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Influence of plant age, temperature, and moisture on *Protaphorura fimata* feeding injury on lettuce in the Salinas Valley of California, USA

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Abstract

Protaphorura fimata Gisin (Collembola: Onychiuridae) is a serious pest of lettuce, *Lactuca sativa* L. (Asteraceae), seedlings in the Salinas Valley of California, USA. Little is known about the growth stages at which lettuce seedlings are vulnerable to *P. fimata* feeding, and how temperature and soil moisture affect symptoms and severity of feeding injury. To determine the most vulnerable lettuce seedling stage to *P. fimata* feeding, sets of lettuce seeds were planted for six consecutive days on the soil within clear plastic cups. On the seventh day, all plants were exposed to 100 *P. fimata* for a week. The results indicate that the number of germinated seeds, fresh weight, and dry weight were significantly lower in 1-day-old seedlings than in 3- to 6-day-old seedlings. To determine effects of soil moisture on *P. fimata* feeding, various amounts of distilled water (1.5, 2.5, 3.5, 4.5, 5.5, or 6.5 ml) were added to 10 g of soil in containers planted with lettuce. Plants were later exposed to 100 *P. fimata* for a week. Results indicate that the seedlings that received 6.5 ml water had significantly lower seed germination and fresh weight, but a greater percentage of injured seedlings and more feeding punctures per germinated seed, than the seedlings that received 2.5, 3.5, 4.5, or 5.5 ml of distilled water. In the temperature assay, 100 *P. fimata* were exposed to lettuce seeds maintained at 5.4, 10.8, 15.5, 21.2, 29.1, or 33.0 °C for 31, 12, 8, 7, 5, and 5 days, respectively, until the first pair of leaves became fully expanded. *Protaphorura fimata* caused feeding injury on the germinating lettuce seeds in all temperature ranges except at 33.0 °C. The number of seeds germinated and fresh weight of seedlings were significantly lower in the seedlings exposed to *P. fimata* than unexposed seedlings at all the temperature ranges.

Introduction

Protaphorura fimata Gisin (Collembola: Onychiuridae) is a ca. 2.5-mm-long soil-dwelling springtail, with no eyes, pigmentation, and furcular. It is a serious pest of lettuce, *Lactuca sativa* L. (Asteraceae), in the northern part of Salinas Valley of California, USA (Joseph et al., 2015). This springtail pest is not reported from other major lettuce-producing regions such as Yuma, Arizona (USA), Imperial Valley in California, China, and India. The value of lettuce is estimated to be ca. US$ 1.4 billion in the Salinas Valley (Monterey County Crop Report, 2014). The direct-seeded young lettuce seedlings in fields with high densities of *P. fimata* show retarded or stunted growth and do not germinate in a synchronous pattern (Joseph et al., 2015). *Protaphorura fimata* is also reported as a pest of sugar maple seedlings (*Acer saccharum* Marsh.) in Oregon (USA) nurseries (EC Bernard, pers. comm.). Several species of onychiurids have been reported to feed on crops (Edwards, 1962; Scott, 1964; Baker & Dunning, 1975; Brown, 1983; Hurej et al., 1992; Boetel et al., 2001). *Protaphorura fimata* is reported to feed on soil fungi, decaying plant materials, and live roots (Crist & Friese, 1993; Jørgensen et al., 2003; Endlweber et al., 2009; Nietschke et al., 2011; Joseph et al., 2015).

Collembola population density or activity is often associated with high soil moisture content through events such

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as precipitation and irrigation (Badejo et al., 1998; Ferguson & Joly, 2002; Lindberg et al., 2002; Lensing et al., 2005). Also, increased fungal activity in response to high soil moisture content is often attractive to soil-dwelling Collembola (Hassall et al., 1986). The behavioral response of *P. fimata* to feeding injury on lettuce under high soil moisture condition has not yet been quantified in the fields of the central coast of California, where the soil organic matter content can be as high as 4% (USDA Soil Conservation Service, 1978) and is heavily irrigated during lettuce seed germination.

Temperature also has a profound impact on lettuce plant development as well as the growth and activities of springtails (Klironomos & Kendrick, 1995). The response of springtails to temperature is species specific (Hopkin, 1997). *Protaphorura fimata* has been found causing crop losses during February to May in the Salinas Valley (SV Joseph, unpubl.). It is not clear why the *P. fimata* problem declines toward the summer and fall (from June to November). Lettuce is primarily direct-seeded in the Salinas Valley and is planted yearlong except during the first 3 weeks in December. Perhaps slower lettuce seed germination and subsequent development during cooler seasons (January to May) predisposes seedlings to *P. fimata* for a longer time frame than during the rest of the year. The relationship between temperature and *P. fimata* feeding of germinating lettuce seeds has not been investigated.

It appears that *P. fimata* attacks the germinating seeds of lettuce (Joseph et al., 2015), but it is not certain whether irregular or inconsistent plant stand is due to persistent feeding by *P. fimata* on both germinating and developing seedling stages of lettuce. Knowing the most vulnerable stage(s) of lettuce to *P. fimata* feeding will help in determining the best timing for control measure intervention to achieve a uniform lettuce stand. Similarly, understanding *P. fimata* feeding habits under various moisture and temperature regimes will help refine *P. fimata* management tactics. The major objectives of this study were to determine: (1) the most vulnerable stages of lettuce seedling to *P. fimata* feeding, and (2) the influence of soil moisture and temperature on *P. fimata* feeding injury of germinating seeds of lettuce.

Materials and methods

The experiments were conducted in the University of California Cooperative Extension Entomology laboratory in Salinas, CA, USA. Field-collected springtails were identified as *P. fimata* using the keys provided in Christiansen & Bellinger (1998), Fjellberg (1998), and Pomorski (1998). The *P. fimata* colony used in Joseph et al. (2015) was reared on Wardley fish food (Hartz Mountain Corporation, Secaucus, NJ, USA) provided biweekly in sealed 473.1-ml clear plastic containers (product no. 9061; Frontier Agricultural Sciences, Newark, DE, USA). In the container, a layer of clay soil (2 cm) was provided and covered with moist paper towel. The springtail containers were sprayed with tap water biweekly. The containers were maintained at 20 °C, ca. 45% r.h., in complete darkness in the laboratory cabinet. A single colony of *P. fimata* was used in all the laboratory experiments. Voucher specimens were deposited in the UC Cooperative Extension Entomology Laboratory (Salinas).

The various assays were conducted in 29.6-ml clear plastic containers (product no. 9051; Frontier Agricultural Sciences). The 'Clear Lake clay' soil, untreated with insecticide or fertilizer for at least 2 months, was collected from a field in Salinas, where *P. fimata* naturally occur, and oven-dried once at ca. 105 °C for 48 h. In all the experiments, 25 unpelleted, untreated 'Little Gem' lettuce seeds (Snow Seeds, Salinas) were used per experimental unit (i.e., a clear plastic container). After inoculating with *P. fimata*, all containers were covered using a clear plastic wrap (Glad Cling Plastic Wrap; Glad Products, Oakland, CA, USA) and sealed using Parafilm (Bemis Company, Oshkosh, WI, USA) around the edge of the containers unless specified.

Effect of plant age on injury

Oven-dried soil (10 g) was added to each container and 25 seeds were planted in a staggered fashion so that there were lettuce seedlings at various stages of development (from 1 to 6 days old) for the experiment. Seeds were planted at the same time of day for six consecutive days. The soil in the container was moistened with 4.5 ml distilled water. All containers were covered with clear plastic wrap. On the 7th day, these plants were inoculated with 0, 50, or 100 *P. fimata*. The assay arena was then covered by inverting another 29.6-ml clear plastic container and edges were sealed using Parafilm strips. The assay arenas were maintained for seven more days in the controlled environmental chamber at ca. 21 °C, L16:D8 photoperiod, and ca. 45% r.h., and evaluated after 7 days. The seedlings planted in successive days (seedling age) were the treatments and each treatment (day) was replicated 5× (five containers) in a randomized complete block design (RCBD). This experiment was repeated two more times with five replications each for a total of 15 replications.

Effect of moisture levels on injury

Distilled water (2.2 ml) was added to 5 g of soil and the total weight was measured. This soil served as the base moist soil layer for the experimental unit. Another 10 g of soil was added on top of the moistened soil. Twenty-five lettuce seeds were placed on the surface of the soil and the treatments (1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 ml of distilled water) were directly pipetted on to the seeds. The smallest water treatment of 1.5 ml barely provided sufficient wetness on the soil surface, whereas 6.5 ml of water provided a greater level of wetness to the soil. The extremes of water treatments were determined based on the preliminary studies which indicated that 4.5 ml water was deemed optimum to provide moisture for *P. fimata* in 10 g oven-dried soil. The containers with soil, seeds, and various water treatments were weighed. Finally, these containers were inoculated with 100 *P. fimata* per container and were covered. The water volume added to the soil served as treatments and each treatment was replicated 5× in an RCBD. This experiment was repeated 3×. The containers were maintained in a controlled environmental chamber at ca. 21 °C, 16L:D8 photoperiod, and ca. 45% r.h., and evaluated after 7 days.

Effect of temperature on injury

Oven-dried soil (10 g) was added to each container and 25 lettuce seeds were placed on the surface of the soil. Distilled water (6.5 ml) was pipetted evenly on to the soil surface. After adding water, the soil was inoculated with 100 *P. fimata* per container and was covered. The experimental plastic containers were placed on a tray and the tray was placed in a 35 × 26 × 17 (deep) cm dark blue plastic box (Rubbermaid, Atlanta, GA, USA) with a lid. The plastic box did not allow light to pass through it. A 1.1-W LED light (Gransta LED; Inter IKEA Systems, Helsingborg, Sweden) was attached to the lid so that light was pointing down from the ceiling of the Rubbermaid box. The light source was provided for the lettuce seedlings to grow throughout the experiment. A temperature data logger (Lascar EL-USB-1; MicroDAQ, Contoocook, NH, USA) was placed in the center of the box to record the air temperature. The boxes were maintained at various air temperature ranges (mean ± SE): 5.35 ± 0.04, 10.75 ± 0.05, 15.54 ± 0.00, 21.20 ± 0.09, 29.09 ± 0.07, and 33.02 ± 0.03 °C and were exposed to *P. fimata* for 31, 12, 8, 7, 5, and 5 days, respectively. Lettuce is grown year round and seeds are sown starting from the last week of December and ending in mid-September in the Salinas Valley, and the daily temperatures substantially vary among growing seasons. In winter and spring months, the selected low temperature ranges are common, whereas the highest temperature range (33.02 ± 0.03 °C) is rarely reached in the Salinas Valley. The time of exposure to *P. fimata* varied because the rate of seedling development differed depending on the temperature. The time of exposure for each temperature treatment was previously determined when 95% of the seedlings had fully expanded the first pair of leaves. The temperature range treatments were accomplished by placing the boxes in a refrigerator (model no. RF260BEAESR; Samsung, Ridgefield Park, NJ, USA) for 5.35 (± 0.04) °C, cooler (model no. HBCN05FVS; Haier, Camden, SC, USA) for 10.75 (± 0.05) °C, or controlled environmental chambers for 15.54 (± 0.01), 21.20 (± 0.09), 29.09 (± 0.07), and 33.02 (± 0.03) °C. Before the containers were removed from the temperature treatment unit for evaluation, the soil temperature was recorded from every container using a digital soil thermometer (model no. 6300; Spectrum Technologies, Aurora, IL, USA). Each temperature range treatment was replicated 10× (10 containers) for *P. fimata* exposed and unexposed in an RCBD. This experiment was repeated 2× for a total of 20 replications per treatment.

Evaluation of feeding injury

After the *P. fimata* exposure period, the number of germinated seeds, seedlings with feeding injury sites, and the total number of feeding sites on the germinated seedlings were determined in all three experiments. The springtails were not removed before evaluation. In addition, fresh and dry weights of the lettuce seedlings were recorded. For fresh and dry weights, all the germinated seedlings were severed at the crown area and the soil particles attached to the tip on the stem were removed. All the seedlings per replication were weighed together. The feeding injury sites on the above-soil plant material were evaluated. To determine dry weight, all seedlings in a set were dried in an oven at 60 °C for 24 h before weighing.

Statistical analysis

Because there were no significant differences among repeated trials within plant age, temperature, and moisture experiments, the data were combined for analysis by experiment type (plant age, temperature, and moisture). For the plant age experiment, data were combined by *P. fimata* density. The number of injured seedlings was expressed as percentage of injured seedlings. The number of germinated seeds, the number of feeding punctures per germinated seeds, and total fresh and dry weights of the seedlings were $\ln(x + 1)$-transformed to establish homogeneity of variance. Percentages of injured seedlings were arcsine $\sqrt{x}$-transformed. Transformed data from plant stage and soil moisture assays were subjected to one-way ANOVA using the generalized linear model (Proc GLM) procedure in SAS v.9.4 (SAS Institute, 2012). As the individual trials within plant age experiments (especially, 50 and 100 *P. fimata*) were not conducted simultaneously, the data were not analyzed by two-way ANOVA, where *P. fimata* density and plant age were considered as variables. Thus, plant age data were analyzed separately by *P. fimata* individual density. The data obtained from water volume 1.5 ml were not included in the analysis because <50% of the seeds germinated. Means were separated using Tukey's honestly significant difference (HSD) test (α = 0.05).

For the temperature experiment, transformed number of germinated seeds, percentage of injured seedlings, number of punctures per germinated seed, and total fresh and dry weights with and without *P. fimata* for various temperature ranges were analyzed using the Proc TTEST procedure in SAS (α = 0.05). The temperature range trials were not conducted simultaneously or using a single piece of equipment; thus, the data were not analyzed by two-way ANOVA, where *P. fimata* density (0 and 100 *P. fimata*) and temperature ranges were considered as variables. Means and standard error for the variables were calculated using Proc MEANS procedure in SAS (α = 0.05).

Results

Effect of plant age on injury

When lettuce was exposed to 100 *P. fimata*, fewer seeds germinated in the 1-day-old seedling treatment than in any other seedling treatments ($F_{5,70}$ = 7.2, P<0.001; Table 1). Percentage of injured seedlings was greater on 1-day-old seedling than in any other seedling age treatments ($F_{5,70}$ = 18.4, P<0.001). More feeding punctures were found on 1- and 2-day-old seedlings than on the other ages ($F_{5,70}$ = 19.8, P<0.001; Table 1). Fresh ($F_{5,70}$ = 44.9) and dry weights ($F_{5,70}$ = 4.9, both P<0.001) of seedlings were lower (by ca. 40%) in the 1-day-old seedling treatment than in the other treatments.

When 50 *P. fimata* were added, the number of seeds germinated was not significantly different among treatments ($F_{5,70}$ = 2.2, P = 0.059; Table 1). More seedlings were injured in 1- and 2-day-old seedling treatments than in 5- and 6-day-old seedlings ($F_{5,70}$ = 12.1, P<0.001), and feeding punctures were greater in 1- and 2-day-old seedling treatments than in other treatments ($F_{5,70}$ = 15.2, P<0.001). Fresh weights of seedlings were lower in 1- and 2-day-old seedling treatment than in other treatments ($F_{5,70}$ = 8.0, P<0.001), whereas dry weights of the seedlings were similar among treatments ($F_{5,70}$ = 1.4, P = 0.22).

When no *P. fimata* were present, fresh weight ($F_{5,62}$ = 1.9, P = 0.11) and dry weight ($F_{5,62}$ = 2.2, P = 0.062) of seedlings were not different among treatments, although the number of germinated seeds was lower in 5-day-old seedlings than in other treatments ($F_{5,62}$ = 2.6, P = 0.031). No feeding injury was observed

Table 1 Mean (± SE) parameter values assessed after exposing lettuce seedlings of various ages to various densities of *Protaphorura fimata*

P. fimata density	Seedling age (days)	No. seeds germinated[1]	% seedlings injured[2]	No. feeding punctures per seedling	Fresh weight (g)[3]	Dry weight (g)[3]
100	1	18.9 ± 1.1b	80.3 ± 6.4a	3.15 ± 0.34a	0.264 ± 0.027b	0.014 ± 0.001b
	2	22.9 ± 0.6a	54.2 ± 10.5b	2.42 ± 0.65a	0.569 ± 0.031a	0.023 ± 0.002a
	3	22.4 ± 0.5a	11.0 ± 1.7c	0.25 ± 0.04b	0.641 ± 0.024a	0.022 ± 0.001a
	4	23.1 ± 0.4a	15.7 ± 3.9c	0.28 ± 0.10b	0.667 ± 0.018a	0.019 ± 0.001a
	5	22.9 ± 0.5a	15.2 ± 4.1c	0.34 ± 0.12b	0.652 ± 0.025a	0.021 ± 0.001ab
	6	23.4 ± 0.5a	25.5 ± 4.8c	0.57 ± 0.14b	0.671 ± 0.027a	0.020 ± 0.001a
50	1	23.1 ± 0.6a	44.8 ± 7.3a	0.99 ± 0.20a	0.509 ± 0.026c	0.031 ± 0.005a
	2	21.8 ± 0.6a	26.2 ± 3.9ab	0.53 ± 0.90a	0.551 ± 0.023bc	0.030 ± 0.004a
	3	23.7 ± 0.5a	9.3 ± 2.4bc	0.23 ± 0.06b	0.612 ± 0.020ab	0.038 ± 0.006a
	4	23.7 ± 0.3a	9.3 ± 2.6bc	0.26 ± 0.08b	0.599 ± 0.024ab	0.034 ± 0.006a
	5	23.7 ± 0.7a	9.2 ± 3.2c	0.23 ± 0.09b	0.646 ± 0.029a	0.035 ± 0.007a
	6	23.5 ± 0.6a	7.6 ± 1.9c	0.17 ± 0.05b	0.667 ± 0.017a	0.041 ± 0.008a
0	1	23.5 ± 0.5ab	0	0	0.571 ± 0.039a	0.017 ± 0.001a
	2	24.3 ± 0.4a	0	0	0.707 ± 0.052a	0.021 ± 0.001a
	3	23.7 ± 0.4ab	0	0	0.636 ± 0.056a	0.019 ± 0.002a
	4	22.8 ± 0.5ab	0	0	0.652 ± 0.037a	0.020 ± 0.002a
	5	21.7 ± 1.0b	0	0	0.620 ± 0.032a	0.018 ± 0.000a
	6	23.5 ± 0.7ab	0	0	0.676 ± 0.038a	0.024 ± 0.002a

Means within a column by *P. fimata* density followed by the same letter are not significantly different (Tukey's HSD test: P>0.05). Non-transformed data are presented.

[1]Of 25 unpelleted, untreated lettuce seeds.

[2]With at least one *P. fimata* feeding injury site detected.

[3]Includes the weight of germinated seedlings per experimental unit.s

on lettuce seedlings for all the age treatments when *P. fimata* was not introduced.

Effect of moisture levels on injury

The weights of units before and after the experiment were different among each of the water volumes ($F_{4,56}$ = 66.4 and 66.9, both P<0.001; Table 2). Fewer seeds germinated in 6.5 ml than in 3.5 and 4.5 ml treatments ($F_{4,56}$ = 3.8, P = 0.008). Percentage of injured seedlings was greater in 6.5 ml than in other treatments ($F_{4,56}$ = 14.1, P<0.001), although there was no significant difference in injured seedlings among 2.5, 3.5, 4.5, and 5.5 ml treatments. The number of feeding punctures per seedling was greater in 6.5 ml than in any other treatment ($F_{4,56}$ = 12.5, P<0.001). Also, the number of feeding punctures was greater in 5.5 ml than in 2.5 ml of water. There was no difference in number of feeding punctures among 5.5, 4.5, and 3.5 ml treatments.

Effect of temperature on injury

The time from seed germination to full expansion of the first pair of leaves varied under various temperature ranges (Table 3). At 5.4 °C, seeds took ca. 30 days whereas at other temperatures 10.8, 15.5, 21.2, 29.1, and 33.0 °C they took 12, 8, 7, 5, and 5 days, respectively. The soil temperatures measured at the end of the exposure time tend to be greater than air temperature for all the temperature treatments except 29.1 °C (Table 3).

Fewer seeds germinated when exposed to 100 *P. fimata* at 5.4 (t = 2.7, d.f. = 38, P = 0.011), 10.8 (t = 3.7, d.f. = 58, P = 0.002), 15.5 (t = 3.3, d.f. = 38, P<0.001), 21.2 (t = 3.7, d.f. = 48, P<0.001), and 29.1 °C (t = −0.5, d.f. = 55, P = 0.60) than in the non-exposed control (Table 3). At 33.0 °C, the germinated lettuce seeds and their development were not uniform and all *P. fimata* were found dead (Table 3). Feeding injury on seedlings was not detected in the absence of *P. fimata* but was detected when exposed to *P. fimata* at 5.4 (injured seedlings: t = −28.4; feeding punctures: t = −21.9, both d.f. = 38, P<0.001), 10.8 (injured seedlings: t = −19.4; feeding punctures: t = −15.9, both d.f. = 58, P<0.001), 15.5 (injured seedlings: t = −23.7, d.f. = 47; feeding punctures: t = −16.2, d.f. = 38, both P<0.001), 21.2 (injured seedlings: t = −30.4; feeding punctures: t = −17.4, both d.f. = 48, P<0.001), and 29.1 °C (injured seedlings: t = −20.3; feeding punctures: t = −16.9, both d.f. = 55, P<0.001) (Table 3). The seedlings were not injured when germinating seedlings were exposed to *P. fimata* at 33.0 °C. The fresh weight of seedlings was lower when they were exposed to 100 *P. fimata* at 5.4 (t = 4.1, d.f. = 38), 10.8 (t = 8.0, d.f. = 58), 15.5 (t = 8.7, d.f. = 38), 21.2 (t = 9.2, d.f. = 38), and 29.1 °C (t = 3.2, d.f. = 38, all P<0.001) than when they were not exposed (Table 3). The fresh weight of the seedlings was not significantly different when germinating seedlings were exposed to *P. fimata* at 33.0 °C compared to non-exposed. There was no significant difference in dry weight between *P. fimata* exposed vs. non-exposed treatment in all temperatures. Hence, the seedlings were not injured and their fresh weights were not different from the control.

Discussion

Results demonstrate that germinating seeds or 1-day-old lettuce seedlings were the most vulnerable stages to *P. fimata* feeding, resulting in reduction in seedling growth.

Table 2 Mean (± SE) parameter values assessed after exposing germinating lettuce seedlings to various soil moisture levels and 100 *Protaphorura fimata*

Water volume (ml)[1]	Soil weight (g) 0 days[2]	Soil weight (g) 7 days[2]	No. seeds germinated[3]	% seedlings injured[4]	No. feeding punctures per seedling	Fresh weight (g)[5]	Dry weight (g)[5]
1.5	18.73 ± 0.04	18.36 ± 0.04	10.5 ± 2.6	43.1 ± 11.0	1.98 ± 0.68	0.092 ± 0.027	0.003 ± 0.001
2.5	19.90 ± 0.60e	19.47 ± 0.07d	18.7 ± 1.3ab	31.9 ± 6.4b	0.92 ± 0.29c	0.271 ± 0.041ab	0.011 ± 0.001a
3.5	20.58 ± 0.09d	20.08 ± 0.06d	21.0 ± 1.0a	43.0 ± 5.9b	0.94 ± 0.23bc	0.296 ± 0.029a	0.015 ± 0.001a
4.5	21.48 ± 0.22c	21.05 ± 0.23c	21.3 ± 0.7a	38.7 ± 6.2b	0.94 ± 0.15bc	0.342 ± 0.035a	0.017 ± 0.002a
5.5	22.44 ± 0.16b	22.07 ± 0.15b	19.3 ± 1.1ab	51.9 ± 8.5b	1.80 ± 0.46b	0.281 ± 0.040ab	0.015 ± 0.002a
6.5	23.28 ± 0.23a	22.84 ± 0.21a	15.8 ± 1.4b	81.5 ± 4.4a	2.67 ± 0.31a	0.178 ± 0.027b	0.016 ± 0.004a

Means within a column followed by the same letter are not significantly different (Tukey's HSD test: P>0.05). Non-transformed data are presented.

[1]The water volume added to 10 g of soil.

[2]Includes the weight of soil (5 + 10 g) and 2.2 ml distilled water plus the various amounts of water added, respectively.

[3]Out of 25 unpelleted, untreated lettuce seeds.

[4]With at least one *P. fimata* feeding injury puncture.

[5]Includes the weight of germinated seedlings per experimental unit.

Table 3 Means (± SE) of parameters when germinating lettuce seedlings were exposed to *Protaphorura fimata* and maintained at various temperatures

Air temperature (°C)[1]	Soil temperature (°C)[2]	Mean time of exposure (days)[3]	No. *P. fimata*	No. seeds germinated	% seedlings injured[4]	No. feeding punctures per seedling	Fresh weight (g)[5]
5.35 ± 0.04	7.8 ± 0.2	31	0	24.1 ± 0.4a	0b	0b	0.248 ± 0.013a
			100	22.6 ± 0.4b	90.4 ± 2.2a	3.74 ± 0.30a	0.187 ± 0.007b
10.75 ± 0.05	13.1 ± 0.1	12	0	24.3 ± 0.1a	0b	0b	0.302 ± 0.012a
			100	23.2 ± 0.3b	80.6 ± 4.2a	2.98 ± 0.29a	0.186 ± 0.008b
15.54 ± 0.01	16.7 ± 0.1	8	0	23.3 ± 0.4a	0b	0b	0.349 ± 0.015a
			100	21.4 ± 0.4b	87.9 ± 2.6a	4.07 ± 0.38a	0.178 ± 0.013b
21.20 ± 0.09	22.2 ± 0.1	7	0	23.9 ± 0.4a	0b	0b	0.368 ± 0.014a
			100	21.8 ± 0.4b	93.6 ± 1.7a	3.63 ± 0.34a	0.202 ± 0.012b
29.09 ± 0.07	29.6 ± 0.3	5	0	23.2 ± 0.3a	0b	0b	0.330 ± 0.011a
			100	23.5 ± 0.4a	83.4 ± 4.3a	3.46 ± 0.34a	0.278 ± 0.011b
33.81 ± 0.16	34.0 ± 0.0	5	0	16.1 ± 1.4	0	0	0.192 ± 0.019
			100	19.2 ± 1.4	0	0	0.196 ± 0.015

Means within a column for each temperature treatment followed by the same letter are not significantly different (Tukey's HSD test: $P>0.05$). Non-transformed data are presented.

[1]Temperature recorded in the data logger.

[2]Temperature recorded just before evaluation using a soil thermometer.

[3]No. days exposed to *P. fimata*.

[4]With at least one *P. fimata* feeding injury site detected.

[5]Includes the weight of germinated seedlings per experimental unit.

Thus, it appears that once the shoot emerges out of the soil surface, lettuce is less susceptible to *P. fimata* feeding injury. However, the potential effect of *P. fimata* feeding on the root hairs of seedlings was not evaluated. The effect of feeding on seedlings with established roots in soil may not be captured by the evaluation because only the above-soil structures were evaluated. The feeding that took place on the germinating phase should be evident on the above-soil structures because the germinating seeds are still on the soil surface and easily accessible to *P. fimata*. At the same time, the fresh and dry weights of the seedling should reflect any damage that might have occurred on the roots of established seedlings because roots were exposed to *P. fimata* for an extended time in all the plant age treatments. *Protaphorura fimata* root feeding symptoms such as stunting or yellowing were not noticed on seedlings older than 2 days. As the germinating phase of the plants is more susceptible, monitoring for *P. fimata* presence and activity should start prior to planting the seeds. Previous studies indicated that beet or potato slice baits attract *P. fimata* if placed in the top layer of the soil (Joseph & Bettiga, 2016); thus, these baits could be used for monitoring *P. fimata* activity in the soil. If the soil is not moist, the baits may not capture *P. fimata* and springtail activity may go undetected.

In this study, *P. fimata* feeding activity and damage were evident at temperatures as low as 5 °C. This suggests that although seed germination and seedling development become progressively slow in cooler conditions, *P. fimata* can feed actively if soil moisture is sufficiently high. Also, this suggests that lettuce seedlings might require prolonged protection from *P. fimata* with additional insecticide sprays until the seedlings are established in the lower temperatures of spring and early summer (January to May). In the later part of summer and fall, when even the nighttime temperature is higher than 15 °C, the seeds can germinate, develop, and become less susceptible to *P. fimata* damage quickly. In these circumstances, insecticide applied at planting is likely to provide adequate *P. fimata* control (Joseph et al., 2015). In the experiments conducted at 21 °C, because *P. fimata* were introduced along with the germinating seeds, the time required for *P. fimata* movement and population build up were not accounted for and perhaps these factors play an important role on *P. fimata* feeding at higher temperatures. At 33 °C, the lettuce seed germination was not consistent and the seedling development was not complete. Moreover, all the *P. fimata* in this assay were dead. Using different equipment to retain temperatures may have caused additional variation to the springtail feeding; however, the variation was less than 0.09 °C (SE) for most temperatures except the 33 °C which had the highest variation (0.16 °C SE).

High moisture content in the soil will favor *P. fimata* feeding on the germinating lettuce seeds. In the Salinas Valley, before the lettuce seeds are planted, fields are preirrigated to aid in land preparation and bed shaping. It has been observed that the *P. fimata* density increased from the sub-surface of soil when the field was recently irrigated or after a rain event (SV Joseph, unpubl.). High soil moisture levels at sub-surface profiles may be needed but might be favoring faster buildup of *P. fimata* populations. Joseph et al. (2015) showed that the *P. fimata* captures in bait traps were greater immediately after irrigation. In the moisture assays, a small layer of moist soil was created in the bottom of the assay anticipating that a residual moisture in the base would allow the *P. fimata* to seek refuge in the soil cracks and crevices in the bottom layers. Moisture levels were later manipulated in the upper soil layer to determine response to varying moisture content. There were very low levels of seed germination in the lowest water treatment (1.5 ml); perhaps there was not enough moisture for seed germination. Moreover, onychiurid activity is reduced in low moisture conditions as shown with *Paronychiurus kimi* (Lee) (Choi et al., 2002).

In conclusion, this study clearly demonstrates that early lettuce seed development stages are the most vulnerable to *P. fimata* feeding injury. Moisture has a profound effect on *P. fimata* feeding on germinating lettuce seeds. This study also suggests that *P. fimata* can attack the germinating lettuce seeds at all growing temperatures in the Salinas Valley, although seed germination and subsequent seedling development at lower temperatures would increase the vulnerability of lettuce seeds to *P. fimata* feeding. This information provides insights not only on the timing of protection but also the extent of protection under various temperature ranges. Plants growing under lower temperatures need prolonged protection for *P. fimata* if adequate moisture is present in the top soil of the bed. In the higher temperatures, seed development would occur rather quickly which suggests that prolonged protection against *P. fimata* is not necessary. These results warrant the need for more field studies on protecting lettuce seeds from *P. fimata* in the lower temperatures especially during spring and early summer lettuce plantings in the Salinas Valley.

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