Oversummer Survival of Inoculum for *Colletotrichum* Crown Rot in Buried Strawberry Crown Tissue

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ABSTRACT

Ureña-Padilla, A. R., Mitchell, D. J., and Legard, D. E. 2001. Oversummer survival of inoculum for *Colletotrichum* crown rot in buried strawberry crown tissue. *Plant Dis.* 85:750-754.

The oversummer survival of *Colletotrichum gloeosporioides* in strawberry crown tissue under field conditions was investigated in 1998 and 1999. Strawberry crowns infected naturally with *C. gloeosporioides* were placed inside cloth bags containing field soil, buried in the field at 5 or 13 cm, then recovered over 6 months of each year. The recovered crowns were plated onto a *Colletotrichum* spp. semiselective medium and speciated by colony, spore morphology, and molecular markers with species-specific DNA primers. Pathogenicity of selected isolates was confirmed by greenhouse bioassays on strawberry. Of the 428 isolates of *Colletotrichum* spp. recovered from buried crowns, 96% were *C. gloeosporioides* and 4% *Colletotrichum acutatum*. Following an initial increase in the detection of the fungus, survival of *C. gloeosporioides* was stable for 2 to 3 weeks, then declined. No *Colletotrichum* spp. were detected after burial for 56 days in 1998 and 98 days in 1999. Because the time between crop seasons is typically more than 170 days, these data support the hypothesis that inoculum of *C. gloeosporioides* does not survive in buried plant debris between seasons in Florida and, therefore, oversummering crop debris does not contribute inoculum for epidemics of *Colletotrichum* crown rot in Florida.

Additional keywords: anthracnose, *Fragaria* × *ananassa*

Strawberries are grown as an annual winter crop in west-central Florida. Green-top transplants obtained from nurseries in the northeastern United States or south-eastern Canada are set in late September through early November on methyl bromide-fumigated, plastic-mulched beds. Fruit are harvested in late November through early April (1). At the end of the harvest season, growers destroy plants by applying herbicide or mowing the tops off the plants. Some growers plant spring vegetable crops into the existing beds after killing the strawberry plants. From April to late May, a grain or legume cover crop typically is planted. During August or early September, the cover crop is incorporated and raised beds are formed and fumigated with a mixture of methyl bromide and chloropicrin. In Florida, only the beds are fumigated and strawberries are grown in the same location every season without crop rotation.

Colletotrichum spp. cause a wide range of strawberry diseases. Diseases of fruit, crown, and roots can result in serious yield losses (4,8,10,11). Many of these diseases have been called anthracnose because of the production of typical symptoms, which include limited necrotic lesions and hypoplasia of tissues and fruit (13,18). Plants with symptoms of *Colletotrichum* crown rot exhibit a sudden wilt of the entire plant. Within the crown, reddish brown necrotic streaks develop in the vascular tissue (20), and eventually the entire crown becomes discolored and the plant dies (11). In Florida, crown rot typically kills 2 to 4% of plants each season (D. E. Legard, unpublished data), although losses of up to 80% have occurred when conditions are conducive for serious outbreaks (14). *Colletotrichum acutatum*, *Colletotrichum fragariae*, and *Colletotrichum gloeosporioides* (teleomorph *Glomerella cingulata*) are the three major *Colletotrichum* species reported to cause crown rot in strawberry (2,3,9,11,14,19). Recent *Colletotrichum* spp. crown rot epidemics in Florida have been caused predominately by *C. gloeosporioides* (15, 25).

The source of primary inoculum for epidemics of *Colletotrichum* spp. crown rot of strawberry in Florida is unclear. Transplants are the most likely source of primary inoculum (8,14), and transplants infected or infested with *Colletotrichum* spp. have

been found in California (7), Louisiana (16), and Israel (8,9). Epidemics also originate from other sources of inoculum (7,12,27). In California, *Colletotrichum acutatum* overwintered in strawberry plant debris for up to 9 months in fields that were not fumigated with methyl bromide (7), and in Ohio, it was overwintered in mummified fruit buried in soil between seasons (27). Horn and Carver (12) suggested that inoculum might survive in crown tissue because *C. fragariae* could be isolated from crowns stored at 5°C over 30 weeks. It has been suggested that *Colletotrichum* spp. associated with strawberry are not likely to survive between seasons in soil or buried plant debris in Florida as a result of the hot and wet summer conditions (14). This hypothesis, however, has not been tested experimentally. Because crown debris is the tissue most likely to survive intact between crop seasons, we evaluated the ability of *C. gloeosporioides* to oversummer in crown tissue under field conditions in Florida.

MATERIALS AND METHODS

The oversummer survival of *C. gloeosporioides* was evaluated under field conditions during 1998 and 1999 at the University of Florida Gulf Coast Research and Education Center (GCREC) at Dover. Canadian-grown green-top transplants of cultivar Camarosa were used both years. Plants were set into raised plastic-mulched beds in October of each year and grown and harvested through April. Plants used in 1998 were set in a nonfumigated soil. For the 1999 experiment, plants were set in a field that had been treated with methyl bromide and chloropicrin (98:2 at 350 kg/ha) prior to planting. No foliar fungicides were applied in either season. Other pesticides were applied as needed to control arthropod pests. Severe *Colletotrichum* spp. crown rot epidemics developed in the plots by the end of each fruiting season. In June of both years, 8 months after planting the field, plants without visible symptoms of *Colletotrichum* spp. crown rot were selected from an area with severe crown rot (20 to 35% of plants with crown rot symptoms). These plants were selected because already-wilted plants would have begun to decay before the start of the experiment. At 1 or 2 weeks before collecting plants for the burial studies, 10 to 20

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Florida Agricultural Experiment Station Journal
Series R-07654.

Accepted for publication 24 April 2001.

Publication no. D-2001-0521-01R
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asymptomatic plants were assayed by plating dissected crown tissues onto a *Colletotrichum* spp. isolation medium (CIM) modified from Steiner and Watson (24). The medium contained 16 g of potato dextrose broth (Difco, Detroit), 14 g of agar (Difco), 250 mg of ampicillin, 150 mg of streptomycin sulfate, 5 mg of iprodione (8), 100 µl of Tergitol (Sigma, St. Louis), and deionized water to 1 liter. *Colletotrichum* spp. were detected in most of the crowns of asymptomatic plants.

The plot used for the 1998 burial study had been cropped with blueberry during the previous 4 years, but the stand had been removed and the area converted to fallow without a cover crop 12 months prior to this study. The area was rotovated 24 h prior to crown burial. On 4 June, 80 plants were dug from two adjacent beds (harvest field) and trimmed to clean the primary crown of leaves, roots, and secondary crowns. Two primary crowns were placed inside a porous cloth (Miracloth; Calbiochem, La Jolla, CA) bag with 50 cc of nonfumigated soil and buried at a depth of 13 cm in a different location (burial field). Burial locations for the bags were organized in a randomized, complete block design with four replicates, with sampling time as treatment. At each sampling time (0, 7, 14, 21, 42, 56, 70, 98, 119, and 147 days after burial), one bag was recovered from each of the four replicated plots (eight crowns total). To assess the ability of *C. gloeosporioides* to survive in the buried crowns, crown tissue was plated onto CIM. Transverse segments, approximately 1 mm thick, were cut from the upper and lower portions of the crown and from two segments within the middle portion of the crown with a surface-sterilized scalpel. The segments were surface disinfected for 3 min in a solution of 0.2% sodium hypochlorite, then rinsed by soaking for 3 min in sterile deionized water. The segments were air dried on sterile paper towels, cut in half, and placed on CIM medium. All cultures were incubated for 3 to 5 days at 24°C, with continuous fluorescent light (750 lumens and 4,100 K; Royal Phillips Electronics, Amsterdam).

For the 1999 study, the burial field had been cropped in strawberry the previous season with conventional practices, including fumigation with methyl bromide. The area was rotovated 24 h prior to burial of the crowns. On 11 July, 260 strawberry plants were collected from a field that had been treated with methyl bromide and chloropicrin (98:2 at 350 kg/ha) prior to planting (harvest field) and trimmed as described for the 1998 experiment. Individual crowns were placed inside Miracloth bags with 50 cc of soil from the burial site. One bag was buried at each of two depths, 5 and 13 cm, in the same location, comprising a single sample pair for each time period. Treatments were replicated 10 times and arranged in a randomized com-

plete block design. Pairs of crowns were recovered from 10 burial locations at each sampling time (0, 7, 14, 21, 28, 35, 42, 49, 56, 70, 98, 119, and 140 days after burial). After recovery, crowns were processed as in the 1998 study. In addition, crown tissue remaining after plating sections on CIM medium was cut aseptically into 3 to 6 mm pieces and finely ground with an electric coffee grinder (Braun, Woburn, MA) for 10 s. The instruments were surface sterilized with 95% ethanol and air dried between samples. The ground tissue was mixed with 50 ml of sterile deionized water and diluted tenfold during the first 6 weeks of the experiment and fivefold afterward. Then, 100 µl of the suspension was plated onto CIM medium. Isolation plates were incubated at 24°C for 3 to 5 days under continuous fluorescent light (750 lumens and 4,100 K).

Isolation cultures were evaluated for the presence of *Colletotrichum* spp. with a dissecting microscope and identified on the basis of colony growth rate, coloration, and shape. After 3 to 5 days of incubation, colonies of *Colletotrichum* spp. were fast growing, white or orange, with or without dark centers, and had smooth borders at the edge of the colonies. Clean cultures were grown on potato dextrose agar (PDA) and then speciated microscopically based on spore morphology. *C. gloeosporioides* isolates had cylindrical-shaped spores, and *C. acutatum* isolates had fusiform-shaped spores (21). Monoconidial cultures of representative isolates were characterized further by polymerase chain reaction (PCR) amplification of the ribosomal DNA internal transcribed spacer region (22,23) to confirm speciation. Conidial suspensions produced from cultures grown on PDA were used to inoculate 250-ml flasks containing 100 ml of sterile Emerson medium (4 g of Bacto yeast extract, 15 g of Bacto soluble starch, 1 g of dibasic potassium phosphate, 0.5 g of magnesium sulfate, and 1 liter of distilled water). Mycelia were grown for 4 or 5 days on a shaker at 150 rpm and then rinsed with sterile distilled water and freeze dried with a centrifugal evaporator (Jouan, Winchester, VA). DNA extraction was performed with 0.05 g of dried mycelium and a Fast DNA spin kit (Bio 101, Vista, CA). PCR amplifications were carried out in 20-µl mixtures containing 2 µl of DNA, 2 µl of 10× reaction buffer (500 mM Tris at pH 8.3, 2.5 mg of bovine serum albumin/ml, 20 mM MgCl₂, 5% Ficoll 400, 10 mM Tartrazine [Idaho Technology, Salt Lake City, UT], and 1779 Ficoll/Dye), 1.2 µl of 1.5 mM MgCl₂, 2.0 µl of 2.0 mM deoxynucleoside triphosphate (Pharmacia P-L Biochemicals, Milwaukee, WI), 0.2 µl of *Taq* polymerase (5 U/µl), and 10.6 µl of sterile distilled water. One microliter each of the universal internal primer from the ITS4 region (5'-TCCTCCGCTTATTG-ATATGC-3') and the species-specific primer CaInt2 (5'-

GGGGAAGCCTCTCG-CGG-3'; 10 µM) for *C. acutatum* or the specific primer Cg/f Int1 (5'-GACCCTCCCGGCCTCCCCC-3'; 10 µM) for *C. gloeosporioides* were used for identification (22,23).

The pathogenicity of *Colletotrichum* spp. from buried crowns was evaluated in greenhouse experiments. Twenty isolates (5% of the isolates recovered) were selected randomly among 403 isolates collected during the 1999 study. Seventeen of the isolates were *C. gloeosporioides* and three were *C. acutatum*. Monoconidial isolates were grown on PDA for 5 days at 24°C, with continuous fluorescent light. For each treatment, conidia were harvested and diluted in sterile distilled water to 1 × 10⁶ conidia/ml. Three strawberry plants of "Camarosa" with crowns approximately 0.5 cm in diameter were inoculated with a conidial suspension of a given *Colletotrichum* species with the use of a 1-cc insulin syringe to wound and inject 0.1 ml of the suspension into leaf axils in the crown area. After inoculation, plants were incubated at 24 to 30°C for 48 h in a humidity chamber and then maintained in a greenhouse at the same temperature for 4 weeks. One strawberry isolate of *C. acutatum* from anthracnose fruit rot and one *C. gloeosporioides* isolate from a diseased crown (*Colletotrichum* crown rot) were also included in the test, and sterile distilled water was used as a control. Plants were evaluated weekly for symptoms of disease, which included wilting and plant collapse. The experiment was repeated twice. An isolate was considered to be pathogenic if two of the three inoculated plants wilted and collapsed within 30 days of inoculation. Crown segments from diseased plants were surface sterilized, sectioned, and plated onto CIM for reisolation and confirmation of the pathogen. Weather parameters during field and greenhouse experiments were recorded with dataloggers.

RESULTS

At the time of burial in 1998 (July 4), *C. gloeosporioides* was isolated from surface-sterilized crown segments taken from 87% of the crowns (Fig. 1A). Over the next 3 weeks of burial, the pathogen was isolated from 100% of the crowns. Recovery of *C. gloeosporioides* began decreasing 42 days after burial when *C. gloeosporioides* was recovered from 75% of the crowns. After 56 days of burial, the pathogen was not recovered from any of the samples. All isolates of *C. gloeosporioides* and *C. acutatum* were speciated by spore morphology, and these identifications were confirmed for representative isolates by PCR amplification of a 450- or 490-bp DNA fragment from the ribosomal DNA internal transcribed spacer region.

In 1999, after segments were surface sterilized and plated, *C. gloeosporioides* was recovered from 70% of the crowns at

the time of burial (July 11) at 5 and 13 cm (Fig. 1A). Recovery of *C. gloeosporioides* increased to 100% at 14 days after burial at 13 cm and 21 days after burial at 5 cm. Forty-two days after burial, the pathogen was recovered from 60% of the crowns at each depth. Seventy days after burial, only 10% of the crowns from both depths yielded *C. gloeosporioides*, which was not recovered on crowns at either depth after 98 days or more.

Recovery of *C. gloeosporioides* from ground crown tissue on dilution plates

(Fig. 1B) was similar to recovery from crown segments. *C. gloeosporioides* was recovered from 70 and 80% of the crowns placed at 5 or 13 cm deep, respectively, at the time of burial. The recovery of *C. gloeosporioides* increased to 100% at both depths after 21 days, declined to 20 to 40% after 49 days, and further declined to 10 to 20% after 70 days. The fungus was not recovered from crowns buried for 98 days or more.

When the data sets from both plating methods were combined, the recovery of

C. gloeosporioides from buried crowns was improved in comparison to either method alone (Fig. 1C). This increased recovery of *C. gloeosporioides* was the result of detecting the fungus from some of the crowns with only one of the plating methods. Specifically, in samples taken 49, 56, and 70 days after burial, *C. gloeosporioides* recovery at 13 cm improved from 10% or 20 to 30% recovery of the pathogen when data from both methods were combined.

C. acutatum also was recovered from buried crowns but only in 1999. *C. gloeosporioides* was the predominant *Colletotrichum* species recovered throughout each year of the experiment. The recovery of *C. acutatum* was similar at both burial depths (A. R. Ureña, unpublished data) and was recovered from 60% of buried crowns after 7 and 14 days of burial. The recovery of *C. acutatum* from buried crowns declined to 30% after 28 days and was not recovered from buried crowns after 56 days of burial.

Weekly average precipitation was similar during the experiment in 1998 and 1999 (Fig. 2A). The average weekly precipitation during the burial period was 5.9 mm in 1998 and 5.2 mm in 1999 (5.4 mm is the 30-year average). During the first 50 days of the burial period, precipitation was greater in 1999 than in 1998 (7.8 versus 4.5 mm weekly average precipitation). From August through September (approximately 63 to 119 days after burial), however, average weekly precipitation was higher in 1998 (10.6 mm) than in 1999 (3.9 mm).

Mean weekly air temperatures from June to October were higher in 1998 than in 1999 (27.2 versus 26.4°C) and higher than the 30-year average (25.5°C). Higher temperatures were especially prevalent during the first 35 days of burial in 1998 compared with 1999 (Fig. 2B). Average weekly soil temperatures were 2 or 3°C higher than air temperatures during 1998 and 1999 (Fig. 2B and C). Average weekly soil temperatures were higher during the first 50 days of the burial period in 1998 than in 1999 (Fig. 2C). Soil temperatures were not recorded between 25 July and 16 August, 1998, because of equipment malfunction. Daily soil moisture content (water potential) was recorded in 1999 (Fig. 2D). The lowest water potential (−0.022 Mpa) occurred 42 days after burial and corresponded with the maximum average soil temperature (32.2°C), the highest air temperature (29°C), and reduced precipitation (2 mm) in 1999 (Fig. 2).

Fourteen of seventeen *C. gloeosporioides* isolates obtained from buried crowns and tested under greenhouse conditions were pathogenic and produced typical crown rot symptoms on strawberry (i.e., plant wilting and death). Three isolates of *C. gloeosporioides* and all three crown isolates of *C. acutatum* evaluated were nonpathogenic. Crowns of plants inoculated with pathogenic isolates exhibited

Fig. 1. Frequency of recovery of *Colletotrichum gloeosporioides* from buried strawberry crowns. **A**, Recovery from surface-sterilized crown segments buried at 13 cm in 1998 and 5 and 13 cm in 1999. **B**, Recovery by dilution plating of ground crown tissue buried at 5 and 13 cm in 1999. **C**, Percentage of crowns from which *C. gloeosporioides* was detected from either surface-sterilized segments or dilution plating of ground tissue.

abundant necrosis and reddish brown streaks. Plants inoculated with isolates of *C. gloeosporioides* obtained from diseased crowns developed typical crown rot symptoms, whereas inoculation with *C. acutatum* isolated from diseased fruit did not. No symptoms of *Colletotrichum* crown rot developed in the sterile water-inoculated controls.

DISCUSSION

C. gloeosporioides did not oversummer in buried strawberry crowns long enough to be a major source of primary inoculum for *Colletotrichum* crown rot epidemics in fruit production fields in Florida. Although *C. gloeosporioides* was isolated frequently from strawberry crowns early in the burial period, it declined quickly to undetectable levels within 56 to 98 days of burial. Because the time period between the incorporation of strawberry debris into the soil at the end of one season and transplanting for the subsequent season usually is 120 to 180 days, there is sufficient time for strawberry crowns to decay and *C. gloeosporioides* inoculum to be eliminated.

Following an initial increase in the detection of *C. gloeosporioides* during the first 3 to 4 weeks of burial, recovery of the pathogen decreased. *C. gloeosporioides* was not recovered from buried crowns 56 days after burial in 1998 or 98 days after burial in 1999. These results suggest that *C. gloeosporioides* does not survive in buried strawberry crowns in Florida long enough to be an important source of primary inoculum for crown rot epidemics, even though strawberries are replanted in the same field every year. Several production practices in Florida limit the opportunities for the inoculum of *Colletotrichum* spp. to oversummer in plant debris or soil. Within 1 month after the end of the season (late March or early April), plants typically are killed. Growers may remove the plastic mulch and incorporate plant material into the soil at this time or use the herbicide paraquat to kill the plants and then use the existing beds for a spring vegetable crop. Under these circumstances, strawberry debris would not be incorporated until May, after the vegetable crop is harvested. These experiments simulated a worst-case situation, where strawberry plants would be grown until incorporated at the end of a late vegetable crop in early June. In most commercial situations, the strawberry plants would have been killed 1 to 2 months earlier. After a summer cover crop of grain or legumes is grown and eventually incorporated in August, the fields are fumigated with a mixture of methyl bromide-chloropicrin and beds for the next season are formed in September and October. Fumigation with methyl bromide also eliminates *Colletotrichum* spp. from soil (7).

Previous studies have shown longer survival times for *Colletotrichum* spp. in

strawberry debris. In Louisiana, inoculum of *C. fragariae* was able to survive up to 7 months in crowns of whole strawberry plants kept at 5°C (12). *C. acutatum* survived from November to July in buried runner segments placed 5 to 7 cm deep in northern California (7). Environmental conditions during the summer in west-central Florida, however, are dramatically different from winter in Louisiana or northern California. It is likely that the lower temperatures during the studies in Louisiana and California would extend the

survival of *Colletotrichum* spp. in plant debris. It has been hypothesized that survival of *C. acutatum* may improve at lower temperatures (7). This may be the result of reduced colonization of plant debris by the pathogen and increased colonization by other soilborne microorganisms that would compete for nutrients in the crown tissue at high temperatures (5,7). Hot and wet summers in Florida would facilitate the rapid colonization and breakdown of strawberry plant debris. In our experiments, the pith of buried crowns decom-

Fig. 2. A, Weekly precipitation, B, weekly average air temperature, C, weekly soil temperature, and D, average weekly soil water potential during summer and fall of 1998 and 1999. No soil temperature data was recorded 23 July to 6 August, 1998. Average weekly soil water potential (D) recorded at 5 and 13 cm during the summer and fall of 1999. All observations were made at the University of Florida, Gulf Coast Research and Education Center, Dover.

posed completely by late October and November, leaving a hollow crown with only the woody cortex remaining. Because the pathogen was no longer recovered from crowns at the end of the experiment, this observation supports the suggestion that most of the pith tissue had been colonized and decomposed by the end of the experiments, leaving little substrate for survival of the pathogen.

Soil moisture content may have affected the survival of *C. gloeosporioides* in buried strawberry crowns by affecting fungus activity or by indirectly disrupting the activity of its competitors. The effects of soil moisture content on survival of *Colletotrichum* spp. are documented (5,17). When inoculated, strawberry petioles were buried in an air-dried or flooded soil for 7 weeks at 25°C, and *C. acutatum* was detected from 60 and 0% of the petioles, respectively (5). In Florida, the survival of *C. acutatum* in infected plant debris of leatherleaf fern or in soil increased with the reduction in soil moisture (17). Although the effect of variations in moisture and temperature on the survival of *C. gloeosporioides* in strawberry crowns is unclear, it is likely that it would behave similarly to *C. acutatum* in plant debris or soil.

The lower precipitation recorded from August to September 1999 compared with the same period in 1998 reduced the moisture content of the soil. This situation may have affected the ability of *Colletotrichum* spp. to survive by influencing the activity of other organisms. The competitive saprophytic ability of *Trichoderma harzianum* in buried plant tissues is reduced when the soil is flooded or when there is a drastic reduction in the moisture content of the soil (6). Tissue plated onto CIM in July 1998 produced large numbers of colonies of *Fusarium* and *Trichoderma* spp., with the latter abundantly growing out of segments that were plated at the end of July (A. R. Ureña, unpublished data). In 1999, the increased recovery of these species from crown segments was not observed until late August. In addition to environmental differences between the 1998 and 1999 experiments, the crowns in 1999 were grown and buried in soil that had been fumigated with methyl bromide, whereas in 1998, the sites were not fumigated. Soil fumigation affects the activity of microbes in treated soil (26,28). Thus, the extended survival of *C. gloeosporioides* in 1999 may have been caused by differences in microbial activity resulting from environmental conditions or soil fumigation.

Most isolates of *C. gloeosporioides* caused *Colletotrichum* crown rot symptoms in the greenhouse bioassay, but none of the *C. acutatum* isolates caused crown rot. These results support the observation that *Colletotrichum* crown rot epidemics in

Florida are caused by *C. gloeosporioides* (15,25). It is unclear why some of the *C. gloeosporioides* isolates recovered from buried crowns did not cause crown rot in the bioassay. A possible explanation is that not all of the *C. gloeosporioides* isolates recovered from strawberry tissue are pathogenic on strawberry. The plants used for the burial studies did not have symptoms of *Colletotrichum* crown rot at the time of collection, and the detached crowns could have been colonized by nonpathogenic isolates of *C. gloeosporioides*. Crown rot bioassays of isolates of *C. gloeosporioides* recovered from strawberry petioles also identified isolates that did not cause crown rot (D. E. Legard, unpublished data).

These results show that propagules of *C. gloeosporioides* in buried strawberry crowns are not likely to be an important source of inoculum for *Colletotrichum* crown rot epidemics in Florida. Other sources of inoculum such as infected or infested transplants or alternate hosts may be more important in the epidemiology of *Colletotrichum* crown rot in Florida. As a result of the potential for some survival of inoculum in well-preserved strawberry debris, however, it is prudent that growers use cultural practices that promote the breakdown of crown tissue in the soil. More research is necessary to further elucidate sources of primary inoculum for *Colletotrichum* crown rot epidemics in Florida.

ACKNOWLEDGMENTS

We thank U. Benny, C. L. Xiao, L. Smith, and the field crew and OPS personnel of the GCREC-Dover for their valuable assistance.

LITERATURE CITED

- Aerts, M. J., and Nesheim, O. N. 1997. Strawberry integrated pest management and pesticide use survey for 1995. Pesticide Information Office, IFAS, University of Florida, Gainesville.
- Bosshard, E. 1997. Why is *Colletotrichum acutatum* a quarantine organism, and *C. gloeosporioides* and *C. fragariae* are not? *Acta Hortic.* 439:799-802.
- Brooks, A. N. 1932. A study of strawberry wilt or crown rot. Pages 144-145 in: Florida Agricultural Experimental Station Annual Report, Gainesville.
- Denoyes, B., and Baudry, A. 1995. Species identification and pathogenicity study of French *Colletotrichum* strains isolated from strawberry using morphological and cultural characteristics. *Phytopathology* 85:53-57.
- Eastburn, D. M. 1992. Effects of soil moisture and temperature on the survival of *Colletotrichum acutatum*. *Plant Dis.* 76:841-842.
- Eastburn, D. M., and Butler, E. E. 1991. Effects of soil moisture and temperature on the saprophytic ability of *Trichoderma harzianum*. *Mycologia* 83:257-263.
- Eastburn, D. M., and Gubler, W. D. 1990. Strawberry anthracnose: Detection and survival of *Colletotrichum acutatum* in soil. *Plant Dis.* 74:161-163.
- Freeman, S., and Katan, T. 1997. Identification of *Colletotrichum* species responsible for anthracnose and root necrosis of strawberry in Israel. *Phytopathology* 87:516-521.
- Freeman, S., Nizami, Y., Dotan, S., Even, S., and Sando, T. 1997. Control of *Colletotrichum acutatum* in strawberry under laboratory, greenhouse, and field conditions. *Plant Dis.* 81:749-752.
- Henz, G. P., Boiteux, L. S., and Lopes, C. A. 1992. Outbreak of strawberry anthracnose caused by *Colletotrichum acutatum* in central Brazil. *Plant Dis.* 76:212.
- Horn, N. L., and Carver, R. G. 1963. A new crown rot of strawberry plants caused by *Colletotrichum fragariae*. *Phytopathology* 53:768-770.
- Horn, N. L., and Carver, R. B. 1968. Overwintering of *Colletotrichum fragariae* in strawberry crowns. *Phytopathology* 58:540-541.
- Howard, C. M., and Albregts, E. E. 1984. Anthracnose of strawberry fruit caused by *Glomerella cingulata* in Florida. *Plant Dis.* 68:824-825.
- Howard, C. M., Maas, J. L., Chandler, C. K., and Albregts, E. E. 1992. Anthracnose of strawberry caused by the *Colletotrichum* complex in Florida. *Plant Dis.* 76:976-981.
- Legard, D. E., Whidden, A. J., and Chandler, C. K. 1998. Incidence and occurrence of strawberry diseases in Florida from 1991-1996. *Adv. Strawberry Res.* 16:35-47.
- McInnes, T. B., Black, L. L., and Gatti, J. M., Jr. 1992. Disease-free plants for management of strawberry anthracnose crown rot. *Plant Dis.* 76:260-264.
- Norman, D. J., and Strandberg, J. O. 1997. Survival of *Colletotrichum acutatum* in soil and plant debris of leatherleaf fern. *Plant Dis.* 81:1177-1180.
- Smith, B. J., and Black, L. L. 1987. Resistance of strawberry plants to *Colletotrichum fragariae* affected by environmental conditions. *Plant Dis.* 71:834-837.
- Smith, B. J., and Black, L. L. 1986. First report of *Colletotrichum acutatum* on strawberry in the United States. *Plant Dis.* 70:1074.
- Smith, B. J. 1998. Anthracnose crown rot. Pages 46-48 in: *Compendium of Strawberry Diseases*, 2nd ed. J. L. Mass, ed. American Phytopathological Society, St. Paul, MN.
- Smith, B. J., and Black, L. L. 1990. Morphological, cultural, and pathogenic variation among *Colletotrichum* species isolated from strawberry. *Plant Dis.* 74:69-76.
- Sreenivasaprasad, S., Mills, P. R., Meehan, B. M., and Brown, A. E. 1996. Phylogeny and systematics of 18 *Colletotrichum* species based on ribosomal DNA spacer sequences. *Genome* 39:499-512.
- Sreenivasaprasad, S., Sharada, K., Brown, A. E., and Mills, P. R. 1996. PCR-based detection of *Colletotrichum acutatum* on strawberry. *Plant Pathol.* 45:650-655.
- Steiner, G. W., and Watson, R. D. 1965. Use of surfactants in the soil dilution and plate count method. *Phytopathology* 55:728-730.
- Ureña-Padilla, A., Kistler, H. C., and Legard, D. E. 2000. Etiology and population genetics of *Colletotrichum* spp. responsible for diseases of strawberry. (Abstr.) *Phytopathology* 90:S79.
- Warcup, J. H. 1976. Studies on soil fumigation IV: Effects on fungi. *Soil Biol. Biochem.* 8:261-266.
- Wilson, L. L., Madden, L. V., and Ellis, M. A. 1992. Overwinter survival of *Colletotrichum acutatum* in infected strawberry fruit in Ohio. *Plant Dis.* 76:948-950.
- Yeates, G. W., Bamforth, S. S., Ross, D. J., Tate, K. R., and Sparling, G. P. 1991. Recolonization of methyl bromide sterilized soils under four different field conditions. *Biol. Fertil. Soils* 11:181-189.