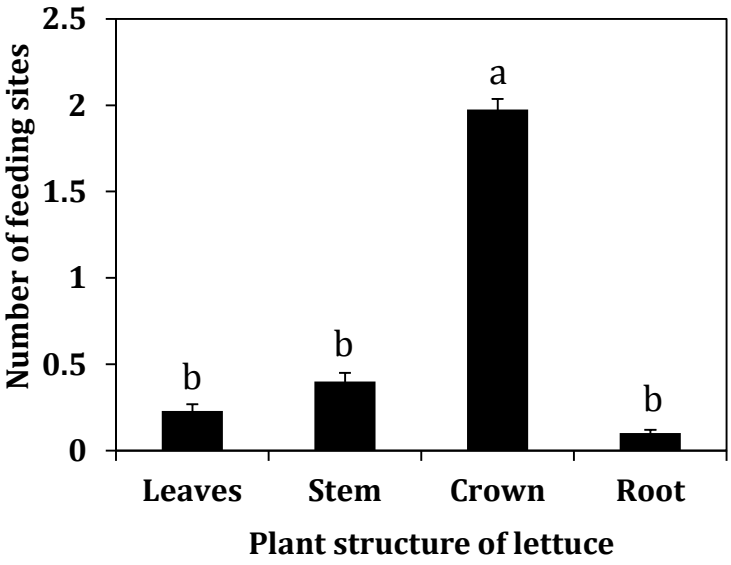

Fig. 3. Number of *P. fimata* feeding injury sites at various parts of the germinated lettuce seedlings after 7-day exposure in a soilless arena. Symbols following means with similar case letters among histograms are not different.

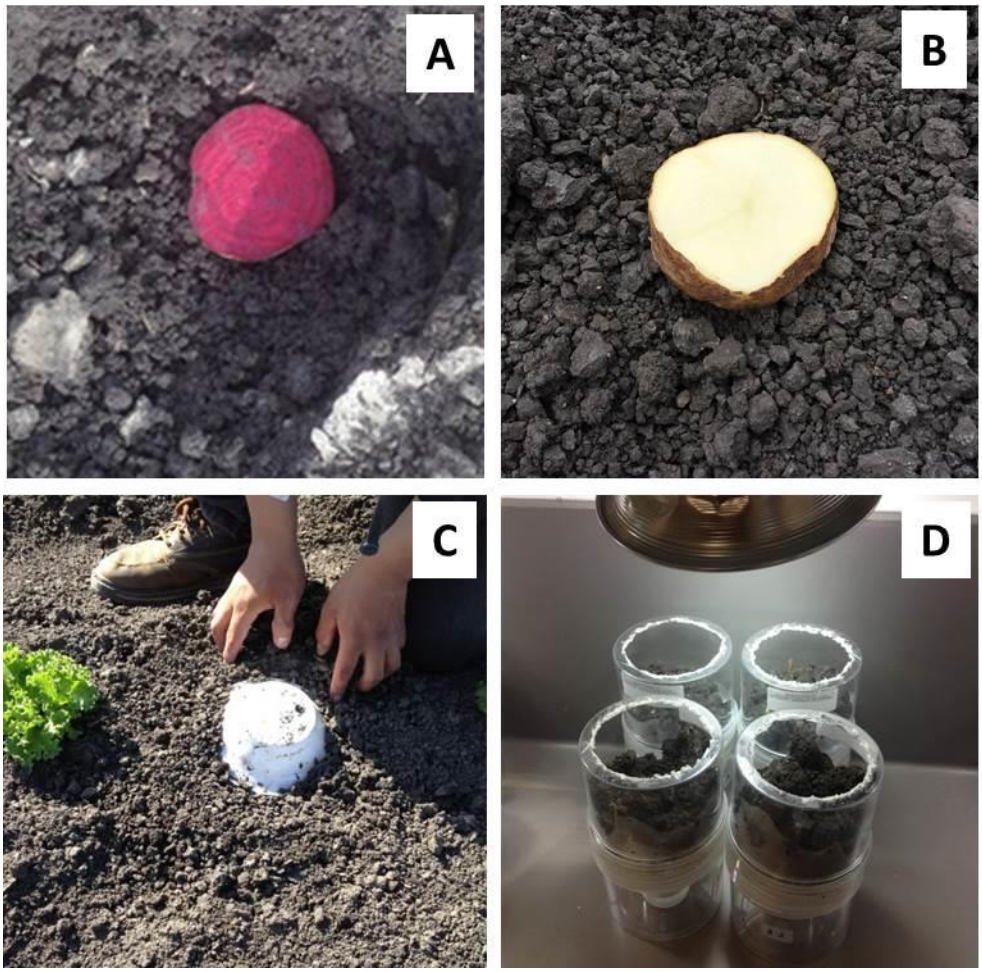

Fig. 4. (A) Beet slice, (B) Potato slice, (C) deployment of beet and potato slices and (D) Berlese funnel.

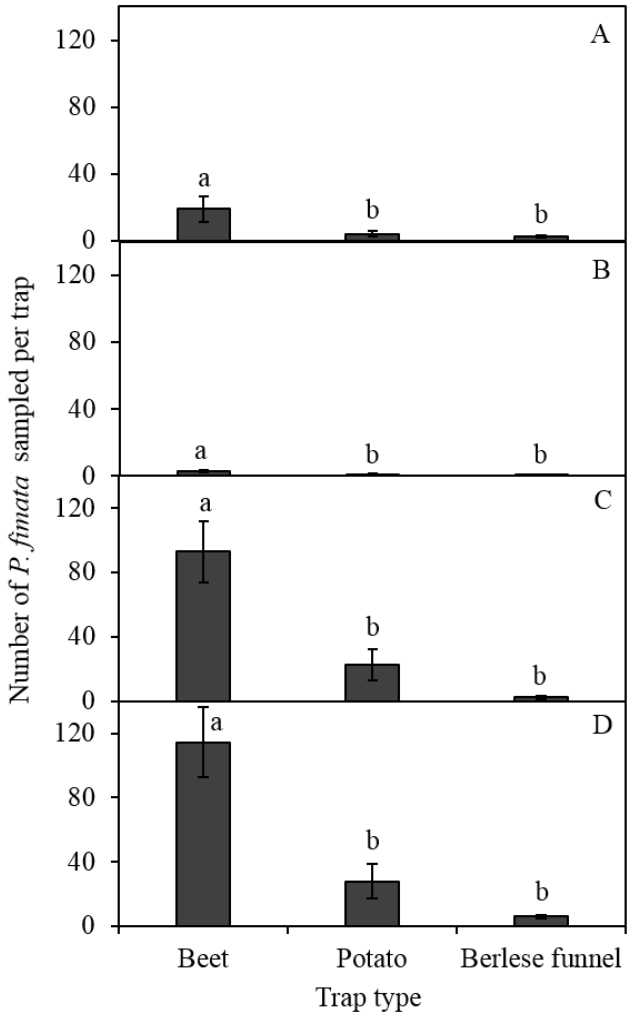

Fig. 5. Number of *P. fimata* captured on beet and potato baits and soil in Berlese funnel in experiment (A) one, (B) two, (C) three, and (D) all experiments combined in 2014. Symbols following means with similar case letters among histograms are not different.

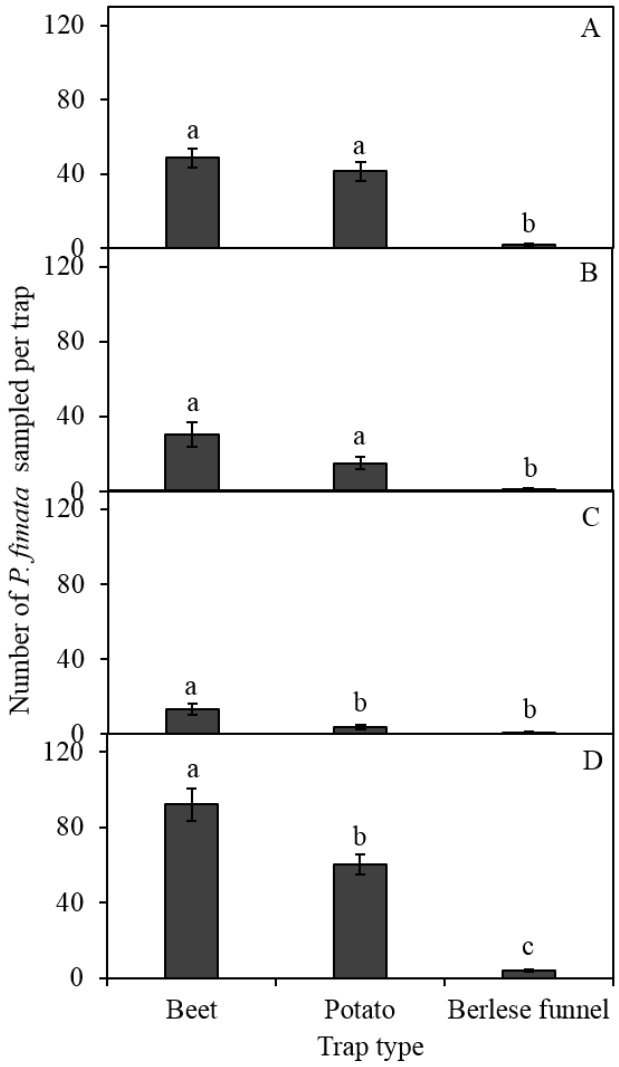

Fig. 6. Number of *P. fimata* captured on beet and potato baits and soil in Berlese funnel in experiment (A) one, (B) two, (C) three, and (D) all experiments combined in 2015. Symbols following means with similar case letters among histograms are not different.

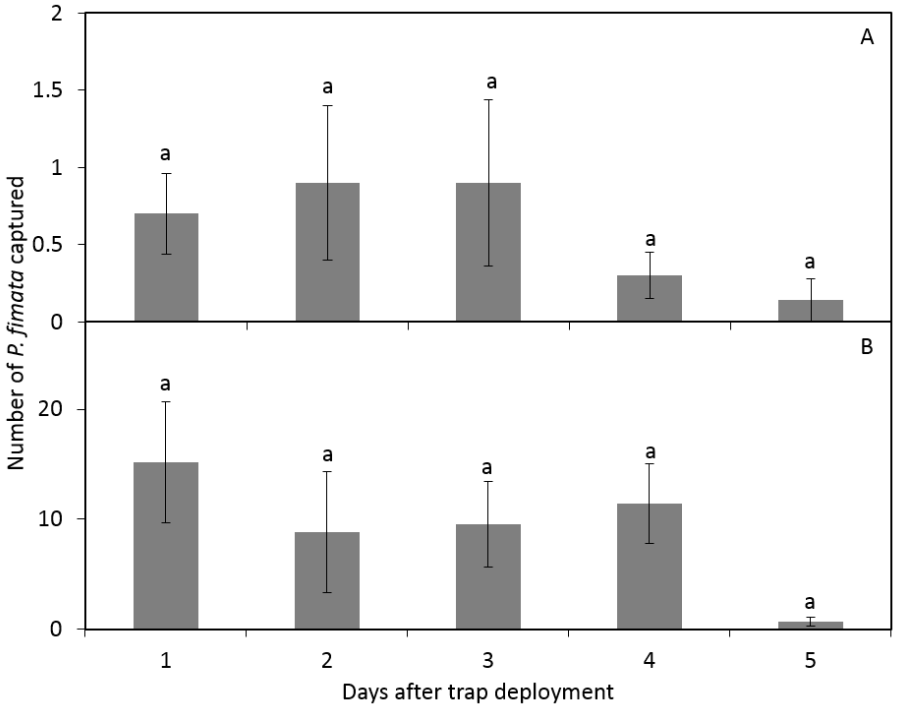

Fig. 7. Number of *P. fimata* captured on beet baits in experiment (A) one, and (B) two in 2015. Symbols following means with similar case letters among histograms are not different.