Disease-Free Plants for Management of Strawberry Anthracnose Crown Rot

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ABSTRACT

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Anthracnose crown rot (ACR), caused by *Colletotrichum fragariae*, limited both plant and fruit production in strawberry summer nursery beds and production fields in Louisiana from 1986 to 1989 on most farms that used locally grown plants. Pathogen-free plants derived from tissue culture and increased in northeast Louisiana were provided to growers in southeast Louisiana for establishment of summer nursery beds. The pathogen-free strawberry plants remained free of ACR in summer nursery beds planted apart from other beds established with locally grown transplants. Once the pathogen was introduced into summer nursery beds (June–October) disease spread of ACR was rapid, but there was no evidence of disease spread in production fields (November–May). The only plants that died from ACR in production fields were those that were already infected when they were transplanted to the field.

Anthracnose crown rot (ACR) of strawberry (*Fragaria* × *ananassa* Duchesne), caused by *Colletotrichum fragariae* A. N. Brooks, can be devastating to strawberries grown in the southeastern United States (17). The geographic distribution of *C. fragariae* includes Louisiana (5), Arkansas (28), Mississippi (24), North Carolina (7,18), Oklahoma (17), and Tennessee (17), within the United States; Argentina (20); Brazil (6); Mexico (19); and India (22). The disease has been particularly damaging in Florida (1,2,3) and Louisiana (5,9,10,11), where it severely limits plant production in the summer nursery beds and causes a plant wilt in production fields in the spring. *C. fragariae* causes petiole lesions (3), crown infections (2,3,10,11), fruit rot (14), and leaf spot (16) of strawberry. In summer nurseries, the pathogen spreads rapidly, girdling stolons and killing runner plants. Symptomless, crown-infected plants transplanted into fruit production fields in the fall are likely to wilt and die during fruiting season the following spring (10,11,17).

The occurrence of ACR in Louisiana was first noted by Carver and Horn in 1960 (5,10,11). They were particularly concerned with the crown rot phase of the disease, which was causing the deaths of plants in summer nurseries and spring production fields. Subsequently, work by Howard (13) and Horn et al (9) led to satisfactory control of the disease through sanitation and the use of the fungicide benomyl (4). Benomyl was effective for several years, but in the past 10–12 yr farmers observed that the benomyl sprays were not controlling the disease. In vitro tests (T. B. McInnes and L. L. Black, *unpublished*) have confirmed observations by Smith (23) that benomyl-resistant strains of *C. fragariae* now exist. Attempts to control ACR through disease resistance have met with limited success because of the difficulties involved in breeding disease resistance into horticulturally acceptable cultivars and because the pathogen is composed of numerous physiological races (8,9,24). Currently, there are no ACR-resistant cultivars that are commercially acceptable to Louisiana producers nor are there any fungicides registered for use on strawberry that are effective against ACR.

C. fragariae survives from one crop to the next in strawberry crowns but not in soil (9,12). Little is known about the host range and the distribution of the fungus in nature apart from cultivated strawberries. A few wild species, *Cassia obtusifolia* L. (15), *Fragaria virginiana* Duchesne (8), *Potentilla canadensis* L. (8), and *Duchesnea indica* (Andr.) Focke (1), have been reported to be hosts of *C. fragariae*. In inoculations in the laboratory, *C. obtusifolia, F. virginiana,* and *P. canadensis* were infected by *C. fragariae*, but the investigators did not consider them likely to play a major role as sources of inoculum in nature (8).

Summer nursery beds located on strawberry production farms have been the traditional source of transplants for production fields in Louisiana (21). The nurseries are established in June by transplanting strawberry plants 0.9 m apart on raised beds 1.8 m wide that have been fumigated with methyl bromide. The source of plant material for the nurseries varies but is frequently runner plants taken from the grower's current year production fields that have been recently abandoned. Strawberry production fields in Louisiana are established annually in October or early November and generally consist of single-row plantings on rows 1.0–1.2 m wide that are shaped into raised beds, fumigated with methyl bromide, and covered with black polyethylene film mulch (21). Traditionally, freshly dug transplants from the grower's nursery or another nursery in the area are used to establish the production fields. The close association of ACR with contaminated plant stock (5,15) and previous accounts of avoiding the disease by use of noninfected plants (9) suggest that the pathogen is not widespread in nature in Louisiana. These observations suggest that ACR could be controlled by beginning with pathogen-free plants and careful cultural management to avoid planting near *C. fragariae*-infected strawberries. The present study was initiated to determine 1) whether plants regenerated by tissue culture could be increased in isolated fields in Louisiana and remain free of the ACR pathogen, 2) whether summer nurseries established with plants from the isolated fields would remain ACR-free, and 3) whether commercial production fields established with this line of plants from summer nurseries would remain ACR-free. For comparison, summer nurseries and production fields established with local sources of plants and out-of-state nursery plants were monitored at the same time for the occurrence of ACR.

MATERIALS AND METHODS

Field increase of ACR-free plants derived from tissue culture. The strawberry cultivar Tangi was obtained in tissue culture from Gene Galletta, USDA, Beltsville, MD, during the spring of 1986. Plants were multiplied in tissue culture and provided to us as small rooted plants by Rhonda Porche-Sorbet of the Louisiana State University Horticulture Department. The plants were grown in Jiffy-Mix (JPA, West Chicago, IL) for 2 mo in the greenhouse before

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being transplanted to the field. The plants were transplanted during August, 1986, into a summer nursery bed that had been fumigated with Terr-O-Gas 67 (67% methyl bromide and 33% chloropicrin; Great Lakes Chemical Corporation, West Lafayette, IN) at the rate of 400 kg/ha. The nursery beds were located on the Louisiana State University Sweet Potato Research Station at Chase, LA, a location remote from the strawberry-growing region. The beds were monitored monthly for symptoms of ACR during the summer of 1986 and the spring of 1987. Runner plants were dug during June, 1987, potted in Jiffy-Mix Plus in 7.6-cm peat pots, and held under a shade screen for 1 wk before being transplanted into commercial summer nursery beds 150 m or more from beds established with local plant sources.

The same procedures were used during 1987-88 and 1988-89 to produce ACR-free transplants. In 1987-88, field increases were made at two locations: Chase, LA, on the Sweet Potato Research Station, and Baton Rouge, LA, on the Louisiana State University Burden Research Plantation. During 1988-89, the field increase of ACR-free plants was made only at Chase.

Summer nurseries. Strawberry summer nursery beds in Tangipahoa and Livingston parishes in southeastern Louisiana were surveyed for the occurrence and severity of ACR from June through October in 1986. ACR severity was determined in each nursery bed by visual estimates of runner plant losses using a disease index of 0–5; in which 0 = no runner plant reduction, 1 = 1–20%, 2 = 21–40%, 3 = 41–60%, 4 = 61–80%, and 5 = 80% or greater runner plant reduction. In each nursery bed, 10 3.5-m cross sections of rows selected at random were rated for runner plant reduction. Isolations to identify the causal agent from representative plants exhibiting ACR symptoms were made on Difco potato-dextrose agar (PDA) (Difco Laboratories, Detroit, MI), using internal crown tissue surface-sterilized with .525% sodium hypochlorite.

In 1987, strawberry summer nursery beds in Tangipahoa parish established with locally grown transplants and with ACR-free plants from Chase were surveyed from June through October for the occurrence of ACR. Summer nursery beds were rated for ACR severity by the same method used in 1986.

Strawberry summer nursery beds in Tangipahoa parish established with locally grown transplants, ACR-free plants from Chase, and greenhouse-grown ACR-free plants from Baton Rouge were surveyed from June through October of 1988 for the occurrence of ACR. A more quantitative evaluation of ACR severity was utilized in 1988. Disease severity evaluations were based on observations of 500 randomly selected stolons from each nursery bed. ACR severity was expressed as the percentage of stolons exhibiting ACR lesions.

Production fields. Commercial strawberry production fields in Tangipahoa and Livingston parishes were surveyed for the occurrence of ACR during the spring of 1986, 1987, 1988, and 1989. In 1986, all surveyed fields were established with locally grown transplants produced from local plant sources. In 1987, surveyed fields included those established with 1) locally grown transplants produced from local plant sources, 2) locally grown transplants produced from the ACR-free plant source at Chase, and 3) transplants from out-of-state nurseries. In 1988 and 1989, surveyed fields included those established as indicated for 1987, plus locally grown transplants produced from a source of greenhouse-grown, ACR-free plants at Baton Rouge. The occurrence of ACR in the production fields was determined by observing 500 plants in each field and noting the number exhibiting wilt symptoms of ACR. One hundred consecutive plants in a row in each quadrant and another 100 plants near the center of each field were selected for the plant counts. Fields ranged in size from 0.2 to 1.2 ha. Isolations from representative plants exhibiting ACR symptoms were made on PDA to identify the causal agent. Standard errors of percent ACR wilted plants in production fields were calculated using a binomial distribution (27).

RESULTS

Field increase of ACR-free plants derived from tissue culture. Only a limited number of runner plants developed during the summer and fall from plants derived from tissue culture transplanted into the field during August, 1986. The plants were kept free of fruit by weekly removal of flowers during the spring of 1987, and runner plants were produced in abundance during May and June. Similar results were obtained during 1987-88 and 1988-89. In 1987-88, a similar field increase of runner plants was made in Baton Rouge. The nurseries were monitored monthly for the occurrence of ACR, but no evidence of the disease was found at any time in any of the plant increase fields.

Summer nurseries. Tangi was the only cultivar being grown in strawberry production fields and summer nursery beds in Tangipahoa and Livingston parishes in 1986. Many of the growers established summer nursery beds in June using plants from their production fields, in which 0.3–30% of the plants were ACR-infected (Table 1). Ten nursery beds in this same area were surveyed on 21 October and all contained ACR-infected plants (Table 2). Disease index ratings ranged from 1.0 to 5.0 among the nurseries surveyed. Plant losses were so great in some nursery beds that growers were forced to purchase plants from out-of-state nurseries in order to have sufficient plants to establish their production fields for 1987.

In 1987, several growers established summer nurseries with local sources of Tangi and Chandler plants. In addition, a few growers established small nursery beds with ACR-free plants from Chase. None of the plants in beds established with the Chase plants exhibited symptoms of ACR (Table 3). All but one (Beauchamp) of the nursery beds established with local plant sources exhibited symptoms when they were surveyed on 28 September 1987. As in 1986, several growers had to purchase out-of-state nursery plants to fill their needs to establish production fields for 1988. A number of growers did not plant summer nurseries and relied entirely on out-of-state nurseries as a source of plants.

In 1988, several growers in Tangipahoa parish established nursery beds using local sources of Tangi and Chandler plants. Five growers established small beds with ACR-free plants from Chase or Baton Rouge. Plants in only one of the five beds established with ACR-free plants exhibited ACR symptoms (Table 4). The single nursery bed (Hoover, L.) in which ACR developed was located adjacent to a bed established with a local source of Chandler plants. Seven of nine surveyed nursery beds that had been established with local plant sources exhibited ACR symptoms (Table 4). Disease severity varied among beds ranging from 10 to 95% of stolons with ACR lesions in the seven beds. The trend toward reliance on out-of-state nurseries for production plants by strawberry growers in Louisiana continued in 1988. Fewer growers attempted to grow plants in summer nurseries, and most of those that did purchased additional plants to satisfy their needs for the 1989 production fields.

Table 1. Incidence of plants wilted by anthracnose crown rot in spring 1986 strawberry production fields of southeastern Louisiana established with transplants from local nurseries

Grower	Cultivar-plant source	Wilted plants (%)[a]
Lavigne	Tangi-local	27.0 ± 2[b]
Hoover, V.	Tangi-local	2.0 ± 0.6
Meyers	Tangi-local	30.0 ± 2
Drude	Tangi-local	22.0 ± 1.9
Hoover, L.	Tangi-local	23.0 ± 1.9
Newman	Tangi-local	30.0 ± 2
Wilson	Tangi-local	8.0 ± 1.2
Cummings	Tangi-local	1.0 ± 0.4
Jenkins	Tangi-local	0.3 ± 0.2
Gregiore	Tangi-local	0.8 ± 0.4

[a] Based on five independent 100-plant counts in different areas of each field. Survey on 29 April 1986.
[b] Standard error.

Production fields. In 1986, the cultivar Tangi was being grown almost exclusively by commercial strawberry growers in Louisiana. Plants for the production fields came from local, on-the-farm, summer nurseries. A survey of production fields on 29 April 1986 showed that ACR occurred in all 10 fields observed with plant losses ranging from 0.3 to 30% (Table 1).

Production fields for 1987 were established with locally grown Tangi plants and Chandler plants grown out of state. ACR occurred in all seven of the surveyed fields established with local plants (Table 2). Disease incidence in these fields ranged from 2 to 60% on 5 May 1987. No plant death due to ACR occurred in the three surveyed production fields that had been established with out-of-state plants (Table 2).

Production fields for 1988 were established with locally grown Tangi and Chandler plants derived from local plant sources, with Tangi plants derived from the ACR-free source at Chase, and with Chandler plants from out-of-state nurseries. ACR occurred in all fields observed that had been established with local plants, ranging in incidence from 2 to 35% (Table 3). ACR did not occur in any of the production fields established with Tangi plants derived from the ACR-free source nor in any fields established with out-of-state nursery plants. A single production field (Meyers) established with an out-of-state source of plants suffered plant losses (Table 3), but in this case *C. acutatum* (and not *C. fragariae*) was isolated from dead and dying plants.

Production fields for 1989 were established with plants that had backgrounds similar to those used in the previous year. ACR occurred in four of the six fields established with local plants derived from local sources (Table 4). ACR incidence in those four fields ranged from 14 to 80% when surveyed on 28 April 1989. The two fields that remained free of ACR were located on the same farm (Beauchamp). As in the previous year, ACR did not occur in any of the production fields established with plants derived from the ACR-free plant source nor in any field established with out-of-state plants.

Table 2. Influence of plant source on occurrence of anthracnose crown rot in summer 1986 strawberry nursery beds and in spring 1987 commercial production fields in southeastern Louisiana

	Nursery beds (summer 1986)		Production fields (spring 1987)	
Grower	Cultivar-source	Disease index[a]	Cultivar-source	Wilted plants (%)[b]
Lavigne	Tangi-local	4	Tangi-local	14 ± 1.6[c]
			Chandler-NC	0
Meyers	Tangi-local	2	Tangi-local	60 ± 2.2
Hoover, L.	Tangi-local	1	Tangi-local	31 ± 2.1
			Chandler-NC	0
Jenkins	Tangi-local	5	Tangi-local	10 ± 1.3
			Chandler-Canada	0
Drude	Tangi-local	5		
Newman	Tangi-local	4	Tangi-local	2 ± 0.6
Cummings	Tangi-local	5	Tangi-local	3 ± 0.8
Battles	Tangi-local	5	Tangi-local	20 ± 1.8
Ardillo	Tangi-local	2		
Lard	Tangi-local	1		

[a] Disease index 0–5: a visual estimate of disease loss in which 0 = no reduction in runner plants and 5 = 80% or greater reduction in runner plants. Values based on estimates from 10 areas per bed chosen at random. Surveyed on 21 October at the time they were being dug to establish production fields.
[b] Based on five independent 100-plant counts in different areas of each field. Surveyed on 5 May 1987.
[c] Standard error.

Table 3. Influence of plant source on occurrence of anthracnose crown rot in summer 1987 strawberry nursery beds and in spring 1988 commercial production fields in southeastern Louisiana

	Nursery beds (summer 1987)		Production fields (spring 1988)	
Grower	Cultivar-source	Disease index[a]	Cultivar-source	Wilted plants (%)[b]
Lavigne	Tangi-local	4		
	Chandler-local	5		
			Chandler-MI	0
Hoover, L.	Tangi-local	1	Tangi-local	17 ± 1.7[c]
	Chandler-local	1	Chandler-local	35 ± 2.1
			Chandler-MI	0
Drude	Tangi-Chase	0	Tangi-Chase	0
			Chandler-MI	0
Corona	Tangi-local	2	Tangi-local	4 ± 0.9
	Tangi-Chase	0	Tangi-Chase	0
			Chandler-MI	0
Jenkins	Tangi-local	5		
			Chandler-MI	0
			Chandler-Canada	0
Vicaro	Tangi-local	1	Tangi-local	2 ± 0.6
Poche	Tangi-local	2	Tangi-local	3 ± 0.8
	Tangi-Chase	0	Tangi-Chase	0
Wells	Tangi-local	2	Tangi-local	20 ± 1.8
			Chandler-MI	0
Wilson	Tangi-local	1		
Ardillo	Tangi-local	4		
Beauchamp	Tangi-local	0		
Liuzza			Chandler-MI	0
Meyers			Tangi-local	16 ± 1.6
			Chandler-local	32 ± 2.1
			Chandler-Canada	0
			Chandler-IN	3[d] ± 0.8

[a] Disease index 0–5: a visual estimate of disease loss in which 0 = no reduction in runner plants and 5 = 80% or greater reduction in runner plants. Values based on estimates from 10 areas per bed chosen at random. Surveyed on 28 Sept. 1987 just before being dug to establish production fields.
[b] Based on five independent 100-plant counts in different areas of each field. Survey on 3 May 1988.
[c] Standard error.
[d] *Colletotrichum acutatum* isolated from these wilted plants.

DISCUSSION

ACR-free plants can be produced in Louisiana in nurseries that are remote from the strawberry production area. By starting with ACR-free Tangi plants derived from tissue culture, ACR-free transplants were produced during three consecutive years on the Sweet Potato Research Station at Chase and one year on the Burden Research Plantation at Baton Rouge. When plants from ACR-free nurseries were used to establish summer nurseries in the strawberry production area, they remained ACR-free in beds separated from contaminated nursery beds by 150 m or more. These results suggest that the primary if not the sole source of ACR inoculum in Louisiana is infected strawberry plants.

The current study has shown that when ACR-free plants are used to establish production fields in the annual planting

system practiced in Louisiana (21), they remain free of the crown rot phase of the disease through the spring fruiting season. Horn et al (9) and Howard (17) concluded that there is little or no movement of ACR in Louisiana and Florida production fields. Observations made in the present study corroborate their conclusion. For example, in commercial production fields where ACR-free plants were used, the crown rot symptom never occurred, even in situations where these fields were planted adjacent to fields with a high incidence of ACR.

Even though plants do not express the crown rot symptoms of ACR in production fields established with ACR-free plants, they are likely to become contaminated with *C. fragariae* if located adjacent to fields with infected plants. To illustrate this point, we observed that when runner plants were taken for nursery bed establishment from production fields showing no ACR symptoms, ACR generally occurred in the nursery beds (see Chandler-local in Tables 3 and 4). Older plants are relatively resistant to the crown rot phase of the disease when newly infected even though they wilt and die suddenly if old quiescent infections are reactivated by high temperatures (26).

During the period of this study, 1986–1989, there was a rapid shift in the Louisiana strawberry industry from the use of local transplants to commercially grown, out-of-state transplants. The shift was initiated due to a shortage of local plants caused by ACR in summer nurseries. The plant shortage forced growers to seek other sources of plants, and they turned to nurseries in North Carolina, Michigan, Indiana, California, and Ontario, Canada. Assays of the plants and surveys of fields established with plants from these sources showed them to be free of ACR during the 1986–1989 growing seasons. There remains some uncertainty, however, in placing total dependence on these commercially-produced plants as sources of ACR-free plants, since Florida growers in the past have experienced losses due to ACR in production fields that were established with plants from some of these areas (17). An additional concern is a similar crown rot caused by *C. acutatum*, which was observed in one field during this study and has been noted by others (25) in fields established with plants from out-of-state nurseries.

Table 4. Influence of plant source on occurrence of anthracnose crown rot in summer 1988 strawberry nursery beds and in spring 1989 commercial production fields in southeastern Louisiana

	Nursery beds (summer 1988)		Production fields (spring 1989)	
Grower	Cultivar-source	ACR severity[a]	Cultivar-source	Wilted plants (%)[b]
Vicaro	Tangi-local	80 ± 1.8[c]		
			Chandler-MI	0
Poche	Tangi-Chase	0	Tangi-Chase	0
	Chandler-local	30 ± 2	Chandler-local	49 ± 2.2[c]
Lavigne	Tangi-BR	0		
	Chandler-local	10 ± 1.3	Chandler-local	80 ± 1.8
			Chandler-MI	0
Hoover, L.	Tangi-Chase	10[d] ± 1.3		
	Chandler-local	15 ± 1.6	Chandler-local	74 ± 2
			Chandler-CA	0
			Chandler-NC	0
Drude	Tangi-local	35 ± 2.1		
	Tangi-Chase	0		
	Chandler-local	23 ± 1.9		
			Chandler-Canada	0
Beauchamp	Tangi-local	0	Tangi-local	0
	Tangi-Chase	0	Tangi-Chase	0
	Chandler-local	0	Chandler-local	0
Wells	Chandler-local	95 ± 1		
			Chandler-Canada	0
Meyers			Chandler-Canada	0
			Chandler-local	14 ± 1.6
Blahut			Chandler-NC	0
			Chandler-CA	0
Liuzza			Chandler-MI	0
			Chandler-Canada	0

[a] ACR severity was determined by randomly selecting a total of 500 stolons from each nursery bed and observing them for the occurrence of ACR lesions. ACR severity was expressed as the percentage of stolons exhibiting ACR lesions. Surveyed on 10 October 1988 at the time they were being dug to establish production fields.

[b] Based on five 100-plant counts in different areas of each field. Surveyed on 28 April 1989.

[c] Standard error.

[d] This plant bed was located adjacent to a bed established with ACR-infected plants. All other beds established with ACR-free plants from Chase were separated from beds established with local plants by 150 m or more.

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