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Title: OPTIMUM TIMING AND USE OF STROBILURIN FUNGICIDES FOR DISEASE CONTROL IN STRAWBERRIES

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Rationale

Strawberries have become an increasingly important crop among fresh fruit crops in the US over the last 20 years, and currently rank second after fresh apples in industry value (Cook, 2002). In Michigan, where most of the strawberries are grown in perennial matted-row fields, about 58 tons of fruit is produced annually with a market value of $5.5 million (Kleweno and Matthews, 2002). For strawberry fruit to be sold fresh, it has to be of the highest quality. Numerous diseases can affect strawberry fruit quality nationwide (Maas, 1998). Most of these diseases also occur in Michigan, including anthracnose fruit rot. Anthracnose fruit rot can be caused by four different species of *Colletotrichum,* i.e., *C. acutatum*, *C. gloeosporioides, C. dematium,* and *C. fragariae* (Maas, 1988; Turechek and Heidenreich, 2001) Anthracnose fruit rot was particularly common at several sites in Michigan in 2002 (Schilder, unpublished data). The identity of the *Colletotrichum* species involved in causing fruit rot in Michigan has not been fully confirmed, since species cannot be identified solely on their morphological characteristics. Information on which species is predominant is important, since *Colletotrichum* species may differ in their sensitivity to fungicides being used.

Strawberry growers rely heavily on the use of fungicides for control of fruit diseases in strawberries. Even though growers are keen to incorporate integrated disease management methods in the production of strawberries, highly effective alternatives for disease management may not be available or cost-effective. Newer fungicides are Quadris (azoxystrobin), and Cabrio (pyraclostrobin). Quadris was registered in 2001, and Cabrio received a registration in 2002. Both Quadris and Cabrio are considered 'reduced-risk' fungicides and have shown good to excellent control of anthracnose as well as other strawberry diseases (Legard et al., 2001). However, there is also a concern about fungicide resistance. Strobilurins have been found to be prone to the development of fungicide resistance because of their single-mode type of action (Olaya et al., 2002). In fact, reduced efficacy of Quadris, presumably due to development of resistance in the anthracnose pathogen, has already been reported in Florida (Dan Legard, personal communication). It is therefore critical that these fungicides are used sparingly and at the optimum time, such that effective control is achieved, target pathogens are not exposed to unnecessary fungicide applications, and the effective lifetime of the fungicide is prolonged. The critical time for protection of the berries can vary. *Colletotrichum* species are able to infect blossoms and fruit at all stages of development (Maas, 1998; Turechek and Heidenreich, 2001). In a trial in annual strawberries in Florida, late-season sprays of Quadris were effective in reducing anthracnose fruit rot (Legard et al., 2001). However, not much information is available on the optimum timing of sprays for anthracnose fruit rot control in perennial strawberries in cooler climates. In addition, not much is know about when berries get infected, nor which *Colletotrichum* species are responsible for anthracnose fruit rot in Michigan.

Objectives

The specific objectives of the project were reduced from four to three due to the reduced funding level:

1) Compare the efficacy of Quadris and Cabrio in controlling anthracnose fruit rot
2) Determine the timing of anthracnose fruit infection in the field
3) Identify *Colletotrichum* species that are found on strawberries.

Materials and methods

1) *Compare the efficacy of Quadris and Cabrio in controlling anthracnose fruit rot*

The experiment was conducted in a 4-yr-old, commercial, matted-row strawberry planting in Onondaga, MI. Rows were spaced 42 in. apart. Treatments were applied to 10-ft sections of row and were replicated four times in a randomized complete block design with no buffer rows. Sprays were applied with an R & D Research CO2 cart-styled sprayer equipped with six bottles (0.8 gal each), a twin gauge Norgren pressure regulator set at 55 psi, and a single XR TeeJet 8002VS nozzle on a 5-ft spray boom. Spray volume was 100 gpa. Spray dates and corresponding phenological stages were as follows: 27 May (bloom), 5 Jun (bloom + ½ in. green fruit), 16 Jun (1 in. green fruit), and 25 Jun (pre-harvest). Rainfall between sprays was 0.92, 0.45, and 0.09 in., respectively. On 26 Jun, 1 Jul and 8 Jul, all berries were hand-harvested from the center 3 ft in each plot. Disposable gloves were used to pick berries and changed between plots to reduce cross-contamination. Twenty-five berries from each plot were placed equidistantly on metal screens in aluminum trays and incubated at 72ºF and 100% RH. After 7 days, berries were visually assessed for fungal sporulation.

2) Determine the timing of anthracnose fruit infection in the field
At the same site, from bloom onwards, blossoms and berries were collected weekly from the unsprayed guard rows on either side of the plot. Four sets of 25 blossoms and/or green berries were treated with paraquat and were then incubated in moist chambers to reveal latent *Colletotrichum* infections. Ripe berries were placed equidistantly on metal screens in aluminum trays and incubated at 25ºC and 100% RH for 3-4 days. After 7-10 days, berries were visually assessed for sporulation of *Colletotrichum* species, which were preliminarily identified based on morphological characteristics. Identification of representative isolates with species-specific primers was done under objective 3.

3) Identify Colletotrichum species that are found on strawberries
Enumeration was done according to Objective 2. For identification, fifteen *Colletotrichum* cultures were isolated from strawberries at the trial site. Two of these were isolated from the same area in 2002. All cultures were grown on potato dextrose agar containing streptomycin and also stored in 15% glycerol water (v/v) at –20 C. Fungal DNA was extracted using a modified method from Hamelin (1996, 2000). Using sterile toothpicks, approximately 2 - 3 mm clumps of mycelia and spores were scraped from culture plates and placed in a sterile eppendorf tube. Six hundred µl of extraction buffer [100mM Tris-HCl, 20 mM EDTA, 2% CTAB (etyltrimethylammonium), 1.4 M NaCl, 1% polyethylene glycol 8000] was added and the mixture ground with a sterile polypropylene pestle for several minutes. DNA was extracted with 600 ul of phenol:chloroform:isoamyl alcohol (25:24:1) and precipitated with cold isopropanol. The resulting pellet was washed with 70% cold ethanol and resuspended in 50 ul Tris-EDTA. DNA was typically diluted 1:10 or 1:00 for use in PCR reactions. All cultures were tested in PCR with two sets of published species-specific primers: one set to identify *Colletotrichum acutatum* (Ca primers) and one set to identify *Colletotrichum gloeosporioides* (Cg primers). There are no primers available for *C. dematium*. However, this fungus is relatively easy to identify based on its appearance due to its acervuli with spines and distinctive sickle-shaped spores.

Results and discussion

1) Compare the efficacy of Quadris and Cabrio in controlling anthracnose fruit rot

Fungicide applications significantly reduced post-harvest anthracnose fruit rot incidence, both of *C. acutatum* and *C. dematium*. Two late applications of Quadris and Cabrio (1-in green fruit) and prior to the first harvest did not work as well as the Quadris/Captec and Cabrio/Captec spray programs, suggesting that infections took place earlier than 1-in green fruit. The best treatment for *C. acutatum* at the first harvest was the Captec/Topsin/Kocide program, which is considered the grower's standard. However, at the second harvest, the standard program did relatively poorly compared to the strobilurins. This may be due to the fact that the ingredients that are active against *C. acutatum* (captan and, to some extent, copper) did not last as long on the fruit surface as the strobilurins. Cabrio tended to be slightly more effective than Quadris when compared singly or in a fungicide program with Captec. However, these differences were not statistically significant.

		Colletotrichum acutatum (%)						*Colletotrichum dematium* (%)[v]					
Treatment, rate/A	Application timing[z]	Harvest 1		Harvest 2		Harvest 3		Harvest 1		Harvest 2			
Untreated		33.3[y]	a	9.0[y w]	a	13.0	ns	4.8	ns	5.0	a		
Quadris F 10 fl oz.....	3, 4	20.3	ab	0.8	bc	3.0		1.3		0.0		c	
Cabrio EG 14 oz.....	3, 4	15.9	b	0.3	c	11.0		0.0		0.0		c	
Quadris F 10 fl oz Captec 4L 2.5 qt.....	1, 3, 4 2,	7.3	c	0.8	bc	7.0		0.0		1.0		bc	
Cabrio EG 14 oz Captec 4L 2.5 qt.....	1, 3, 4 2,	4.1	c	0.8	bc	4.0		2.1		2.6		abc	
Captec 4L 2.5qt +Topsin M 70WP 1 lb +Kocide 2000 2.75 lb....	1, 2, 3, 4	2.1	c	7.2	ab	3.0		1.0		1.2		bc	

[z]Spray dates: 1 = 27 May (bloom), 2 = 5 Jun (bloom + ½ in. green fruit), 3 = 16 Jun (1 in. green fruit), 4 = 25 Jun (pre-harvest).

[y] Values shown are actual means; statistical analysis was performed on Arcsin-transformed data.

[x]Column means followed by the same letter are not significantly different according to Fisher's Protected LSD test (*P*#0.05); ns = not significant.

[w]Column means followed by the same letter are not significantly different according to Fisher's Protected LSD test (*P*#0.063).

[v]No *Colletotrichum dematium* was observed in the harvest three berries.

2) Determine the timing of anthracnose fruit infection in the field

Colletotrichum infection was not detected in flowers. A low level of *Colletotrichum* (both *C. acutatum* and *C. dematium*) infection was first detected in small green berries on June 11 (Figs. 1 and 2). No infection was detected on Jun 19. However, on June 26, at the first harvest, more infections were noted, and even higher infection levels occurred on July 1 at the second harvest. In each case, infection incidence was higher in red berries than in green berries, and *C. acutatum* was more common than *C. dematium.* Very few of the berries actually showed symptoms in the field: most of the infections were latent and became apparent in post-harvest rot tests. The reason for the late appearance of anthracnose infections is thought to be the relatively cool growing season (Figure 3).

Figure 1. Incidence of *Colletotrichum acutatum* in strawberry ('Jewel') flowers and fruit, Onondaga, MI, in 2003.

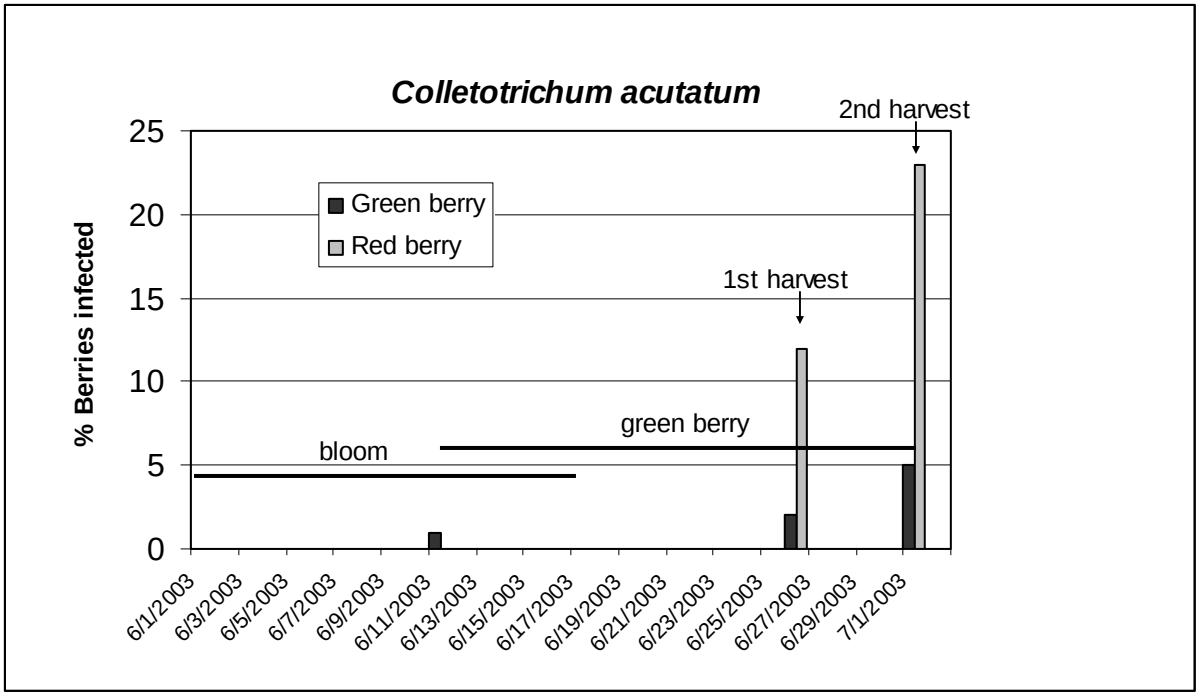


Figure 2. Incidence of *Colletotrichum dematium* in strawberry ('Jewel') flowers and fruit, Onondaga, MI, 2003.

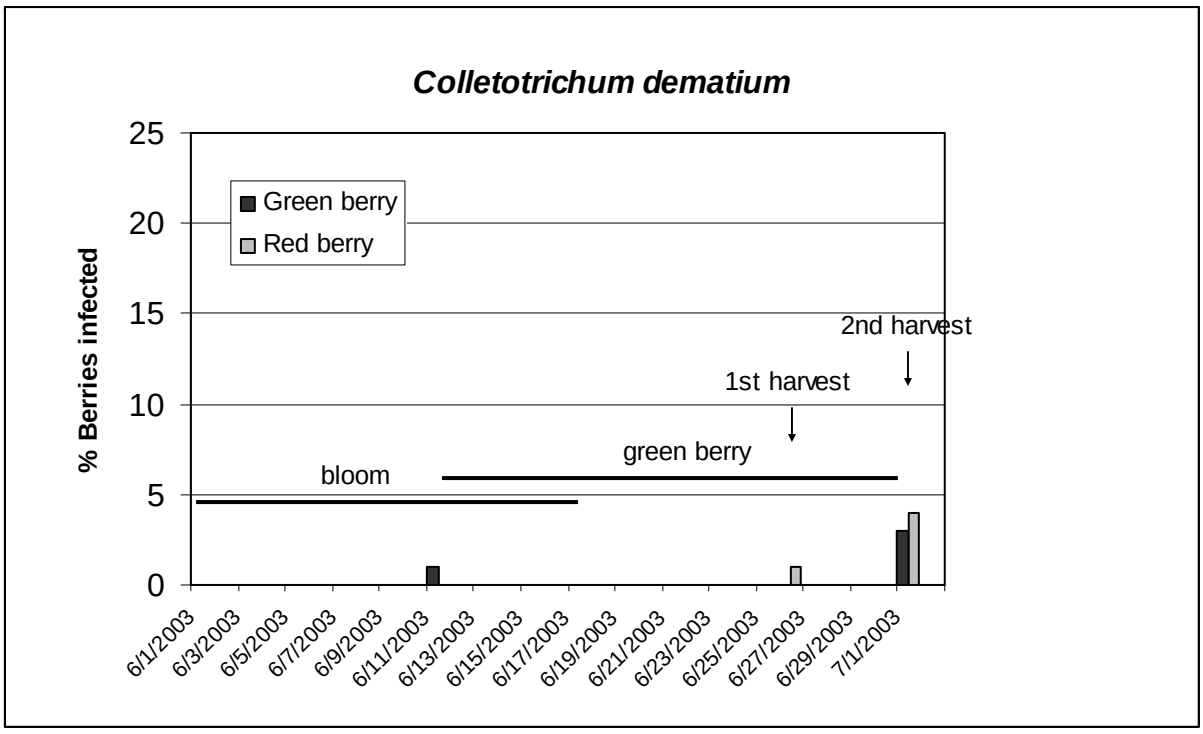
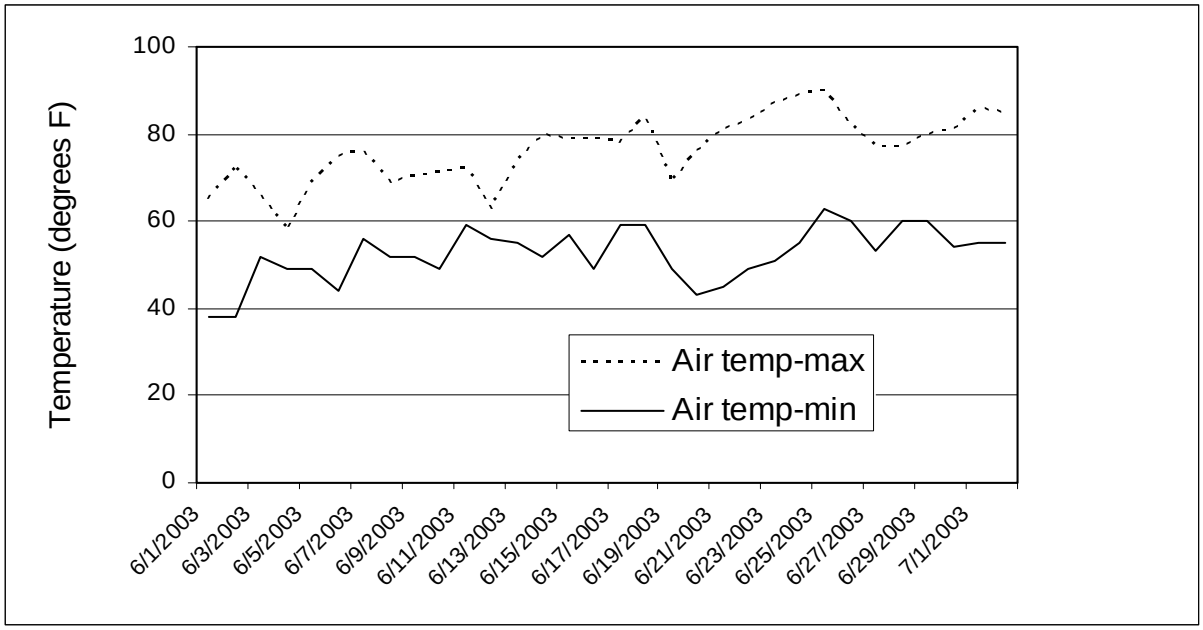


Figure 3. Air temperature during 2003 growing season, East Lansing, MI.



3) Identify Colletotrichum species that are found on strawberries

Of the 15 isolates tested with species-specific PCR primers, 11 produced appropriate PCR products with the Ca primers, indicating positive identification as *Colletotrichum acutatum*. Five of these 11 isolates were also exposed to the Cg primers but resulted in no PCR product. The other four *Colletotrichum* isolates exhibited characteristic *Colletotrichum* morphology in culture, but purified DNA from these cultures did not react with either set of primers. This was presumably due to impurities in the DNA. Based on their cultural similarity to other isolates, we assume that they are in fact *C. acutatum* but they would have to be retested to be sure. *Colletotrichum dematium* was also detected on strawberry fruit and was identified based on morphology.

Table 2. Summary of PCR reactions on *Colletotrichum* isolates from strawberry fruit:

Culture number	Year isolated	Plant part from which isolated	Reaction to Ca primers	Reaction to Cg primers	Identification based on primers
007	2002	Fruit	+	not tested	*C. acutatum*
025	2002	Fruit	+	-	*C. acutatum*
099	2003	Fruit	+	not tested	*C. acutatum*
100	2003	Fruit	+	not tested	*C. acutatum*
101	2003	Fruit	+	not tested	*C. acutatum*
102	2003	Fruit	-	-	unknown
104	2003	Fruit	-	-	unknown
105	2003	Fruit	-	-	unknown
107	2003	Fruit	+	-	*C. acutatum*
109	2003	Fruit	-	-	unknown
110	2003	Fruit	+	-	*C. acutatum*
111	2003	Fruit	+	-	*C. acutatum*

113	2003	Fruit	+	-	*C. acutatum*
114	2003	Fruit	+	not tested	*C. acutatum*
115	2003	Fruit	+	not tested	*C. acutatum*

Conclusions

The research showed that, like in many other states, the primary pathogen causing anthracnose fruit rot is *Colletotrichum acutatum*. However, *C. dematium* was also found and may be more common in certain years, locations or cultivars. No blossom infection was observed, but both green and red fruit were infected. Infection incidence increased closer to harvest and red fruit supported more infections than green fruit. The strobilurins were effective in reducing post-harvest anthracnose fruit rot, however, two late sprays may not be sufficient for disease control. It is recommended to include Captan in the spray program, preferably for the earlier sprays such that the total number of strobilurin sprays can be reduced to limit the risk of resistance development. Using the strobilurins just prior to harvest may not keep berries from that harvest from developing post-harvest rot, but may help keep berries healthy at later harvests. Cabrio tended to be slightly more effective than Quadris at the rates tested. Price of these materials will probably dictate grower choice.

References cited

Cook, R. 2002. Strawberries. PDF file on website: rics.ucdavis.edu/postharvest2/Pubs/ strawberriesfinal1Sept02.pdf.

Hamelin, R.C., Berube, P., Gignac, M., and Bourassa, M. 1996. Identification of root rot fungi in nursery seedlings by nested multiplex PCR. Applied and Environmental Microbiology 62 (11): 4026-4031.

Hamelin, R.C., Bourassa, M., Rail, J., Dusagenyagasani, M., Jacobi, and Laflamme, G. 2000. PCR detection of *Gremmeniella abietina*, the causal agent of *Scleroderris* canker of pine. Mycological Research 104 (5): 527-532.

Kleweno, D. D., and Matthews, V. 2002. Michigan Agricultural Statistics 2001-2002. Michigan Department of Agriculture, Lansing, MI. 94 pp.

Maas J. L. 1998. Compendium of strawberry diseases. APS Press, St. Paul, MN, 98 pp.

Legard, D.E., Mertely, J.C., Xiao, C.L., and Duval, J.R. 2001. Evaluation of fungicides to control anthracnose fruit rot of strawberry, 2000. Fungicide and Nematicide Test Reports 56 (electronic version).

Olaya, G., Avila-Adame, C., and Koeller, W. 2002. Resistance of *Colletotrichum graminicola* to Qo inhibitors. Phytopathology 92: S61 (abstr.)

Turechek, B., and Heidenreich, C. 2001. Strawberry anthracnose. Tree Fruit and Berry Pathology Website, Cornell University.http://www.nysaes.cornell.edu/pp/extension/tfabp/santsmf.shtml