

## **Project Title**

Influence of plant age, temperature, and moisture on springtail feeding injury on lettuce

## **Project Investigator**

Dr. Shimat Villanassery Joseph  
IPM Entomology Advisor  
University of California Cooperative Extension Monterey County

## **Summary**

Springtail is a serious pest of lettuce seedlings in the Salinas Valley. Little is known about the growth stages at which lettuce seedlings are vulnerable to springtail feeding, and how temperature and soil moisture affect severity of feeding injury. The results indicate that germinated seeds and their weights were lower in 1-day-old seedlings than in 3- to 6-day old seedlings. The seedlings that received the most (6.5 mL) water had lower seed germination and lower weights than the seedlings that received 2.5, 3.5, 4.5, or 5.5 mL of distilled water. Springtail feeding injury on the germinating lettuce seeds was evident in all temperature ranges tested (at 40-80°F) except at 90°F.

## **Objectives**

The major objectives of the present study were to determine: (1) the most vulnerable stages of lettuce seedlings to springtail feeding, (2) the influence of soil moisture and (3) temperature on springtail feeding injury of germinating seeds of lettuce.

## **Procedures**

Field-collected springtails were identified as *Protaphorura fimata*. A springtail colony was maintained at UC Cooperative Extension Entomology Laboratory. The various assays were conducted in clear plastic containers. The 'Clear Lake clay' soil, untreated with insecticide or fertilizer for at least 2 months, was collected from a field in Salinas. In all the experiments, 25 un-pelleted, untreated lettuce seeds were used per experimental unit (i.e., a clear plastic container). After inoculating with springtails, all containers were covered using a clear plastic wrap and sealed using Parafilm around the edge of the containers unless specified otherwise.

### ***Effect of plant age on injury***

Oven-dried soil (10 grams) was added to each container and 25 seeds were planted in a staggered fashion so 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 springtails. The assay arena was then covered by inverting another 29.6-ml clear plastic container and the edges were sealed using Parafilm strips. The assay arenas were maintained for seven more days in the controlled environmental chamber and evaluated after 7 days. The seedlings planted in successive days (seedling age) were the treatments and each treatment (day) was replicated 5 times (five containers) in a randomized complete block design (RCBD). This experiment was repeated two more times with 5 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 springtail in 10 g oven-dried soil. The containers with soil, seeds, and various water treatments were weighed. Finally, these containers were inoculated with 100 springtails per container and were covered. The water volume added to the soil served as treatments and each treatment was replicated 5 times in an RCBD. This experiment was repeated 3 times. The containers were maintained in a controlled environmental chamber 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 springtail per container and was covered. The experimental plastic containers were placed on a tray and the tray was placed in a dark blue plastic box with a lid. The plastic box did not allow light to pass through it. A LED light 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. Air temperature was controlled. The boxes were maintained at various air temperature ranging from 40, 50, 60, 70, 80 and 90°F and were exposed to springtails 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 (90°F) is rarely reached in the Salinas Valley. The time of exposure to springtails 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 the first pair of leaves had fully expanded. Each temperature range treatment was replicated 10 times (10 containers) for springtail exposure and non-exposure in an RCBD. This experiment was repeated 2 times for a total of 20 replications per treatment.

### ***Evaluation of feeding injury***

After the springtail 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 before weighing.

## Results

### *Effect of plant age on injury*

When lettuce was exposed to 100 springtails, fewer seeds germinated in the 1-day-old seedling treatment than in any other seedling treatments (Table 1). Percentage of injured seedlings was greater on 1-day-old seedling than in any other seedling age treatments. More feeding punctures were found on 1- and 2-day-old seedlings than on the other ages (Table 1). Fresh and dry weights of seedlings were lower (by ca. 40%) in the 1-day-old seedling treatment than in the other treatments.

When 50 springtails were added, the number of seeds germinated was not significantly different among treatments (Table 1). More seedlings were injured in 1- and 2-day-old seedling treatments than in 5- and 6-day-old seedlings, and feeding punctures were greater in 1- and 2-day-old seedling treatments than in other treatments. Fresh weights of seedlings were lower in 1- and 2-day-old seedling treatment than in other treatments, whereas dry weights of the seedlings were similar among treatments.

When no springtails were present, fresh weight and dry weight of seedlings were not different among treatments, although the number of germinated seeds was lower in 5-day-old seedlings than in other treatments. No feeding injury was observed on lettuce seedlings for all the age treatments when springtails were 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 (Table 2). Fewer seeds germinated in 6.5 ml than in 3.5 and 4.5 ml treatments. Percentage of injured seedlings was greater in 6.5 ml than in other treatments, 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. 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 40°F, seeds took ca. 30 days whereas at other temperatures 50, 60, 70, 80, and at 90°F, they took 12, 8, 7, 5, and 5 days, respectively. The soil temperatures measured at the end of the exposure time tended to be greater than air temperatures for all the temperature treatments except 80°F (Table 3).

Fewer seeds germinated when exposed to 100 springtails at 40, 50, 60, 70, 80 and 90°F than in the non-exposed control (Table 3). At 90°F, the germinated lettuce seedling development was not uniform and all springtails were found dead (Table 3). Feeding injury on seedlings was not detected in the absence of springtail but was detected when exposed to springtails at 40, 50, 60, 70, and 80°F (Table 3). The seedlings were not injured when germinating seedlings were exposed to springtail at 90°F. The fresh weight of seedlings was lower when they were exposed to 100 springtails at 40, 50, 60, 70, and 80°F than when they were not exposed (Table 3). The fresh weight of the seedlings was not different when germinating seedlings were exposed to springtail at 90°F compared to non-exposed. There was no significant difference in dry weight between springtails 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 springtail feeding, resulting in reduction in seedling growth. Thus, it appears that once the shoot emerges out of the soil surface, lettuce is less susceptible to springtail feeding injury. However, the potential effect of springtail 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 springtail. 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 springtails for an extended time in all the plant age treatments. Springtail root feeding symptoms such as stunting or yellowing were not noticed on seedlings older than 2 days. Because the germinating phase of the plants is more susceptible, monitoring for springtail presence and activity should start prior to planting the seeds. Previous studies indicated that beet or potato slice baits attract springtails if placed in the top layer of the soil; thus, these baits could be used for monitoring springtail activity in the soil. If the soil is not moist, the baits may not capture springtail and springtail activity may go undetected.

In this study, springtail feeding activity and damage were evident at temperatures as low as 40°F. This suggests that although seed germination and seedling development become progressively slower in cooler conditions, springtail can feed actively if soil moisture is sufficiently high. Also, this suggests that lettuce seedlings might require prolonged protection from springtail 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 60°F, the seeds can germinate, develop, and become less susceptible to springtail damage quickly. In these circumstances, insecticide applied at-planting is likely to provide adequate springtail control. In the experiments conducted at 70°F, because springtail were introduced along with the germinating seeds, the time required for springtail movement and population build up were not accounted for and perhaps these factors play an important role on springtail feeding at higher temperatures. At 90°F, the lettuce seed germination was not consistent and the seedling development was not complete. Moreover, all the springtail in this assay were dead.

High moisture content in the soil will favor springtail feeding on the germinating lettuce seeds. In the Salinas Valley, before the lettuce seeds are planted, fields are pre-irrigated to aid in land preparation and bed shaping. It has been observed that the springtail density increased from the sub-surface of soil when the field was recently irrigated or after a rain event. High soil moisture levels at sub-surface profiles may be needed but might be favoring faster buildup of springtail populations. Springtail 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 residual moisture in the base would allow the springtails 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 because there was not enough moisture for seed germination.

## **Conclusion**

This study clearly demonstrates that early lettuce seed development stages are the most vulnerable to springtail feeding injury. Moisture has a profound effect on springtail feeding on germinating lettuce seeds. This study also suggests that springtail 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 springtail feeding. This information provides insights not only on the timing of protection but the extent of protection under various temperature ranges. Plants growing under lower temperatures need prolonged protection from springtail 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 springtail is not necessary. These results warrant the need for more field studies on protecting lettuce seeds from springtail in the lower temperatures especially during spring and early summer lettuce plantings in the Salinas Valley.

**Table 1** Mean parameter values assessed after exposing lettuce seedlings of various ages to springtail

| Springtail density | Seedling age (days) | No. seeds germinated <sup>1</sup> | % seedlings injured | No. feeding punctures per seedling | Fresh weight (g) | Dry weight (g)  |
|--------------------|---------------------|-----------------------------------|---------------------|------------------------------------|------------------|-----------------|
| 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  |

**Table 2** Mean parameter values assessed after exposing germinating lettuce seedlings to various soil moisture levels and 100 springtail

| Water volume (ml) <sup>1</sup> | Soil weight (g) |               | No. seeds germinated | % seedlings injured | No. feeding punctures per seedling | Fresh weight (g) | Dry weight (g) |
|--------------------------------|-----------------|---------------|----------------------|---------------------|------------------------------------|------------------|----------------|
|                                | 0 days          | 7 days        |                      |                     |                                    |                  |                |
| 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 |

**Table 3** Means of parameters when germinating lettuce seedlings were exposed to springtails and maintained at various temperatures

| Temperature (°F) | Mean time of exposure (days) | No. springtail | No. seeds germinated | % seedlings injured | No. feeding punctures per seedling | Fresh weight (g) |
|------------------|------------------------------|----------------|----------------------|---------------------|------------------------------------|------------------|
| 40               | 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   |
| 50               | 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   |
| 60               | 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   |
| 70               | 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   |
| 80               | 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   |
| 90               | 5                            | 0              | 16.1 ± 1.4           | 0                   | 0                                  | 0.192 ± 0.019    |
|                  |                              | 100            | 19.2 ± 1.4           | 0                   | 0                                  | 0.196 ± 0.015    |