# Effect of Sodium Tetrathiocarbonate on Growth and Viability of *Sclerotinia minor* and *S. sclerotiorum* and Development of Lettuce Drop

M. E. MATHERON, Extension Plant Pathologist, and J. C. MATEJKA, Research Specialist, University of Arizona, Yuma Agricultural Center, Yuma 85364

**ABSTRACT**

Matheron, M. E., and Matejka, J. C. 1993. Effect of sodium tetrathiocarbonate on growth and viability of *Sclerotinia minor* and *S. sclerotiorum* and development of lettuce drop. Plant Dis. 77:995-998.

Studies were initiated to determine the effect of sodium tetrathiocarbonate (STTC) on mycelial growth and viability of sclerotia of *Sclerotinia minor* and *S. sclerotiorum* and to evaluate the efficacy of this material for control of Sclerotinia leaf drop of lettuce. No growth of mycelia from agar disks containing either *S. minor* or *S. sclerotiorum* was apparent 72 hr after exposure to STTC at 122 μg/ml, but mycelia resumed growth when placed on potato-dextrose agar (PDA). Mycelia of *S. minor* and *S. sclerotiorum* were killed following exposure at rates of 245 and 1,225 μg/ml, respectively. There was a significant linear relationship between the concentration of STTC applied to soil in which sclerotia of *S. minor* or *S. sclerotiorum* were buried and the subsequent percentage of sclerotia that germinated. Only 3% of sclerotia of *S. minor* in soil treated with STTC at 3,675 μg/ml subsequently germinated when placed on PDA. In contrast, the germination rate for sclerotia of *S. sclerotiorum* treated with STTC at the same rate was 95%. For sclerotia of *S. minor*, a significant linear relationship was observed between the duration of exposure to STTC at 2,450 μg/ml and subsequent germination. Germination ranged from 75 to 5% after exposure to STTC for 8 and 96 hr, respectively. When compared with nontreated soil containing sclerotia of *S. minor*, a preplant drench with STTC at 3,675 μg/ml resulted in a significant increase in plant survival and fresh weight of leaf tissue of lettuce plants subsequently seeded and grown. These investigations demonstrate the potential utility of STTC for control of leaf drop of lettuce caused by *S. minor*.

Sclerotinia leaf drop of lettuce (*Lactuca sativa* L.), caused by *Sclerotinia minor* Jagger and *S. sclerotiorum* (Lib.) de Bary, occurs annually in the Yuma County production area of southwestern Arizona. Although disease incidence within the 18,000 ha of lettuce grown in this region varies from year to year, disease severity in affected fields has been high and of economic importance. The lettuce production season begins with initial seeding in late August and ends with final harvest by mid-April. During this time, the incidence of lettuce drop is highest during cool, wet periods (4).

The efficacy of the dicarboximide fungicides iprodione and vinclozolin against species of *Sclerotinia* has been demonstrated (14,20,24,26). These materials can provide effective control of lettuce drop in Arizona (14). However, recent studies have documented the in vitro development of resistance to dicarboximide fungicides by *S. minor* (6,22,23), a situation that suggests the need for continued testing of new materials and methods for disease control.

The activity of sodium tetrathiocarbonate (STTC) on the sporulation and growth of six species of *Phytophthora* in vitro (13) and the control of Phytophthora root rot of citrus (15) recently has been reported. When added to water and applied to soil, STTC releases carbon disulfide, a known biocide (5,7,8,18). The objectives of this study were to determine the effect of STTC on mycelial growth and viability of sclerotia of *S. minor* and *S. sclerotiorum* and to ascertain the efficacy of this material for control of Sclerotinia leaf drop of lettuce. A partial account of this study was reported earlier (16).

Accepted for publication 21 June 1993.

## MATERIALS AND METHODS

**Mycelial growth and viability.** Sclerotia of *S. minor* and *S. sclerotiorum* were collected from naturally infected lettuce plants in Yuma County. Several sclerotia of *S. minor* and *S. sclerotiorum* were surface-disinfested by agitation in a 5.25% solution of NaClO (undiluted household bleach) for 3 min, rinsed in sterile distilled water, then plated onto petri dishes containing potato-dextrose agar (PDA). Growth and colony morphology of all isolates from sclerotia of *S. minor* or *S. sclerotiorum* were similar, so one isolate of each species was selected to be used in all subsequent in vitro tests.

*S. minor* and *S. sclerotiorum* were grown on PDA for 5 days at 24 C. Five replicate 6-mm-diameter agar disks were removed from the edge of an actively growing culture of each pathogen and placed in a similar number of 9-cm-diameter plastic petri dishes containing 20 ml of 5% clarified V8 juice broth amended with STTC (Enzone 3.4E) at concentrations of 24, 122, 245, 612, and 1,225 μg/ml. V8 juice broth was prepared by centrifuging V8 juice for 10 min at 1,000 *g*, followed by dilution of the supernatant with sterile distilled water and adjustment to an initial pH 7 with KOH. Control petri dishes contained only broth. Petri dishes containing similar concentrations of STTC were incubated in sealed plastic containers at 26–28 C. After 72 hr, radial growth of mycelia was measured from the edge of each inoculum disk. To ascertain the effect of STTC on the viability of mycelium, agar disks without visible mycelial growth were transferred to PDA. After 96 hr at 24 C, the disks were observed for mycelial growth. This dosage-response test was performed three times.

**Germination of sclerotia.** Sclerotia of *S. minor* were produced according to the method of Patterson and Grogan (21). Briefly, potatoes with skins removed were cut into 1-cm cubes, and 10–15 pieces were autoclaved (121 C, 102 kPa, 15 min) in 125-ml Erlenmeyer flasks twice in 24-hr intervals. A disk of agar containing mycelium of *S. minor* was then added to the potato cubes in each flask, and the flasks were incubated at 20 C for 4–6 wk. Mature sclerotia were then separated from residual liquefied potato tissue by washing the contents of each flask in running tap water within a soil sieve. Sclerotia were air-dried and stored in a glass container at room temperature until needed. Sclerotia of *S. sclerotiorum* were produced by inoculating 9-cm-diameter petri dishes containing PDA with the pathogen and incubating the dishes at 24 C for 2 wk. Sclerotia, which formed at the edge of the petri dishes, were collected, air-dried, and used in subsequent tests.

Ten sclerotia of *S. minor* or *S. sclerotiorum* were placed between two layers of Whatman No. 4 filter paper on a 2.5-cm layer of nonsterile field soil (sandy loam [81% sand, 7% silt, 12% clay], used in all laboratory and greenhouse tests) in a 10-cm-diameter × 10-cm-deep plastic pot, then covered with an additional 5-cm layer of field soil. The soil in each pot was drenched with water containing 0, 612, 1,225, 2,450, or 3,675 μg/ml of STTC in sufficient quantities to thoroughly wet the soil. Five replicate pots were established for each treatment. Pots were allowed to drain freely, then incubated for 7 days at 26–28 C in the laboratory. Sclerotia were then removed from the soil, rinsed in tap water, blotted dry, and placed onto petri dishes containing PDA. After 4–5 days, the number of sclerotia that germinated were recorded. This experiment was performed three times.

To determine the effect of duration of exposure to STTC on viability of sclerotia of *S. minor*, 10 sclerotia were placed between two layers of filter paper in field soil within a plastic pot as described earlier. The soil in each pot was drenched with water containing 2,450 μg/ml of STTC in sufficient quantities to thoroughly wet the soil. Pots were allowed to drain freely, then incubated at 26–28 C. Control pots were drenched with water only. At 8 hr, 16 hr, and 1, 2, and 4 days after treatment, the sclerotia from five pots treated with STTC and five control pots were removed from the soil, rinsed in tap water, blotted dry, and placed onto petri dishes containing PDA. After 4–5 days, the number of sclerotia that germinated were recorded. This test was conducted twice.

**Effect of preplant drench on disease development.** A sandy loam soil (350 $cm^3$) was added to 10-cm-diameter × 10-cm-deep plastic pots. Approximately 150 sclerotia of *S. minor*, produced on potato cubes as described earlier, were sprinkled on the soil surface and covered with an additional 1-cm layer of soil. Water containing 3,675 μg/ml of STTC was added to each pot in sufficient amounts to thoroughly wet the soil. Control pots were drenched with water only and included soil with and without sclerotia. Six replicate pots were established for each treatment. Pots were allowed to drain freely, then maintained in the greenhouse. One week after treatment, each pot was drenched with enough tap water to thoroughly wet the soil, and 15 seeds of lettuce cv. Vanguard 75 were placed on the soil surface of each pot, covered with a 5-mm layer of sterile sand, and fertilized with water-soluble Miracle-Gro fertilizer. After emergence, lettuce seedlings were thinned to a uniform stand of 10 plants per pot. Plants were maintained in the greenhouse, fertilized weekly with Miracle-Gro fertilizer, and observed for the development of symptoms of lettuce drop. Final determinations of plant growth and disease development were made 3 mo after seeding. The average minimum/maximum air and soil temperatures in the greenhouse during these studies were 15/32 C and 14/26 C, respectively. This experiment was performed three times, with comparable results. Because variances between data from each run of the experiment were homogeneous, the data presented here were derived from the combined results of the three experiments.

Linear regression analysis was used to detect a significant relationship between the concentration of STTC and the resultant germination of sclerotia as well as the relationship of duration of exposure to STTC and germination of sclerotia of *S. minor*. Data from disease control tests were subjected to analysis of variance. When a significant *F* test was achieved, Duncan's multiple range test (10) was used to determine significant differences among treatments. Data were processed with the MSTAT-C statistical software package (17).

## RESULTS

**Mycelial growth and viability.** No growth of mycelia from agar disks containing either *S. minor* or *S. sclerotiorum* was apparent 72 hr after exposure to STTC at 122 μg/ml (Table 1). Although growth was inhibited at this concentration of STTC, subsequent placement of treated agar disks onto PDA revealed that the mycelia within the agar disks were still viable. Exposure of mycelia to STTC at concentrations of 612 and 1,225 μg/ml were necessary to kill *S. minor* and *S. sclerotiorum*, respectively (Table 1).

**Germination of sclerotia.** There was a significant linear relationship between the concentration of STTC applied to soil in which sclerotia of *S. minor* or *S. sclerotiorum* were buried and the subsequent percentage of these treated sclerotia that germinated (Fig. 1). In these tests, 91% of sclerotia of *S. minor* germinated after treatment with STTC at 612 μg/ml, whereas only 3% of sclerotia treated with STTC at 3,675 μg/ml subsequently germinated when placed onto PDA. In contrast, germination rates for sclerotia of *S. sclerotiorum* were 98 and 95% after exposure to STTC in soil at rates of 612 and 3,675 μg/ml, respectively.

When sclerotia of *S. minor* were buried in soil and drenched with STTC at 2,450 μg/ml, a significant linear relationship was observed between duration of exposure to the chemical and the subsequent percentage of treated sclerotia that germinated (Fig. 2). Germination ranged from 75 to 5% after exposure to STTC at 2,450 μg/ml for 8 and 96 hr, respectively.

**Effect of preplant drench on disease development.** A preplant soil drench with STTC at 3,675 μg/ml resulted in a significant increase in plant survival and fresh weight of leaf tissue for lettuce

plants seeded and grown in treated soil containing sclerotia of *S. minor* (Table 2). Growth and survival of lettuce plants in soil infested with *S. minor* and treated with STTC were not significantly different from growth and survival of plants in noninfested soil. No evidence of phytotoxicity was observed on lettuce plants grown in soil treated 1 wk before seeding with STTC at 3,675 μg/ml.

## DISCUSSION

The ultimate utility of any fungicide for control of lettuce drop is determined by the effect of the material on the life stages of *S. minor* and *S. sclerotiorum* and the proper placement of the material in the field to maximize its activity against a pathogen. Infection of lettuce with *S. minor* can occur either at the soil line through senescent lower leaves or belowground through stem tissues (12). The majority of infections begin from sclerotia within the top 2 cm of soil (1,2,11) and result from myceliogenic germination of these fungal propagules (12). Apothecia rarely have been observed for *S. minor*, and ascospores are not regarded as important propagules in the epidemiology of disease development with this pathogen (3,12). In contrast, infection of lettuce with *S. sclerotiorum* primarily occurs at ground level on lower leaves, originating from mycelia or ascospores arising from germinating sclerotia (12). Mycelial germination from sclerotia of *S. sclerotiorum* is common in Arizona (27) but infrequent in the eastern United States (1).

For effective control of leaf drop of lettuce originating from mycelial germination of sclerotia, fungicides currently are applied in the field with the objective of placing the material around the crown and upper roots to form a protective layer between the soil surface and lower leaves (20). STTC at appropriate concentrations inhibited growth of *S. minor* and *S. sclerotiorum* in our studies, but the relatively short time that carbon disulfide remains in soil after application (13) suggests that the current formulation of this material will probably not provide long-term control of lettuce drop by preventing growth of mycelia. On the other hand, STTC was lethal to sclerotia of *S. minor* in soil, which suggests that the tested formulation of this compound has potential utility as a preplant soil drench for control of lettuce drop caused by this pathogen.

The in vitro development of resistance by *S. minor* to dicarboximide fungicides (6,22,23), a class of compounds that includes iprodione and vinclozolin, is a demonstration of the potential for development of resistance in the field. The mode of action of dicarboximides is not clear even though it has been studied extensively (25). As a general nonselective biocide, however, the carbon disulfide released by STTC may be equally lethal to all isolates of *S. minor*, whether they are susceptible or resistant to dicarboximides. In this case, STTC could play a role in resistance management strategies to prevent or delay development of resistance by *S. minor* to dicarboximides in the field.

Sclerotia of *S. minor* apparently are not killed rapidly when STTC is applied to soil containing these fungal structures. In our studies, 75% of sclerotia removed from soil 8 hr after treatment germinated, whereas a treatment period of 96 hr was necessary to reduce germination of sclerotia to 5%. Perhaps a formulation of STTC that released carbon disulfide at a slower rate than the tested formulation would maintain a lethal concentration of the biocide in the soil for a longer period of time, which in turn might shorten the time needed to kill sclerotia of *S. minor* in soil. A slow-release formulation could

**Table 1.** Growth and viability of mycelia of *Sclerotinia minor* and *S. sclerotiorum* in V8 juice broth after 72 hr in the presence of sodium tetrathiocarbonate (STTC)[z]

| STTC (μg/ml) | Mycelial growth (mm) | | Agar disks with viable mycelia (%) | |
|---|---|---|---|---|
| | *S. minor* | *S. sclerotiorum* | *S. minor* | *S. sclerotiorum* |
| 0 | 10 | 12 | 100 | 100 |
| 24 | 1 | 2 | ... | ... |
| 122 | 0 | 0 | 100 | 100 |
| 245 | ... | ... | 80 | 100 |
| 612 | ... | ... | 0 | 40 |
| 1,225 | ... | ... | 0 | 0 |

[z] Each value is the mean of five replicate measurements from one representative experiment. This experiment was conducted four times.

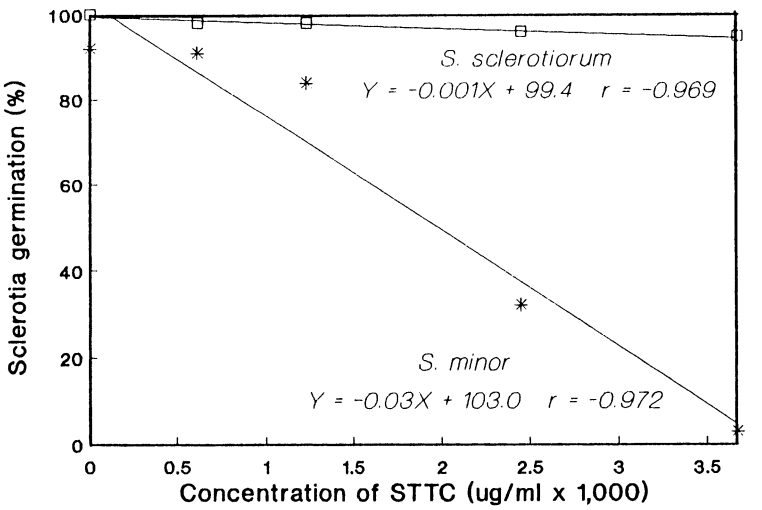


**Fig. 1.** Significant linear regressions ($P = 0.01$) of dosage-reponse effects of sodium tetrathiocarbonate (STTC) on myceliogenic germination of sclerotia of *Sclerotinia minor* and *S. sclerotiorum*.

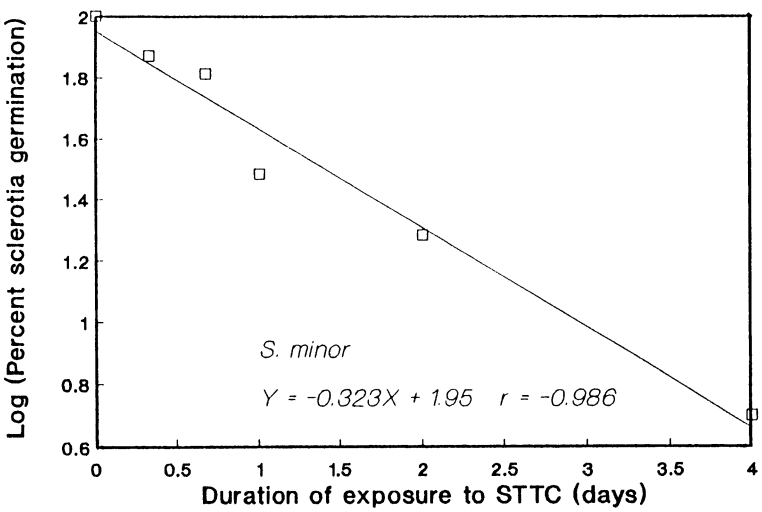


**Fig. 2.** Significant linear regression ($P = 0.01$) of duration of exposure to sodium tetrathiocarbonate (STTC) on myceliogenic germination of sclerotia of *Sclerotinia minor*.

**Table 2.** Effect of preplant treatment with sodium tetrathiocarbonate (STTC) on subsequent growth of lettuce and development of lettuce drop in soil artificially infested with sclerotia of *Sclerotinia minor*[x]

| Treatment | Fresh weight of leaves (g) | Plant survival[y] (%) |
|---|---|---|
| Infested soil | | |
| No STTC | 21 b[z] | 31 b |
| STTC, 3,675 μg/ml | 78 a | 98 a |
| Noninfested soil | 68 a | 92 a |

[x] Each value is the mean of 18 replicate pots from three experiments, with 10 lettuce plants per pot.

[y] Percentage of lettuce plants alive at the termination of the experiment.

[z] Numbers in each column with a different letter are significantly different ($P = 0.05$) according to Duncan's multiple range test.

also maintain an adequate level of carbon disulfide in the soil for a sufficient duration to kill the larger sclerotia of *S. sclerotiorum*. The tested formulation of STTC did not reduce the germination of sclerotia of *S. sclerotiorum*.

The use of carbon disulfide as a partial soil sterilant first was recorded in 1894 (9,19). Since then, this material has been reported to have biocidal effects on *Armillaria mellea* (5,8,18), *Trichoderma viride* (18), *Clitocybe tabescens* (7), species of *Phytophthora* (13,15), and plant-parasitic nematodes (7). The biocidal effects of carbon disulfide released from STTC on mycelium of *S. minor* and *S. sclerotiorum* and sclerotia of *S. minor* demonstrate the potential utility of this compound for disease control. Additional studies are needed to determine the spectrum of activity of STTC in various soil types and at different soil temperatures.


## ACKNOWLEDGMENTS

This research was supported by grants from the Arizona Iceberg Lettuce Research Council, the Unocal Corporation, and state and Hatch funds allocated to the Arizona Agricultural Experiment Station.


## LITERATURE CITED


1. Abawi, G. S., and Grogan, R. G. 1979. Epidemiology of diseases caused by *Sclerotinia* species. Phytopathology 69:899-904.
2. Adams, P. B., and Tate, C. J. 1975. Factors affecting lettuce drop caused by *Sclerotinia sclerotiorum*. Plant Dis. Rep. 59:140-143.
3. Adams, P. B., and Tate, C. J. 1976. Mycelial germination of sclerotia of *Sclerotinia sclerotiorum* on soil. Plant Dis. Rep. 60:515-518.
4. Anonymous. 1985. Integrated pest management for cole crops and lettuce. University of California Statewide Integrated Pest Management Project. Publ. 3307.
5. Bliss, D. E. 1951. The destruction of *Armillaria mellea* in citrus soils. Phytopathology 41:665-683.
6. Brenneman, T. B., Phipps, P. M., and Stipes, R. J. 1987. Control of Sclerotinia blight of peanut: Sensitivity and resistance of *Sclerotinia minor* to vinclozolin, iprodione, dicloran, and PCNB. Plant Dis. 71:87-90.
7. Chandler, W. A. 1969. Reduction in mortality of peach trees following preplant soil fumigation. Plant Dis. Rep. 53:49-53.
8. Filip, G. M., and Roth, L. F. 1977. Stump injections with soil fumigants to eradicate *Armillariella mellea* from young-growth ponderosa pine killed by root rot. Can. J. For. Res. 7:226-231.
9. Girard, A. 1894. Recherches sur l'augmentation des recoltes par l'injection dans de sol du sufure de carbone a doses massives. Soc. Nat. Agric. France Bull. 54:356-363.
10. Gomez, K. A., and Gomez, A. A. 1984. Statistical Procedures for Agricultural Research. 2nd ed. John Wiley & Sons, New York.
11. Imolehin, E. D., and Grogan, R. G. 1980. Factors affecting survival of sclerotia, and effects of inoculum density, relative position, and distance of sclerotia from the host on infection of lettuce by *Sclerotinia minor*. Phytopathology 70:1162-1167.
12. Johnston, S. A., and Lorbeer, J. W. 1986. Field evaluation of fungicides for control of lettuce drop. Pages 187-189 in: Methods for Evaluating Pesticides for Control of Plant Pathogens. K. D. Hickey, ed. American Phytopathological Society, St. Paul, MN.
13. Matheron, M. E., and Matejka, J. C. 1988. In vitro activity of sodium tetrathiocarbonate on sporulation and growth of six *Phytophthora* species. Phytopathology 78:1234-1237.
14. Matheron, M. E., and Matejka, J. C. 1989. In vitro and field comparison of six new fungicides with iprodione and vinclozolin for control of leaf drop of lettuce caused by *Sclerotinia sclerotiorum*. Plant Dis. 73:727-730.
15. Matheron, M. E., and Matejka, J. C. 1991. Effect of sodium tetrathiocarbonate, metalaxyl, and fosetyl-Al on development and control of Phytophthora root rot of citrus. Plant Dis. 75:264-268.
16. Matheron, M. E., and Matejka, J. C. 1991. Biocidal activity of sodium tetrathiocarbonate on *Sclerotinia minor* and *S. sclerotiorum*. (Abstr.) Phytopathology 81:1138.
17. MSTAT Development Team. 1988. MSTAT-C, a Microcomputer Program for the Design, Management, and Analysis of Agronomic Research Experiments. Michigan State University, East Lansing.
18. Munnecke, D. E., Kolbezen, M. J., and Wilbur, W. D. 1973. Effect of methyl bromide or carbon disulfide on *Armillaria* and *Trichoderma* growing on agar medium and relation to survival of *Armillaria* in soil following fumigation. Phytopathology 63:1352-1357.
19. Oberlin, C. 1894. Bodenmudigkeit und Schwefelkohlenstoff. Mainz, Zabern.
20. Patterson, C. L., and Grogan, R. G. 1985. Differences in epidemiology and control of lettuce drop caused by *Sclerotinia minor* and *S. sclerotiorum*. Plant Dis. 69:766-770.
21. Patterson, C. L., and Grogan, R. G. 1988. Relationship of growth media and drying and of age of sclerotia to eruptive germination and infection by *Sclerotinia minor*. Plant Dis. 72:1046-1048.
22. Porter, D. M., and Phipps, P. M. 1985. Effects of three fungicides on mycelial growth, sclerotium production, and development of fungicide-tolerant isolates of *Sclerotinia minor*. Plant Dis. 69:143-146.
23. Porter, D. M., and Phipps, P. M. 1985. Tolerance of *Sclerotinia minor* to procymidone and cross tolerance to other dicarboximide fungicides and dicloran. Peanut Sci. 12:41-45.
24. Reilly, C. C., and Lamoureux, G. L. 1981. The effects of the fungicide, iprodione, on the mycelium of *Sclerotinia sclerotiorum*. Phytopathology 71:722-727.
25. Sisler, H. D. 1988. Dicarboximide fungicides: Mechanisms of action and resistance. Page 52 in: Fungicide Resistance in North America. C. J. Delp, ed. American Phytopathological Society, St. Paul, MN.
26. Springer, J. K., and Johnston, S. A. 1982. Fungicides for lettuce drop. Plant Dis. 66:281.
27. Troutman, J. L., and Matejka, J. C. 1982. Establishing and maintaining a lettuce drop nursery. Plant Dis. 66:415.