Final Project Report to the NYSIPM Program, Agricultural 1PM 2003-2004

Title: Evaluation of Strawberry Nursery Stock for the Presence of Anthracnose and Angular Leaf Spot.

Project Leader:
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Type of grant:
Cultural methods; sanitation; physical controls.

Project location:
All of NY.

Abstract:

Most strawberry growers in New York establish commercial fields with runner plants purchased from local or out-of-state nurseries. Two important diseases of strawberry in New York, anthracnose and angular leaf spot, are known to be associated with nursery plants. Even though these pathogens are capable of surviving in plant debris and other hosts, nurseries are often blamed as the source of the pathogen when new plantings develop disease. Anthracnose can travel on senescent tissue or on soil particles on runner transplants when shipped or, possibly, within crown tissue; Angular leaf spot is known to reside within the crown tissue. Currently, nurseries are not required to certify their plants free of anthracnose or angular leaf spot. The objective of this experiment was to determine the presence and importance of these pathogens on nursery stock. Strawberry plants of several commonly-grown varieties were purchased from nurseries across North America. Half of the plants were potted and grown in a greenhouse to quantify viability and to observe if any disease symptoms would develop. Isolations from the crown tissue of the remaining plants were done to screen for the two pathogens. With one exception, all plants were free from anthracnose. Suspect bacteria were isolated from many crowns and PCR analysis to identify the pathogen has been completed for one quarter of the isolates; all tested negative. From the limited sampling that we have done, it appears that contamination from either of these pathogens is not, at least, a perennial problem of the nursery.

Background and justification:

Strawberries are grown on 2000 acres in New York with an annual production value of approximately $7 US million. Most strawberry growers in New York establish commercial fields with runner plants purchased from local or out-of-state nurseries. Two important diseases of strawberry in New York, anthracnose *(Colletotrichum acutatum)* and angular leaf spot *(Xanthomonas fragariae),* have been shown to be associated with infected nursery plants. Even though these pathogens are capable of surviving in plant debris and other hosts, nurseries often get the blame when new plantings develop disease.

Anthracnose is a general term used to describe diseases caused by fungi (generally) in the genus ***Colletotrichum.*** Although at least four species of ***Colletotrichum*** are known to cause anthracnose on strawberry, the most prevalent species in New York is *C* ***acutatum.*** This pathogen primarily causes fruit rot but can also infect the petioles, leaves and crown of the plant. Introduction of the disease on infected plants can cause significant yield losses when warmer temperatures and rainfall prevail during fruit set and harvest. Once the disease is established in the field, the pathogen can overwinter on infected and mummified fruit and is difficult to eradicate (Wilson et al., 1992). Recently, it has been shown that *C* ***acutatum*** can survive and reproduce on green leaf tissue without causing symptoms through a process· called conidiation (Leandro et al., 2001). These latent infections go undetected through inspections of transplants and are potential sources of inoculum under favorable conditions.

Dissemination of the bacterium ***Xanthomonas fragariae*** in transplant material has been shown to be the most significant source of inoculum for angular leaf spot. In Minnesota, the pathogen has been shown to overwinter in plant debris and to cause disease symptoms on plants the next year (Kennedy and King, 1962). In Florida, the pathogen was found to survive in low populations on nursery transplants during high summer temperatures (Roberts et al., 1996). The ability of this pathogen to survive in the field under diverse conditions indicates that clean nursery stock is essential for control of this disease.

Objectives:

The objective of this project was to determine the incidence of ***Colletotrichum acutatum*** and ***Xanthomonas fragariae*** on runner transplants from a sample of nurseries that supply New York strawberry growers. Results of this survey will help to determine if contamination or infection of nursery plants is a perennial problem in the nursery industry or are sporadic occurrences. Results will help us to advise growers about the precautions they may need to take when purchasing plants.

Procedures:

Nine hundred and fifty nursery plants were obtained from twelve nurseries located in Indiana, Maryland, Massachusetts, Michigan, New York, North Carolina, Oregon, Washington, and Ontario, Canada. The cultivars included Allstar, Earliglow, Guardian, Honeoye, Kent, Jewel, and Seascape. Twelve plants of each cultivar were planted in soilless mix and grown under standard greenhouse conditions. Care was taken to disinfect working area between planting each set of 12 plants. In order to eliminate cross contamination by overhead watering, each group of 12 plants were placed at least one meter apart. After two months of growth, disease was assessed. Up to ten of the remaining crowns from each cultivar and nursery combination were subjected to isolation methods described below.

Crown tissue from dormant plants was excised and internal tissue was plated on ***Colletotrichum*** isolation media (Legard and Mertley, 2002), potato dextrose agar containing antibiotics and Wilbrink's media. Fungal isolates were observed based on morphology and microscopic examination of conidia. Bacterial isolates with morphology similar to X. ***fragariae*** were stored in 40% glycerol at -20C. These isolates will be identified to genus using PCR with primers in the

hrp coding region (Leite, .et al., 1994) and to species using nested PCR methods described by Roberts et al., 1996.

Results and Discussion:

Of the 456 plants grown in the greenhouse, 155 died of undetermined cause within the two month period (Table 1). Of the cultivars sampled Jewel, Allstar and Earliglow had the best viability (88, 82 and 84 percent survival, respectively). Plants of the Honeoye cultivar had 50% and Kent had 42%. Seascape and Guardian had the lowest viability (27 and 17 percent survival, respectively). The relatively low viability of cultivars Honeoye (50%) and Kent (42%) in addition to the very low viability of Seascape (27%) and Guardian (17%) warrants further research. In an unrelated study, we observed that the complete removal of plant material surrounding the inner crown reduced the percent death in greenhouse grown plants. Further research attempting to culture pathogens from outer plant material may provide information on pathogen presence in nursery stock plants. Because both of these diseases are difficult to manage once in a field, elimination of inoculum sources at planting is essential.

Colletofrichum acutatum was detected in one crown of cultivar Earliglow from a California grower (nursery 8). This pathogen was not detected in any other crown tissue. Ninety-six bacterial colonies with morphology similar to X.*fragariae* were isolated from crown tissue and stored for further identification (Table 2). One isolate from each cultivar of each nursery (25% of total number of isolates) were subjected to PCR amplification of the *hrp* coding region. None of these isolates tested were found to be a *Xanthomonas* species. The remaining bacterial isolates will be tested for identification to genus using *hrp* primers. Additionally, isolates identified as *Xanthomonas* will be further screened for identification as X.*fragariae* with nested PCR procedures.

In summary, our current findings indicate that C. *acutatum* (anthracnose) or angular leaf spot (X. *fragariae)* is not a perennial problem of the nursery industry. Further study should be done to determine the likelihood of external plant material on dormant transplants of harboring these pathogens as a possible source of field inoculum. These studies, however, are much more difficult to conduct.

References:

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Table 1

Percent viability of strawberry plants grown under.standard greenhouse conditions. Each percentage is based on twelve plants.

	Percent Viability of Greenhouse Plants by Nurser,												Average
Cultivar	**1**	**2**	3	4	5	6	7	**8**	9	**10**	**11**	**12**	
Jewel	100	100	100	83	92	75	100			50			88
Allstar	100		100	75	92	50	100	100		42			82
Earliglow		67	83	83	83	92	100		83				84
Honeoye		**42**	67	100	25		100	8			8		50
Kent				25			92			8			42
Seascape		33								0	25	50	27
Guardian						17							17
Average	100	61	88	73	73	59	98	54	83	25	17	50	

Table 2

Number of bacterial colonies isolated from strawberry crown tissue that have been stored for PCR identification.

	Number of bacterial colonies stored												
Cultivar	**1**	2	**3**	4	5	6	**7**	**8**	9	**10**	**11**	**12**	**Total**
Jewel	**1**	2	3	3	0	2	2			1			14
.Allstar	4		5	4	4	5	0	9		0			31
Earliglow		6	3	1	2	2	1		2				17
Honeoye		3	**5**	1	6		**5**	0			4		**24**
Kent				0			3			0			3
Seascape		2								0		4	6
Guardian						1							1
Total	5	13	16	9	12	10	**11**	9	2	1	4	4	**96**