

Strawberry Anthracnose: Detection and Survival of *Colletotrichum acutatum* in Soil

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ABSTRACT

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Propagules of *Colletotrichum acutatum* were detected in soil from a recently fallowed strawberry nursery plot. A survey of strawberry nursery and production fields showed that *C. acutatum* was present in soils from sites with a recent history of strawberry anthracnose but not in fumigated soils. Isolates of *C. acutatum* from soil were culturally similar to isolates from plant tissue and were equally pathogenic in assays using detached fruit. Soil naturally infested with *C. acutatum* initiated disease development on strawberry plants in the greenhouse. *C. acutatum* survived in buried strawberry tissue for 9 mo, but soil population densities gradually declined over an 11-mo period. Results suggest that soilborne propagules, especially those in soil attached to planting stock, may be a source of inoculum in California.

Additional keywords: *Colletotrichum fragariae*, overwintering

Colletotrichum fragariae Brooks, *C. acutatum* Simmonds, *C. dematium* (Pers.) Grove, and *C. gloeosporioides* Penz. have been reported to cause anthracnose on strawberry (1,2,6,9). Strawberry anthracnose was first identified in California in 1983 (12) and at that time was attributed to *C. fragariae*, the species most commonly reported in the southeastern United States. In 1986 an isolate from California was identified as *C. acutatum* (10), and in an extensive survey of California strawberry fields in 1986 and 1987, only *C. acutatum* was isolated from infected plants (3).

In 1968, Horn and Carver (4) reported that healthy strawberry plants grown in soil that previously contained plants infected by *C. fragariae* did not develop anthracnose, and they concluded that *C. fragariae* did not survive in soil. Although their work focused on *C. fragariae*, their conclusion was apparently transferred to other species of *Colletotrichum* that cause strawberry anthracnose, because no further attempts were made to look for a soilborne phase of the disease cycle. This view has persisted despite research showing that *C. acutatum* f. sp. *pinnae*, the cause of terminal crook of *Pinus radiata*, can survive in soil for up to 2 yr (7).

C. acutatum was recently detected in soil from a strawberry field that had been fallow for several weeks. This detection prompted further study to determine the incidence of *C. acutatum* in field soil planted to strawberry, whether cultures isolated from soil were pathogenic to

strawberry, whether soilborne propagules could serve as a source of inoculum, and how long *C. acutatum* could survive in soil. Preliminary results of this research have been previously reported (3).

MATERIALS AND METHODS

Field survey. Soil samples were taken from 44 fields, including strawberry nursery and production fields (planted and fallow sites), sites with and without a recent history of anthracnose, and sites that had and had not been fumigated since the last strawberry planting. Five to 10 subsamples from each site were combined, thoroughly mixed, and assayed for *C. acutatum* by the spread plate technique. Soil samples (15 g) were homogenized in 150 ml of 0.1% water agar in a blender for 2 min. Suspensions were diluted to give soil concentrations of $1:10^2$ and $1:10^3$ (w/v). An aliquot (0.5 ml) of each dilution was then spread onto the surface of modified dextrose peptone yeast extract agar (DPYA) (8) containing benomyl (5 mg a.i./L). Five replicates were used per dilution. Plates were incubated at 24 C for 6–8 days under cool-white fluorescent light and were then evaluated for formation of colonies of *C. acutatum*. Representative colonies were transferred to potato-dextrose agar (PDA) to verify identification.

Soils washed from strawberry crowns from 24 plots in two northern California nurseries also were assayed for *C. acutatum*. Plants were harvested, trimmed, and stored at 3 C for 2 wk following standard commercial nursery procedures. Roots were removed from five crowns from each plot, and the five crowns were placed in 150 ml of sterile distilled water and soaked for 2 hr with

occasional agitation. Aliquots (0.5 ml) of undiluted and diluted (1:10) crown washings were spread onto the surface of DPYA + benomyl. Five replicates were used per dilution. Plates were incubated and evaluated as described previously.

Cultural characteristics. Four cultures of *C. acutatum* isolated from soil were compared with five cultures of *C. acutatum* isolated from strawberry fruit, runners, and crowns. Cultures were compared on the basis of colony morphology on PDA, size and shape of conidia, and diameter of colonies on PDA after 7 days of growth at 24 C. Three replicates were used per isolate. The temperature responses of three isolates (isolate 0001 from a fruit lesion, isolate 0080 from soil, and isolate 0097 from a petiole lesion) were measured as colony diameter after 7 days of growth on PDA at 9, 12, 15, 18, 21, 24, 27, 30, and 33 C.

Pathogenicity tests. The ability of soilborne propagules of *C. acutatum* to infect susceptible strawberry cultivars was tested by spray-inoculating plants with a suspension of naturally infested field soil with a population density of *C. acutatum* of 10^4 colony-forming units (cfu) per gram. Soil was suspended in sterile distilled water and homogenized in a blender for 30 sec to give an inoculum concentration of 100 cfu/ml. Six-week-old plants of the susceptible cultivars Pajaro and E25 were spray-inoculated to the point of runoff (11), sealed in clear polyethylene bags, and placed in a growth chamber at 27 C. Sterile water was sprayed on a second group of plants as a control. After 3 days, plants were removed from the plastic bags, placed under a mist system at 24 C, and monitored for symptom development on petioles, blossoms, and peduncles. This experiment was performed two times.

Soil isolates of *C. acutatum* were tested for their ability to cause lesions on detached strawberry fruit. Berries were rinsed with tap water, blotted dry, placed in moist chambers, and inoculated; a 20- μ l drop of a 10^5 conidial suspension was placed on the fruit surface, and the fruit was then pierced with a sterile dissecting probe through the drop to a depth of 1–2 mm. Three replicates per isolate were used. Berries were rated for lesion development after incubation at 24 C for 8 days. Berries inoculated with sterile water were used as controls. This

experiment was performed two times.

Survival tests. A strawberry nursery in Red Bluff, CA (Colombian fine sandy loam soil) was planted to strawberry in the spring of 1986. By August, the level of anthracnose was so high that the plants were tilled into the soil before harvest. To evaluate the ability of *C. acutatum* to survive in soil in the absence of strawberry, soil from the field was assayed monthly for the presence of *C. acutatum* beginning in late September. The site remained fallow throughout the course of the study. Populations of *C. acutatum* were determined by the spread plate method described previously.

In a second survival test, runners showing typical anthracnose lesions were

collected from a nursery plot of cultivar Pajaro. The infected runners were placed in nylon mesh bags (five segments per bag). Healthy runner segments were included as controls. Bags were buried 5–7 cm deep at five randomly selected sites in a fallow nursery plot. One bag from each of the five sites was recovered monthly for 9 mo. Recovered runner segments were washed in running tap water for 15 min, cut in half, immersed in 0.5% NaOCl for 30–45 sec, rinsed in sterile distilled water, and blotted dry. Segments were placed on acidified PDA and on PDA containing benomyl (5 mg a.i./L), streptomycin (30 mg/L), and tetracycline (30 mg/L) and were incubated at 24 C for 3–4 days. The number of segments yielding *C. acutatum* on either medium was then recorded.

RESULTS

Field survey. *C. acutatum* was detected in 17 of 44 soils assayed (Table 1). Detection of the fungus in soil was associated closely with the incidence of anthracnose symptoms in the current or most recent strawberry crop. The pathogen, however, was never isolated from soils that had been fumigated after the removal of the last strawberry crop. *C. acutatum* also was detected in soil washed from strawberry crowns from 16 of 24 nursery sites.

Cultural characteristics. *C. acutatum* colonies on DPYA + benomyl were small (5–10 mm in diameter) and white, with appressed margins and small amounts of tufted hyphae near the center. The most distinguishing feature of these colonies, however, was the presence of salmon-colored masses of conidia. Conidia produced on DPYA + benomyl were morphologically similar to conidia produced on PDA, so they were used to verify the identity of questionable

colonies.

Cultures of *C. acutatum* isolated from soil were morphologically indistinguishable from cultures isolated from strawberry tissues showing anthracnose symptoms. Colonies grown on PDA under fluorescent light at 24 C were composed of white, floccose aerial mycelium and were white to tan in reverse. Salmon-colored masses of conidia were produced either in small clumps or spread over most of the colony surface. Darkly pigmented stromatic structures were occasionally formed on the colony surface, which gave the colony a "peppered" appearance. Colony diameters averaged 61.5 mm after 7 days of growth at 24 C.

The hyaline, one-celled conidia varied in shape from oblong to fusiform, but most tended to be pointed at both ends. The conidia varied somewhat in size but averaged $15 \times 4 \mu\text{m}$.

The temperature response curves of one isolate of *C. acutatum* from soil and two isolates from strawberry tissue were essentially identical (Fig. 1). The optimal temperature for all three isolates was 24 C, and no growth occurred after 7 days at 9 or 33 C.

Pathogenicity tests. Plants inoculated with suspensions of naturally infested soil developed blighted blossoms and sunken black lesions on young petioles and peduncles, similar to symptoms observed in the field (Table 2). *C. acutatum* infection was verified by isolation on acidified PDA and identification using morphological characteristics. Lesions also developed on a few control plants, presumably as a result of contamination.

In a second pathogenicity test, an isolate of *C. acutatum* from soil caused lesions on detached strawberry fruit. The lesions were indistinguishable in size and appearance from lesions caused by isolates recovered from infected strawberry plants.

Survival tests. *C. acutatum* was detected in soil from the fallow nursery plot from September 1986 through May 1987 but was not detected in the sample taken in July 1987 (no sample was taken in June). *C. acutatum* was recovered

Table 1. Prevalence of *Colletotrichum acutatum* in strawberry nursery and production field soils in California

Field status	Number of sites	
	Sampled	With <i>C. acutatum</i>
Currently in strawberry production, anthracnose symptoms present	17	14
Currently in strawberry production, anthracnose symptoms absent	15	1
Fallow, ^a fumigated ^b	9	0
Fallow, ^a not fumigated	3	2

^aFallow fields had been in strawberry production up to 12 mo before sampling but were not planted during the season when the study was conducted.

^bFields were fumigated after the most recent strawberry crop was removed. In standard fumigations, a mixture of methyl bromide (66%) and chloropicrin (33%) is injected at a depth of 20 cm, and the soil is then covered with a polyethylene tarp for at least 48 hr.

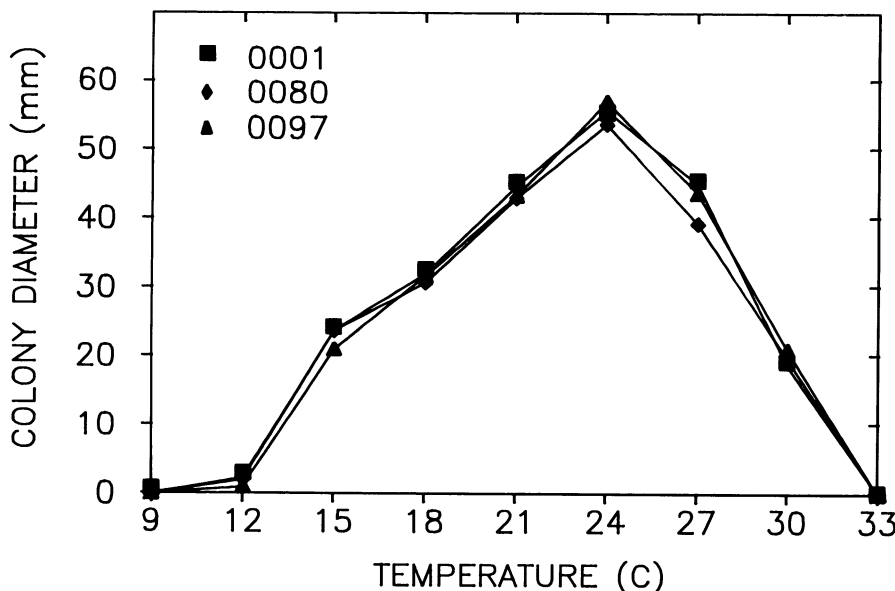


Fig. 1. Temperature-growth response curves for three isolates of *Colletotrichum acutatum*. Isolates 0001 and 0097 were isolated from strawberry tissue, and isolate 0080 was isolated from soil.

Table 2. Anthracnose symptom development on plants treated with a suspension of soil naturally infested with *Colletotrichum acutatum*

Cultivar	Treatment ^a	Number of plants	
		Treated	Symptomatic
Pajaro	Inoculated	25	8
	Not inoculated	25	1
E25	Inoculated	20	9
	Not inoculated	20	3

^aInoculated plants were treated with an aqueous suspension of soil infested with *C. acutatum*. Plants not inoculated were treated with sterile water.

from almost 100% of the runner segments buried in soil for up to 5 mo (Fig. 2). The recovery percentage declined sharply in April, however, and stayed low for the remainder of the study.

DISCUSSION

In a survey of nursery and production fields, *C. acutatum* was detected in soil after anthracnose occurred in the current or most recent strawberry crop. Only one field without symptomatic plants was infested with *C. acutatum*, and it was adjacent to a field with plants showing symptoms. This suggests that soilborne inoculum originates from the current season's infected strawberry plants.

It is common practice in both nursery and production systems to fumigate soils with methyl bromide and chloropicrin before planting. *C. acutatum* was not isolated from any field that had been fumigated after the last strawberry crop was removed, so propagules free in the soil probably do not survive fumigation to act as inoculum in the following seasons.

Although routine soil fumigation before planting appears to eliminate inoculum in the field, the importance of soilborne inoculum became evident when *C. acutatum* was detected in soil adhering to stored planting stock. Harvested plants are coated with soil, which remains on the plants during storage and replanting. Propagules of *C. acutatum* in this soil would, therefore, escape fumigation and could serve as a source of primary inoculum. Strategies aimed at eliminating this source of inoculum, such as use of clean stock or preplant treatments, may be necessary to control the disease.

Cultures of *C. acutatum* isolates obtained from soil were indistinguishable

from those of isolates obtained from infected strawberry tissues, and pathogenicity tests showed that isolates from soil and plant tissues were equally virulent on detached strawberry fruit. Thus, it is unlikely that isolates found in soil represent a subgroup of *C. acutatum* distinct from isolates pathogenic on strawberry.

Soilborne inoculum is capable of initiating infection on petioles, runners, and blossoms, because young plants inoculated with solutions of soil naturally infested with *C. acutatum* became diseased. The control plants that became infected were contaminated either in the nursery or while they were being handled in the greenhouse. It is interesting that lesions formed only on petioles that were not fully developed at the time they were inoculated; thus, it appears that some tissues become resistant to infection as they mature.

C. acutatum was detected in soil from a strawberry nursery plot that was tilled with a disk before harvest because of high levels of anthracnose. The pathogen was initially detected 4 wk after tilling, indicating that short-term survival in soil is possible. Although the sampling technique used was fairly crude and the accuracy of colony counts was questionable, the population density of *C. acutatum* in this soil tended to decline in subsequent samplings. However, the fungus was detected 9 mo after tilling, indicating that *C. acutatum* can survive in soil for an extended period of time.

The pathogen also was able to survive for 9 mo in strawberry tissue that had been buried at depths of 5–7 cm. Thus, *C. acutatum* can survive in soil for a minimum of 9 mo. The abrupt decline in the percentage recovery of *C. acutatum* from buried tissue in April, 6

mo after burial, coincided with the onset of warmer spring temperatures, which may have contributed to the decline. If increases in soil temperature are detrimental to survival, then *C. acutatum* may survive better in debris buried at greater depths.

Results from the field studies, although based on 1-yr experiments, indicate that long-term survival of *C. acutatum* in soil is possible. The results are reported here as supporting evidence for the conclusions from the other repeated experiments. Additional work needs to be done to verify the rates of decline in population density.

The detection of *C. acutatum* in soil suggests a new dimension in the disease cycle of strawberry anthracnose, in that propagules may overwinter in soil and act as a source of inoculum. Most previous work on strawberry anthracnose has focused on *C. fragariae*, a closely related species that is the primary cause of strawberry anthracnose in the southeastern United States (5,6). The belief that *C. fragariae* cannot survive in soil came from the absence of disease symptoms on plants grown in soil from fields with a recent history of anthracnose (4). However, apparently no attempt has been made to detect *C. fragariae* in soil directly.

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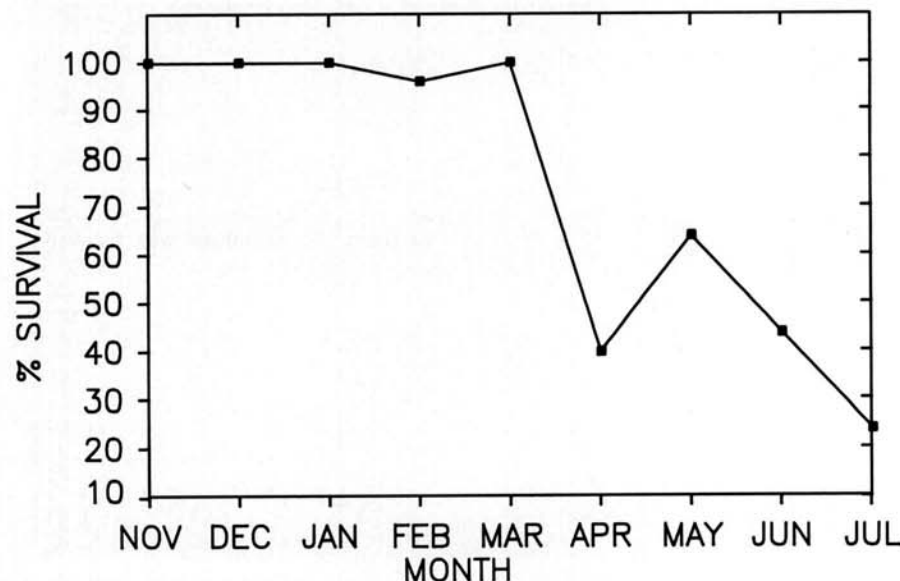


Fig. 2. Survival of *Colletotrichum acutatum* in strawberry tissue buried in a fallow strawberry nursery plot. Survival was measured as the percentage of recovered pieces that yielded *C. acutatum*.