Effects of Soil Moisture and Temperature on the Survival of *Colletotrichum acutatum*

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ABSTRACT

Eastburn, D. M., and Gubler, W. D. 1992. Effects of soil moisture and temperature on the survival of *Colletotrichum acutatum*. Plant Dis. 76:841-842.

Sections of strawberry petioles inoculated with *Colletotrichum acutatum* were buried in unsterilized soil and subjected to a range of soil temperatures (10, 25, and 40 C) and soil moistures (flooded, moist [10.6%], and dry). Sections were recovered weekly and assayed for the presence of *C. acutatum*. Survival of *C. acutatum* was highest under cool, dry soil conditions. Survival decreased with increases in both soil temperature and moisture.

Strawberry anthracnose was first observed in California in 1983 (15), and the pathogen was subsequently identified as *Colletotrichum acutatum* J. H. Simmonds (13). Infections by the pathogen have resulted in significant reductions of marketable fruit, especially in excessively wet seasons (7,8). Anthracnose also is a problem for the strawberry nursery industry because the pathogen infects crowns and produces lesions on immature petioles and stolons (3,13,14).

In a study on the overwintering of *C. fragariae* A. N. Brooks, another species causing strawberry anthracnose, it was concluded that *C. fragariae* did not have the ability to survive in soil (9). However, *C. acutatum* f.sp. *pinea* Dingley & Gilmour was found to survive in soil for 2 yr (12). Isolates of *C. acutatum* that were pathogenic on strawberry survived in soil for more than 7 mo (7). Levels of survival were at or near 100% during the winter months, from November through March, but quickly declined in the spring and early summer. It was hypothesized that the reduction in survival was associated with changing soil conditions, primarily soil temperature and moisture.

This study was undertaken to evaluate the effects of soil temperature and moisture on the survival of *C. acutatum* in unsterilized field soil under controlled conditions.

MATERIALS AND METHODS

Yolo fine sandy loam soil was collected, thoroughly mixed, and stored in sealed plastic bags at 2 C until used. Some of the soil was air-dried at room temperature for 3 days prior to use. Soil moisture content of the nondried soil was determined gravimetrically at the time of use and found to average 10.6 percent, which corresponds to a water potential (ψ) of approximately −0.3.5 MPa (5).

Petioles of fully expanded leaves taken from strawberry plants grown in the greenhouse were air-dried for 5–7 days, cut into 1-cm segments, and autoclaved at 121 C for 45 min. Conidia were collected from 7-day-old cultures of *C. acutatum* grown on potato-dextrose agar (PDA). The conidia were suspended in sterile distilled water, and the spore concentration was adjusted to 10^6 conidia per milliliter. The resulting conidial suspension was poured into petri dishes containing the autoclaved petiole pieces. After 1 hr the liquid was poured off, and the dishes were sealed with Parafilm and placed in an incubator at 24 C for 3 days. The Parafilm was then removed, and the pieces of petioles were allowed to dry slowly for 3 days.

Colonized petiole pieces were placed in nylon mesh bags, five pieces per bag, and the bags were buried in the unsterile soil in 945-ml glass jars, seven bags per jar. Bags were buried in the middle of each jar with approximately 5 cm of soil above and below the bags. Nylon string was attached to each bag, so that an individual bag could be removed without removing the remaining bags. Three levels of soil moisture were evaluated: air-dried soil; moist soil (10.6% moisture); and flooded soil, in which water was added to the jars and maintained at a height 1 cm above the soil surface. Jars were then sealed with plastic film to minimize changes in soil moisture during the course of the experiment.

Jars were immersed in water baths that were adjusted to temperatures of 10, 25, and 40 C (± 1 C). Bags containing the petiole pieces were recovered from each of the jars every 7 days for 7 wk. Recovered pieces were rinsed in running tap water for 10–15 min, soaked in 0.5% NaOCL for 30 sec, and rinsed in sterile distilled water. Pieces were then cut in half, and one half was placed on acidified PDA and the other on PDA containing benomyl (5 mg a.i/L), streptomycin (30 mg/L), and tetracycline (30 mg/L). After incubation at 24 C for 3–4 days, the number of pieces yielding colonies of *C. acutatum* on either medium was recorded. The initial percent colonization level (time = 0 wk) was determined using unburied, colonized petiole pieces, which were washed and cultured as described above.

Jars were placed in the water baths according to a complete randomization scheme of the replicated soil moisture treatments. There were three replications of each soil moisture treatment within each of the three water-temperature baths, and the experiment was performed twice. Results were statistically analyzed using the analysis of covariance procedure, with experimental trial, temperature, and moisture entered as class variables and time entered as a continuous variable. Because there were no true replications of temperature within a trial, the trial × temperature interaction was used as the mean square error term to evaluate the significance of temperature. The residual error term was used to evaluate all other components of the model. Bartlett's test for homogeneity of variances (10) was used to test the assumption of homogeneity of variances of the analysis of variance procedure.

RESULTS

Analysis of covariance showed that the variables of temperature, moisture, and time all had a highly significant effect on percent survival of *C. acutatum*, with P values of 0.0169, 0.0001, and 0.0001, respectively. The time × temperature interaction also was highly significant ($P > F = 0.0001$). The R^2 value for the model was 0.6474.

Under dry soil conditions, survival remained at 100% over a 7-wk period at 10 and 25 C (Fig. 1A). At 40 C, survival had dropped to 20% by the 7th wk in the first run of the experiment but remained at 100% throughout the study in the second run. Survival in moist soil was extremely temperature-dependent. Survival remained above 80% throughout the study at 10 C. Survival fluctuated at 25 C and rapidly declined at 40 C (Fig. 1B). In the flooded soil, survival declined most rapidly at 25 and 40 C (Fig. 1C), and at 10 C survival declined sharply after 3 wk.

Variation was lowest for data points near 0, or 100% survival, and highest for data points with levels of survival near

Accepted for publication 14 February 1992.

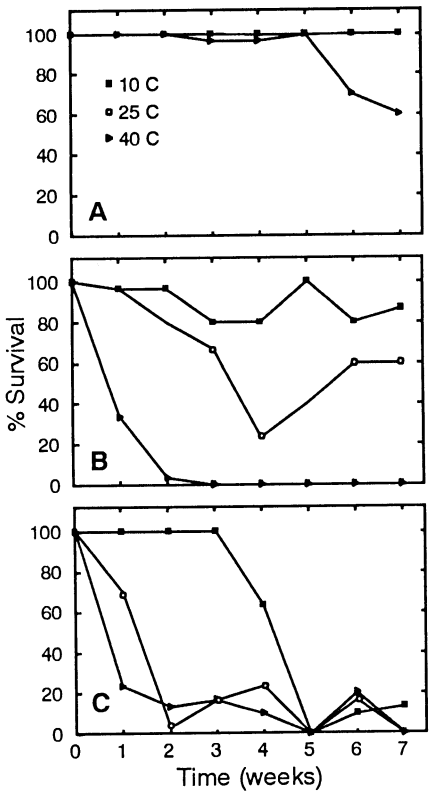

Fig. 1. Percent survival of *Colletotrichum acutatum* on pieces of inoculated strawberry petioles buried in **(A)** air-dried soil, **(B)** moist soil, and **(C)** flooded soil and incubated at 10, 25, and 40 C. Values are averages of two experiments.

50%. Data were transformed using the arcsine transformation in an effort to satisfy the equal variance assumption of the analysis of variance procedure. However, using Bartlett's test for homogeneity of variances, it was found that the arcsine transformation did not result in more uniform levels of variation. Adjusted chi-square values increased after transformation, so statistical evaluations were made using nontransformed data.

Recovery of fungi other than *C. acutatum* was highest from the pieces buried in moist soil at 25 C and lowest from the pieces buried in dry soil at 10 C. The problem of contaminant fungi masking the presence of *C. acutatum* was reduced by dividing samples and using two isolation media. Colonies of *C. acutatum* grew more slowly on the medium containing benomyl. However, colonies were easily distinguished on this medium, and contaminants were less of a problem.

DISCUSSION

Although the optimal temperature for growth of *C. acutatum* is in the range of 24–28 C (7,14,16), the pathogen was found to survive best in soils that were cool and dry. The reduced activity of *C. acutatum* at lower temperatures may correspond with a reduction of nutrient utilization, so that the fungus uses the colonized substrate more slowly. Activities of competing or antagonistic soilborne organisms are likely to be reduced under cool and dry conditions as well (1,2,4,6,11). As temperatures and moisture levels increased, the ability of *C. acutatum* to survive was reduced, and this may be a result of an increase in the activities of other soilborne microorganisms. In the plating process for the detection of *C. acutatum*, the most contamination from other fungi developed on the segments that had been buried in moist soil. Those segments that were buried in dry soil were relatively free of contamination. The results of this study may explain why, in a field-survival study (7), *C. acutatum* on tissue buried in the soil was able to survive well during the winter and early spring but declined rapidly as soil temperatures increased in the late spring and summer.

Difficulties were encountered when analyzing the data, because levels of variation for survival values near 0 and 100% were at or near 0. Thus, the assumption of equal variance for the analysis of variance procedure was not satisfied. Attempts to minimize the problem by data transformation were not successful. However, results of the analysis of variance procedure are reported here as a best measure of significance, with the understanding that the assumptions of the test have not been satisfied, and that the results may not be valid.

Most commercial strawberry fields in California, both nursery and production, are fumigated prior to planting. Preliminary experiments and a survey of field soils (7) have indicated that *C. acutatum* does not survive the soil fumigation process. Nonfumigated soil, however, enters the field on the surfaces of planting stock, and propagules in this soil may be an important source of primary inoculum. Between the times when plants are dug from the nursery and planted in production fields, planting stock is often kept in refrigerated storage. The results of this study indicate that levels of survival are highest at low temperatures. Therefore, the short-term refrigeration of planting stock may favor the survival of the pathogen.

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