

ABSTRACT

SHUMAN, JOEL LEE. Anthracnose Fruit Rot Resistance in Strawberry.

(Under the direction of James R. Ballington.)

The purpose of this research has been to determine the nature of strawberry fruit resistance to anthracnose fruit rot. Production in the United States and North Carolina is heavily dependent upon two cultivars, Chandler and Camarosa. Both cultivars are susceptible to anthracnose fruit rot (AFR), caused by the imperfect fungus *Colletotrichum acutatum*. Results from this research will be used to further our understanding of the strawberry-*C. acutatum* system and to control AFR through breeding strategies or cultural practices. Components of resistance in strawberry to AFR were studied under field and controlled environment conditions; five strawberry genotypes were inoculated with conidia of *C. acutatum* under field conditions and seven genotypes were inoculated with either five inoculum concentrations or three isolates of *C. acutatum* in growth chambers. Strawberry genotypes responded differently to *C. acutatum* in the field and in a controlled environment. Components of resistance to AFR included rate-limiting resistance, reduced percent lesion, reduced probability of lesion formation due to fruit age and genotype, and plant canopy architecture. Rates of disease progress were different among genotypes, inoculum concentrations, isolates, and the genotype x isolate interaction. Susceptible genotypes and virulent isolates had faster rates of disease progress. Differences were observed among genotypes for yield, percent by weight, and number of berries with AFR. Plant canopy architecture influenced the hours of fruit

wetness and the yield of berries with AFR; a loose open canopy had fewer hours. Young and old fruit were less susceptible to AFR than fruit of median age.

ANTHRACNOSE FRUIT ROT RESISTANCE IN STRAWBERRY

by

JOEL LEE SHUMAN

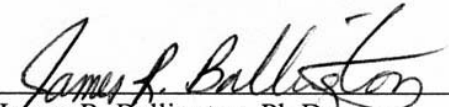
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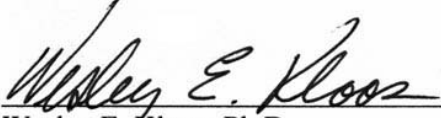
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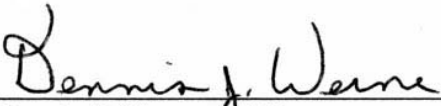
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DEDICATION

This thesis is dedicated to my parents who brought me here and loved me and to my closest friend, Holly Scoggins, for her patience, understanding, and humor during my tenure in the Department of Horticultural Sciences at the North Carolina State University, Raleigh, NC.

BIOGRAPHY

Joel Lee Shuman was born in Seoul, South Korea on 20 April, 1968. One of his earliest memories is winging his way over the other “big pond” and trying to eat an orange, peel and all, to meet his new, adoptive, Pennsylvania Dutch/English parents. He traded in his kim-chi and pork barbecue for kraut, steak, and potatoes.

During his childhood he labored in the family vegetable and fruit garden, a primary food source for a family of six. He also worked in a small, family-owned greenhouse and truck farm in Lancaster County, PA during his teenage years. He somehow survived his middle school days at Londonderry M.S., PA where his teachers told him to duck under the “lead-lined” desk when the folks at Three Mile Island hollered from 4 miles away. He graduated from Lower Dauphin High School in Hummelstown, PA and decided that JoePa needed a Korean linebacker. Unfortunately, he was still only 5’7” and a soaking wet 125 pounds.

Instead, he decided to enroll in a Plant Science program at The Pennsylvania State University. He graduated with a B.S. under the Socratic guidance of Dr. Felix Lukezic in May 1991. He couldn’t escape the clutches of Happy Valley and decided to continue his education with Drs. Barb Christ and Stan Pennypacker in Plant Pathology. He graduated in August 1995. He enrolled in the Horticulture Department at North Carolina State University in August 1995.

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Chapter One

GENERAL INTRODUCTION

Strawberry

Taxonomy and Origin

Strawberries are members of the family Rosaceae, subfamily Rosoideae, and genus *Fragaria*. Closely related genera include *Duchesnea*, the mock strawberry, and *Potentilla*, the cinquefoils. *Fragaria* species can be grouped by ploidy: there are nine diploids, two tetraploids, one hexaploid, and four octoploids. Diploids ($2n=14$) include *F. vesca* Duch., *F. viridis* Duch., *F. nilgerrensis* Schlect., *F. daltoniana* J. Gray, *F. nubicola* Lindl. ex Lacaite., *F. iinumae* Makino, *F. yezoensis* Hara, *F. nipponica* Makino, and *F. mandschurica* Staudt (Staudt, 1989). The alpine strawberry, *F. vesca*, is the most geographically widespread. Cytogenetic studies indicate that this species may be a diploid progenitor of the octoploid strawberries. The two tetraploids ($2n=28$) are *F. orientalis* Losinsk and *F. moupinensis* (Franch.) Card. The lone hexaploid ($2n=42$), *F. moschata* Duch., or musky strawberry, is found in northern and central Europe into eastern Russia. This species was domesticated in the early 1600's and fruit were commonly known as 'Hautbois' or 'Hautboy'. Cultivated plantings still exist in Europe.

Four octoploids ($2n=56$) are known: *F. iturupensis* Staudt, *F. chiloensis* (L.) Duch., *F. virginiana* Duch., and *F. ×ananassa* Duch. *F. iturupensis* is found in Iturup Island of the Kuril Islands (northeast of Japan) (Staudt, 1973). Taxonomic characteristics include obovate sub-glaucous leaves (similar to *F. iinumae*), hermaphroditic flowers, and almost spherical fruit. The beach or Chilean strawberry, *F. chiloensis*, is found along the Pacific coast from Alaska down through central California, along the beaches of Chile and inland to the Andes Mountains, and on top of mountains in Hawaii. They were once extensively cultivated in Chile, Peru, and possibly Ecuador. Wild populations are primarily dioecious although hermaphrodites have been found in California (Hancock and Bringham, 1979; Darrow, 1966). Plants are low-spreading, vigorous, and produce many runners. Leaves are thick, dark-green and very glossy. Fruit are dull to bright red, firm, white fleshed, pungent, and large. Four subspecies are recognized based on morphology and distribution (Staudt 1962): ssp. *chiloensis* (South America), ssp. *lucida* (Washington to California), ssp. *pacifica* (California to Aleutian Islands), and ssp. *sandwicensis* (Hawaii).

The scarlet or Virginia strawberry, *F. virginiana*, is found North America from the Southeastern U.S. north to Newfoundland and as far west as the Yukon Territory into Alaska, oftentimes in meadows. Plants are slender, tall, and have many runners. Only females and hermaphrodites are observed in the eastern U.S. while all three sexes are equally found in western populations (Luby and Stahler, 1993; Stadler et al., 1988). Fruit are soft, round, up to 1.5 cm diam, light red, aromatic, with deeply embedded seeds, and

white flesh. Both plant and fruit characters are highly variable. Four subspecies are recognized by Staudt (1989): ssp. *virginiana* Duch. (Eastern U.S. to Newfoundland and west to Yukon Territory); ssp. *glauca* (Wats.) Staudt [southern Arizona through the Rocky Mountains into northwest Canada and central Alaska, probably equivalent to *F. ovalis* (Darrow, 1966) due to lack of hybridization barriers and intermediate characters]; ssp. *platypetala* (Rydb.) Staudt (California to British Columbia and in the Rocky Mountains in Colorado and Wyoming); and ssp. *grayana* (E. Vilmorin ex Gay) Staudt (Texas through Louisiana, Alabama and north to New York).

However, this classification scheme by Staudt has undergone considerable debate. New evidence suggests that these four “subspecies” are too closely related to be considered infraspecific taxa. Welsh et al. (1987) suggested that ssp. *glauca* and ssp. *platypetala* completely intergrade and should be referred to as a single taxa var. *glauca*. Hokanson et al. (1993) suggested that strawberries in the Black Hills and eastern front ranges of the Rocky Mountains may be introgressive swarms between ssp. *glauca* and ssp. *grayana*. Finally, Harrison et al. (1997), using multivariate analysis, found that these strawberries from the Black Hills were morphologically intermediate between collections of eastern ssp. *virginiana* and western ssp. *glauca*. Furthermore, when multivariate analysis was done using RAPD data, this population of strawberries from the Black Hills was part of a large cluster group that encompassed the eastern ssp. *virginiana* and western ssp. *glauca*.

The dessert or pineapple (Ananas) strawberry, *F. ×ananassa*, is the most important cultivated strawberry worldwide. It arose as a chance hybrid of *F. chiloensis* x

F. virginiana within European gardens during the mid-1700's. Naturally occurring hybrids have been found in coastal areas of southwest British Columbia, Washington, Oregon, and northern California. Staudt (1962) recognized these hybrids as *F.*

×*ananassa* nm. *cuneifolia* (Nutt. Ex Howell). Many of the dessert strawberry's traits are intermediate to its parents. Plants have large fruit, high yields, and vigor.

Production

Most strawberry production (98%) is located in the northern hemisphere but production is expanding in the southern hemisphere. The two major worldwide production systems are annual hill and matted row. Features of the matted row system include: spring planting, flat beds, straw mulch, summer renovation, overhead irrigation, and two to five production seasons. Matted row is being quickly supplanted by the annual hill system in the Southeastern U.S. and worldwide. Features of the annual hill system include summer or late autumn planting, raised beds, plastic mulch, trickle irrigation, usually one to two production seasons, and sometimes overhead irrigation for frost protection. Other production systems include tunnels and greenhouses.

The U.S. produces about 20% of the world's strawberries, followed by Spain, Japan, Poland, Italy, and the Korean Republic (Hancock, 1999). Hectarage in the U.S. has increased from 18,482 ha in 1997 to 19,870 ha in 2000 (Agricultural Statistics Board, 2001). Leading states in 2000 were: 1) California at 11,169 ha; 2) Florida at 2,550 ha; and 3) Oregon at 1,416 ha. Other leading states are North Carolina, Washington, and New York with between 607 to 688 ha. Production within the U.S. increased from

738,358 t in 1997 to 838,057 t in 2000.

North Carolina ranked 4th nationally in 2000 for strawberry production, with about 10,478 t produced on 728 ha with a value of \$17.3 million (Agricultural Statistics Board, 2001). Acreage has remained consistent from 1997 to 2000 but production from this acreage has fluctuated between 7,983 to 10,478 t. Value of the crop ranged from \$12.6 million in 1997 to \$17.3 million in 2000. Most of the North Carolina strawberry acreage is planted to the annual hill system (plasticulture). The latest estimate in 1997 was 500 ha (Hancock, 1999). The figure has most likely increased since then.

Plasticulture, as practiced in North Carolina, is the annual hill training system where freshly dug plants or plugs are planted in early fall in double rows on fumigated, raised beds covered with black plastic mulch. Plasticulture became feasible in North Carolina because of the California strawberry ‘Chandler’. ‘Chandler’ is early maturing, tolerant of cool temperatures, possesses a long harvest period (usually 6 weeks but up to 2 months), and can be harvested 7-8 months after planting in the annual hill system, compared to about 12 months for matted row (MR). Another California strawberry with increasing importance in plasticulture is ‘Camarosa’. It is now the most widely planted cultivar in the world (Hancock, 1999).

The typically humid conditions experienced in the Southeast are especially conducive to fruit rot. The fruit of both ‘Chandler’ and ‘Camarosa’ often become severely infected with the fungus *Colletotrichum acutatum* Simmonds, resulting in ripe rot or anthracnose fruit rot (AFR). It has been stated that, “Anthracnose, caused by *C. acutatum*, is the major limiting factor to strawberry production in North Carolina, and the

long term survival of the strawberry industry in the state appears literally dependent on development of anthracnose resistant cultivars” (Ballington and Milholland, 1993). At present, there are no effective fungicides available for fruit rot control. The emergency Section 18-C registration for Difolatan (Captafol) was lost in 1987. Captafol had consistently provided the best general control of strawberry diseases and its use gave the highest marketable yields of all fungicides tested (Delp and Milholland, 1980). The other fungicides generally have constraints that limit their use. Many fungi have become resistant to the benzimidazoles such as Benlate and Topsin-M. Dodine (Cyprex) is registered for strawberries but requires a 14-day preharvest interval and has a limit of 12.5 L/ha/season. Vinclozilin (Ronilan) and iprodione (Rovral) control grey mold but provide little protection against other diseases. Finally, copper fungicides often prove phytotoxic. New formulations have shown promise at controlling AFR. Both captan (Captan) + thiophanate-methyl (Topsin-M) and azoxystrobin (Quadris) significantly reduced AFR compared to other treatments (none, Captan, Switch, Cygnus, and Elevate) (Louws, et al., 1998). Quadris has been labelled (section 18) for use on strawberries in North Carolina. Fungicide use increases production costs and contributes to ground-water contamination, limiting the sustainability of such a practice. Therefore, genetic resistance is the most sustainable, cost-efficient, and practical means of disease control.

Colletotrichum

Anthracnose is defined as a disease that appears as black, sunken lesions on

leaves, stems, or fruit and is caused by fungi that produce their asexual spores in an acervulus (Agrios, 1988). Two important genera of imperfect fungi that incite anthracnose on many plant hosts are *Colletotrichum* and *Gloeosporium*. Species of *Colletotrichum* can cause disease on a wide variety of plants such as: cereals and grasses; cucurbits; tomatoes and other vegetables; azalea; and legumes like soybean and sweet. Other diseases include bitter rot of apple and cranberry, dieback and canker of *Camellia* and privet, and ripe rot of grape and other fruits. Furthermore, a single species can infect several plant hosts. For example, *C. acutatum* can infect almond, apple, blueberry, and strawberry (Adaskaveg and Hartin, 1997; Bernstein et al., 1995; Smith et al., 1996). Some imperfect fungi with *Colletotrichum* or *Gloeosporium*-type conidia also reproduce sexually via ascospores produced in perithecia. If a teleomorph is formed, *Colletotrichum* species generally produce *Glomerella* or occasionally *Physalospora*-type asci. *Gloeosporium* species typically form *Glomerella* or *Gnomonia*-type asci. However, not all imperfect fungi are known to reproduce sexually. Teleomorphs were produced *in vitro* for the first time for several isolates of *C. acutatum* from apple and one from blueberry (Guerber and Correll, 1997). No teleomorphs have been reported for isolates of *C. acutatum* from strawberry. Other genera of ascomycetous fungi that cause anthracnose-type infections include *Diplocarpon*, *Elsinoe*, and *Gnomonia*.

The three most important *Colletotrichum* species causing anthracnose on strawberry are *C. fragariae* Brooks, *C. gloeosporioides* (Penz.) Penz. et Sacc, and *C. acutatum*. These fungi can infect most plant tissue but the two economically devastating phases are crown rot and fruit rot. Crown rot is usually associated with *C. fragariae* and

sometimes *C. gloeosporioides*. Fruit rot is usually associated with *C. acutatum*. The species can be distinguished by morphological, cultural, and molecular traits (Freeman and Katan, 1997; Simmonds, 1965; Smith and Black, 1990). Smith and Black (1990) reported that *C. fragariae* could be identified by cylindric conidia, beige to olive to dark gray colonies, and no asci in culture. Isolates of *C. gloeosporioides* (teleomorph: *Glomerella cingulata*) could be differentiated from other species by gray or olive-gray colonies, dark gray to dark olive in reverse, cylindric conidia, and asci in culture. Isolates of *C. acutatum* could be identified by pink, orange, rose, or beige colonies, cream, pink, or rose in reverse, fusiform conidia, and no asci. The presence or absence of setae has also been used to aid in the identification of *Colletotrichum* species (Gubler and Gunnell, 1991).

In North Carolina, anthracnose fruit rot or ripe rot of strawberry is caused by *C. acutatum* (Grand et al., 1990). It has also become the dominant species affecting strawberry fruit in other regions of the U.S. such as California (Eastburn and Gubler, 1992) Ohio (Ellis and Madden, 1993); it has even been reported as far north as Connecticut (Lamondia, 1991) and was able to overwinter in Ohio (Wilson et al., 1992). The incidence and severity of AFR are increased in the humid conditions of the Southeast. Conidia of *C. acutatum* require free moisture to germinate conidia (Wilson et al., 1990). Conidia are splash-dispersed and were shown to be dispersed more effectively on black plastic mulch than either bare soil or straw (Madden et al., 1993; Yang et al., 1990).

Preliminary data on fruit infection (B.J. Smith, personal communication) indicate

that *C. acutatum* is a generalist pathogen with a subcuticular intramural invasion strategy while *C. fragariae* fits the pattern of a specialist pathogen with an intracellular hemibiotrophic invasion strategy. *C. acutatum* invades fruit by growing extensively through the fruit cell walls and entering the cells usually after their death.

Resistance

Limited work has been done determining the mechanisms and heritability of resistance to anthracnose in various strawberry plant parts including crowns, foliage, petioles, and runners. Resistance varies among plant parts and among strawberry genotypes. It has been incorporated into strawberry cultivars using locally adapted *F. ×ananassa* and *F. virginiana* ssp. *virginiana* in state Agricultural Experiment Station breeding programs (Baton Rouge, La.; Dover, Fla.; and Raleigh, N.C.) and USDA breeding programs (Poplarville, Miss. and Beltsville, Md.). Reports are often conflicting and probably dependent upon a complex set of factors including environment, species and isolate (race) of *Colletotrichum* or *Gloeosporium*, plant part inoculated, inoculation and incubation method, and strawberry genotype. For example, crown and petiole resistance may not be controlled by the same mechanism but inoculation technique could prevent the accurate determination of such resistance. Spraying plants until runoff could allow conidia to be deposited into the crown. Therefore, both petiole and crown resistance are being assayed, rather than just petiole resistance.

Plant/Petiole

Delp and Milholland (1981) found that selections of *F. virginiana* ssp. *virginiana* from the Blue Ridge Mountains and Wake County, N.C. were not resistant to *C. fragariae*. Two commercially grown cultivars, 'Apollo' and Sequoia, were rated resistant. Petioles were inoculated with 3×10^6 conidia·ml⁻¹ of *C. fragariae*: isolates CF-7, CF-14 from Louisiana and CF-4, -9 to -12, -15, and -16 from North Carolina. They also reported that resistance to *C. fragariae* exists in petioles but not crowns. Two Louisiana selections (L-2556 and L-6632) were very resistant to petiole infection but 75% and 100% of the plants, respectively, died after crowns were injected. An alternative explanation may be that crown resistance and petiole resistance are not necessarily based upon the same resistance mechanism(s).

Some work has been done studying the inheritance of resistance. Gupton and Smith (1991) crossed strawberry genotypes with differing responses to *C. fragariae*. Petioles of seedlings from these crosses were sequentially spray-inoculated until runoff at 1.5×10^6 conidia·ml⁻¹ with *C. fragariae* isolates CF-63, followed by LA-1, and FLA-1 and then *C. acutatum* Goff or Mil-1. Frequency distribution of disease severity ratings was bimodal, suggesting major gene action. Narrow-sense heritability estimates were 0.37 and 0.26. Mean disease severity ratings were generally less for *C. acutatum* than for *C. fragariae* but no genotype x *Colletotrichum* sp. interaction was reported. The authors suggested that strawberry genotypes respond in a similar manner to both species. However, they did not rule out the possibility of genotype x isolate or genotype by species interactions as they did report genotype x *C. fragariae* isolate interactions in the

same study. Smith and Black (1990) also found that most cultivars responded similarly to both species.

Winterbottom (1988) reported that plant resistance to *C. acutatum* was conferred by a major, dominant gene because of a bimodal segregation for resistance and susceptibility. Neither inoculum nor plant tissue(s) inoculated was specified. Less definitive results were found in a later study by Simpson, Winterbottom, and others (1994): standard deviations for symptom expression were variable and often large. Their most pathogenic isolate, *C. acutatum* IMI351247-1, was spray-inoculated at 1.5×10^6 conidia·ml⁻¹ onto all plant parts. Plants were grown in a glasshouse. Five genotypes were rated as highly resistant to anthracnose: ‘Elvira’, ‘Honeoye’, EM99, EM224, and EM255. However, ‘Honeoye’ was reported to be susceptible by Denoyes-Rothan et al. (1999) to *C. acutatum* isolate 1276b. Six genotypes were rated as having useful levels of resistance: ‘Cambridge Favourite’, ‘Gorella’, ‘Pandora’, ‘Pantagruella’, EM17, and EM290.

Inheritance of resistance to *C. acutatum* 1267b was investigated in seedlings from 3x3 factorial crosses using 6 cultivars: Elsanta, Darestival, and Rosanne as female parents and Pajaro, Sequoia, and Dover as male parents (Denoyes-Rothan, B. 1997). A 0 to 5 disease rating scale was used from 0 = no lesion to 5 = dead plant. However, rated seedlings were then separated into two classes: R for resistant seedlings (0 to 0.5 rating) and S for susceptible seedlings (1 to 5 rating). In essence, a quantitative rating scale was reduced to a bimodal scale, possibly influencing the conclusions drawn. Seedlings from selfed resistant ‘Sequoia’ and ‘Dover’ fell into 3R:1S and 1R:0S ratios, respectively.

Seedlings from crossing susceptible x resistant cultivars fell into 1R:1S ratios. Based on these results, the author concluded that resistance to *C. acutatum* 1276b is controlled by a single gene and that resistance is dominant to susceptibility. 'Sequoia' is heterozygous for this gene while 'Dover' is homozygous. These results must be interpreted cautiously. Resistance to *C. acutatum* may be inherited by one or a few dominant genes but a two-class rating system could predispose one to fit data to a qualitative, Mendelian model. However, these results agree with those of Gupton and Smith (1991) and Winterbottom (1993).

Runner/Crown

Ballington and Milholland (1993) observed differences in lesion length of runners inoculated with *C. acutatum* CA-1 among 31 strawberry genotypes. They concluded that high levels of runner resistance may be important in reducing inoculum and AFR. The inheritance of resistance to *C. acutatum* on runners was reported to be quantitatively inherited (Gimenez, 1997). This was based on the frequency distribution of lesion lengths within plant progenies using two half-diallel mating designs. Both additive and non-additive effects were important in the inheritance of resistance.

Smith and Spiers (1982) reported that several strawberry lines demonstrated resistance to crown rot. They injected inoculum into crowns and also spray inoculated seedlings with *C. fragariae*, making no attempt to keep inoculum out of crowns. Galletta et al. (1993) also reported that four strawberry clones (US70, US159, US292, and US438) were resistant to crown rot.

Fruit

Little is known about resistance of strawberry fruit to *C. acutatum*. Is there genetic resistance to AFR in strawberry fruit? If so, how is it inherited? Hancock et al. (1990) did not identify any resistance to fruit rot in *Fragaria*. Maas and Howard (1985) reported that strawberry fruit were uniformly susceptible to AFR. Based on pathogenicity tests, Ballington and Milholland (1993) suggested that there is no genetic resistance to fruit infection by *C. acutatum*, particularly on ripe fruit. However, field observations suggest that some cultivars and genotypes possess moderate levels of resistance to AFR (Ballington and Milholland, 1993; Denoyes-Rothan et al., 1999; Olcott-Reid and Moore, 1995b; Maas, 1978). Differing levels of field susceptibility to anthracnose fruit rot, gray mold (*Botrytis cinerea* Pers. ex Fr.), and leather rot [*Phytophthora cactorum* Leb and Cohn (Schroet.)], were observed among various cultivars and selections in Arkansas (Olcott-Reid and Moore, 1995a). Within-genotype and environmental correlations between the individual fruit rots were often positive, indicating that clones with greater amounts of one disease often had greater amounts of another.

Galletta et al. (1995) reported that both plants and fruit of 'Delmarvel' were resistant to anthracnose. Fruit loss due to anthracnose was similar to 'Sweet Charlie'. Smith et al. (1998) reported that all plant parts of 'Pelican' were resistant to anthracnose. Plants of 'Sweet Charlie' have been reported to be resistant to anthracnose (Chandler et al., 1997). But, plant resistance is not always associated with fruit rot resistance. The foliage, runners, and petioles of 'Sweet Charlie' are susceptible to anthracnose but the

fruit often ‘escapes’ severe rotting in North Carolina (Gimenez, 1997). Conversely, plants of ‘Tribute’ and ‘Tristar’ are moderately resistant but the fruit are susceptible to anthracnose fruit rot. In the same vein, plants of ‘Apollo’ are somewhat resistant (Smith and Spiers, 1982) but the tender-skinned fruit often become rotted (Smith and Black, 1990). These contradictions indicate that other fruit characteristics may be related to this apparent field resistance.

Fruit resistance has also been reported in controlled environments. Denoyes-Rothan et al. (1999) found resistance to AFR in 26 strawberry genotypes. Detached strawberry fruit were inoculated with 2×10^4 conidia·ml⁻¹ of *C. acutatum* isolate 1276b. A 15- μ l drop was deposited on each fruit and lesion size was followed from 4 to 8 d after inoculation. Mean fruit lesion diameter ranged from 0.4 mm for US438 to 14.4 mm for ‘Pajaro’. ‘Chandler’ had a mean lesion diameter of 3.3 mm and disease incidence of 57%. Disease incidence was highly correlated with lesion size ($R^2=0.89$). They postulated that disease incidence may provide a quick and easy means to evaluate fruit rot resistance.

Some studies also suggest that there is an isolate x cultivar interaction. Hancock et al. (1990) summarized data indicating an isolate by cultivar interaction for each isolate of *C. acutatum*, *C. fragariae*, and *C. gloeosporioides*. Ballington and Milholland (1993) did not find an interaction with respect to percentage of AFR among five genotypes inoculated with two isolates of *C. acutatum* (CA-1 and CA-8). Both samples were collected in North Carolina: CA-1 was isolated from a strawberry runner while CA-8 was isolated from a strawberry fruit. The percent AFR was higher for green fruit inoculated

with CA-1 compared to CA-8 for all genotypes: indicating that CA-1 is more virulent than CA-8. Ripe fruit that were inoculated with CA-1 and CA-8 had 100% AFR for all genotypes. These results indicate that ripe fruit are uniformly susceptible to AFR, regardless of isolate. Isolates from hosts other than strawberry were weakly virulent to almost nonpathogenic on runners. All isolates developed lesions on green and ripe, attached fruit of 'Oso Grande' and 'Titan'. Smith and Black (1990) reported genotype x isolate interactions for *C. fragariae* with respect to stolon and plant resistance. They suggested that there may be a race-cultivar system.

Fruit characteristics have been associated or correlated with reduced anthracnose fruit rot. Fruit firmness and calyx and neck ratings were correlated with field fruit rot reactions to anthracnose (*C. gloeosporioides*), leather rot, and gray mold of 9 strawberry cultivars harvested 3 times/week over 13 dates (Olcott-Reid et al., 1995b). Skin toughness and flesh firmness did not vary over the season, except immediately after a heavy rain where firmness increased. Greater skin toughness was not highly correlated with reduced levels of any fruit rot but greater fruit flesh firmness was correlated ($r^2 = 0.26$ to 0.37) with reduced anthracnose fruit rot. Subsequent field trials verified the greenhouse study results (Olcott-Reid et al., 1995a).

In a similar study, fruit quality at picking (firmness, compression strength and skin color) was tested over five years on a number of U.S. and Italian cultivars and selections (Testoni and Lovatti, 1989). Data were reported on 15 cultivars and 9 selections. Firmness and compression strength did not vary greatly among the cultivars. A high negative correlation was found between the compression strength (flesh firmness)

and the incidence (%) of rots during storage. The compression test appeared to be more reliable than the pressure test in predicting the susceptibility to rotting. Nevertheless, the ‘Cesena’ and ‘Dana’ berries showed a low percentage of rots, despite being rather soft at harvest and susceptible to bruising.

Fruit acidity may be a component of AFR resistance. However, its role in resistance remains unclear. Both ‘Sweet Charlie’ and ‘Pelican’ show reduced susceptibility to AFR but ‘Sweet Charlie’ is not acid while ‘Pelican’ is acid. This doesn’t necessarily eliminate acidity as a component of resistance. ‘Pelican’ may be less susceptible to AFR because of increased acidity while ‘Sweet Charlie’ may be less susceptible because of another resistance component or components. However, it should also be noted that many fungi are able to grow in high acid conditions.

Chemical composition of strawberry fruit, uninfected and infected by *Colletotrichum* species, was compared among susceptible and resistant seedlings (Wang and Galletta, 1997). Determination of resistance, inoculation techniques, or *Colletotrichum* species used or present was not reported. The authors report that fruit tissues from resistant genotypes contained more soluble solids content, titratable acid, sucrose, total sugar, ascorbic acid, putrescine, spermidine, and spermine than those from susceptible genotypes. Infected fruit had lower titratable acid but higher soluble solids content, fructose, glucose, and total sugar than uninfected fruit. More research and details are necessary to determine the importance and applicability of their findings.

Pre-harvest calcium treatments have also been shown to reduce post-harvest fungal disease development (possibly through delaying onset) (Cheour et al., 1990, 1991;

Makus and Morris, 1989; Smith and Gupton, 1993). Greenhouse-grown ‘Tangi’ was treated with various pre-harvest calcium applications to determine their effect on anthracnose infection of ripe fruit (Smith and Gupton, 1993). In the first study, two application methods (soil drench and foliar spray) of three calcium sources [Ca(NO₃)₂, CaCl₂, CaSO₄] were applied. Treatments were applied 3 times weekly before harvest and through harvest (13 weeks total). Ripe fruit from treated plants were harvested and incubated with a conidial suspension of *C. acutatum* (1.5x10⁵ conidia·ml⁻¹). Fruit treated with CaSO₄ had the lowest incidence of anthracnose and foliar applications of CaCl₂ were better than the drench.

Sources of Resistance

There is little information available on sources of anthracnose resistance within *Fragaria* species. Several genotypes of *Fragaria virginiana* appear to be susceptible to petiole infection in *C. fragariae* in studies by Delp and Milholland (1981). Later work by Ballington et al. (In press) indicated that resistance to *C. acutatum* and *C. fragariae* in petioles can be found in *F. virginiana* from Southeastern U.S. states. Other sources of resistance are species from closely related genera like *Duchesnea* and *Potentilla*. Delp and Milholland (1981) found that *D. indica* did not become infected after two inoculations with *C. fragariae* and that *P. canadensis* showed fleck-type lesions on the stolons and girdling, sharply defined lesions on petioles. Intergeneric *Fragaria* x *Potentilla* crosses have been made (Ellis, 1962; MacFarlane-Smith and Jones, 1985; Sayegh and Hennerty, 1993). It may be possible to incorporate resistance from related

genera using traditional breeding methods, tissue culture and/or molecular tools.

We know a little about inheritance of resistance to anthracnose in plants. We know virtually nothing about the mechanisms and inheritance of resistance to anthracnose in fruit. Similarly, we know little about the interaction, from both a physical and genetic perspective, between strawberry fruit and the pathogen(s). Progress is slowly being made in the U.S. and elsewhere. Results presented herein should enhance our understanding of the strawberry-*C. acutatum* and provide insights on breeding for AFR resistance.

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Chapter Two

**FIELD RESISTANCE OF STRAWBERRY TO ANTHRACNOSE
FRUIT ROT**

(In the format appropriate for submission to HortScience)

For: HortScience

Plant Pathology

Field Resistance of Strawberry to Anthracnose Fruit Rot

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***Abstract.* Field resistance of strawberry (*Fragaria ×ananassa* Duchesne) to anthracnose fruit rot (AFR) was characterized by inoculating five genotypes with *Colletotrichum acutatum* isolate CA-1 and determining the number of rotten fruit at harvest in 1998 and 1999. The experimental design was a split plot. Fruit in one whole plot were inoculated with conidia of *C. acutatum* and fruit in the other whole plot were not inoculated. Differences in yield, percent by weight, and number of berries with AFR were observed among genotypes in both years. The five cultivars ranked from highest to lowest AFR in 1998 were as follows: Chandler, Camarosa, Apollo, Sweet Charlie, and Pelican. Four cultivars and one breeding selection ranked from highest to lowest AFR in 1999 as follows: Chandler, Camarosa, NCR 95-08, Sweet Charlie, and Pelican. Severity of AFR was influenced by rainfall**

amount; severe AFR and high rainfall occurred in 1998 and low amounts of rainfall and AFR occurred in 1999. Reduced AFR severity did not necessarily result in reduced incidence or severity of other fruit rots. Plant source of ‘Camarosa’ and ‘Chandler’ (nursery vs. anthracnose-infested field) affected most yield components, including AFR, for ‘Camarosa’ but did not for ‘Chandler’. Daily air temperature and hours of leaf wetness within the plant canopy were measured for ‘Sweet Charlie’ and ‘Camarosa’. The AFR susceptible ‘Camarosa’ consistently had longer hours of leaf wetness per day than the AFR resistant ‘Sweet Charlie’. These results indicate that field resistance to AFR may be related to an open canopy architecture. These results also confirm that strawberry genotypes respond differently to anthracnose fruit rot under field conditions.

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North Carolina (N.C.) ranked 4th nationally in 2000 for strawberry production (Agricultural Statistics Board, 2001). In 2000, about 10,478 t were produced on 728 ha with a value of \$17.3 million. The principal production system is the annual hill training system (plasticulture) (Poling, 1993). Main components of this system include: raised, fumigated, offset, double beds; drip tape; and black plastic mulch over the beds. Plasticulture is profitable in N.C. because of the California strawberry ‘Chandler’. This cultivar is early maturing, tolerant of cool winter temperatures, and fruits over a long period (6 to 8 weeks).

However, the fruit of ‘Chandler’ are susceptible to anthracnose fruit rot (AFR) (Ballington and Milholland, 1993), caused by *Colletotrichum acutatum* Simmonds in N.C. (Grand et al., 1990). Mean fruit rot can be as high as 70% in plasticulture (Ballington and Milholland, 1993). Conidia of *C. acutatum* have been shown to be rain-dispersed more effectively on black plastic compared to either soil or straw (Madden et al., 1993; Yang et al., 1990). Thus, change to plasticulture from the matted row system provided increased yield potential but also increased crown rot, stolon lesions, and AFR. Furthermore, the humid conditions of the Southeast increase the incidence and severity of AFR. ‘Camarosa’ is another California strawberry with increasing commercial value to the N.C. strawberry industry but its fruit are also susceptible to AFR (Ballington et al., 1998). ‘Sweet Charlie’ (Chandler et al., 1997) and ‘Pelican’ (Smith et al., 1998) have been reported to be resistant to anthracnose. Sweet Charlie’ is primarily being used for early winter/spring fruiting, especially in Florida.

Field observations suggest that some cultivars and clones possess moderate levels

of resistance to AFR (Ballington et al., 1998; Maas, 1978; Olcott-Reid and Moore, 1995a, b). Olcott-Reid and Moore (1995b) showed that fruit firmness was correlated with AFR. Hancock et al. (1990) did not identify any resistance to AFR in *Fragaria*. Maas (1978) and Maas and Howard (1985) report that strawberry fruit appear to be uniformly susceptible to wound inoculation. Finally, plant resistance is not necessarily correlated with fruit rot resistance. ‘Sweet Charlie’ has susceptible foliage, runners, and petioles but the fruit usually ‘escapes’ severe rotting (Ballington, personal communication; Gimenez, 1997). Smith and Spiers (1982) reported that ‘Apollo’ plants are somewhat resistant but the fruit often display AFR (Smith and Black, 1990). These reports indicate that there may be genetic resistance of fruit to AFR but that its identification may be confounded by other factors including fruit characteristics, pathogen by cultivar interactions, and plant architecture.

The objectives of this study were to characterize field resistance of strawberries to anthracnose fruit rot and to compare disease development on plants infected with natural inoculum with those artificially inoculated.

Materials and Methods

The experimental design was a split plot. Inoculation was the whole plot. Fruit in one whole plot were inoculated with conidia of *Colletotrichum acutatum* isolate CA-1 [isolated by Milholland (Ballington and Milholland, 1993)]. Fruit in the other whole plot were not artificially inoculated but were exposed to natural inoculum. Cultivars were the

subplot and were arranged in a randomized complete block design with four replicates. The experiment was performed in 1998 and 1999.

Plants. In 1998, cultivars were Apollo, Camarosa, Chandler, Pelican, and Sweet Charlie. Runner tips were collected from plots at the Upper Piedmont Research Station (Reidsville, N.C.) for ‘Apollo’, ‘Camarosa’, ‘Chandler’, and ‘Pelican’. Additional plants of ‘Sweet Charlie’, ‘Camarosa’, and ‘Chandler’ were obtained from commercial nurseries in Eastern N.C. The “anthracnose clean” nursery plants of ‘Camarosa’ and ‘Chandler’ were compared to those collected from anthracnose infested fields at the Upper Piedmont Research Station (UPRS). Plants of ‘Camarosa’ and ‘Chandler’ from the nursery were designated ‘Camarosa’ (N) and ‘Chandler’ (N) while those from the UPRS field were designated ‘Camarosa’ (F) and ‘Chandler’ (F).

In 1999, the four cultivars were Camarosa, Chandler, Pelican, and Sweet Charlie. There were not sufficient runner tips of ‘Apollo’ to include it in the study. The breeding selection, NCR 95-08, was substituted for ‘Apollo’ because it had demonstrated reduced AFR compared to ‘Chandler’ and ‘Camarosa’ in elite breeding trials (Ballington et al., 1998). This selection, which resulted from the cross (FL 87-210 x ‘Delmarvel’), was developed and selected by the strawberry breeding program in the Horticultural Science Department at North Carolina State University.

In the 1998 study, crowns of ‘Apollo’, ‘Camarosa’ (F), ‘Chandler’ (F), and ‘Pelican’ were collected from the UPRS in July 1997, transported in coolers filled with ice, and stored for 2 d at 3 °C. Runner tips of ‘Sweet Charlie’, ‘Camarosa’ (N), and ‘Chandler’ (N) were obtained from the two nurseries and stored at 3 °C until planting.

Runner tips were removed from storage and transplanted into 40-cell plug trays containing peat substrate (Fafard Mix #4-P). The trays were placed into mist beds (5 sec/5 min) for approximately 12 d. Plants were then removed from the mist beds and placed on greenhouse benches until planting. Plants were watered daily and fertilized weekly with 20-20-20 (8.06 g/l of water).

In the 1999 study, runner tips of ‘Camarosa’, ‘Chandler’, and NCR 95-08 were obtained from the Mountain Horticultural Crops Research Station (Fletcher, N.C.) July 1998, transported in coolers filled with ice, and stored for 2 d at 3 °C. Runner tips of ‘Sweet Charlie’ were obtained from a commercial nursery in Western N.C. in July and stored at 3 °C. Plugs of ‘Pelican’ were obtained from Barbara Smith (USDA, Poplarville, Miss.) in August. Runner tips were rooted and treated as above.

Plants were transplanted into four raised beds at the UPRS on 15 September, 1997 for the 1998 harvest season and on 17 September, 1998 for the 1999 harvest season. The raised beds were fumigated with methyl bromide (224 kg·ha⁻¹) and covered with black plastic mulch. Each experimental unit was 1.53 m long by 76 cm wide and 15 to 20 cm high. Two rows of five plants each (10 total) were planted within each experimental unit (EU). In 1998, limited quantities of ‘Camarosa’ (F) made it necessary to distribute only five plants within the two rows of each EU, rather than 10. Plants were spaced 0.3 m apart within a row. A 30-cm border separated each EU. Rows of raised beds were on 1.5 m centers. Rows were oriented perpendicular to the slope of the field. ‘Pelican’ was used as a border around the entire experiment. A 1.5 m, two row plot of ‘Pelican’ was planted at the ends of the two split plots and between the two split plots in an effort to

limit spread of the pathogen.

Inoculation. One whole plot relied on natural inoculum to initiate infections. The other whole plot was inoculated with *C. acutatum* isolate CA-1. Cultures were maintained on potato dextrose agar (PDA) at 25 °C under fluorescent lights 12 hr each day. Cultures were transferred approximately 7-10 d before inoculation. To produce inoculum, conidia from approximately 1-wk-old cultures were washed from the dishes, vortexed for 1.5 min, filtered through cheesecloth, and suspended in 200 ml sterile, distilled water with two drops Tween 20 as a surfactant. The spore concentration was adjusted to 1×10^6 conidia·ml⁻¹ with the aid of a hemocytometer. Inoculations were performed in the early evening to maximize conditions for optimal infection. Fruit were sprayed until runoff. Inoculations were made weekly from 27 April to 1 June, 1998 and from 26 April to 31 May, 1999.

Environment. Environmental data were obtained from the Rockingham National Climatic Data Center, National Oceanic & Atmospheric Administration, located at Reidsville, N.C. (latitude 36°23' north, longitude 79°42' west, elevation 890'). Minimum and maximum air temperature (1.2 m above ground level) and rainfall were observed manually by personnel at the station each morning at 800 hours. Additionally, one temperature/humidity recorder (Spectrum Technologies, Plainfield, IL) was placed within a plot of 'Camarosa' and 'Sweet Charlie'. Each recorder was mounted on a 1 cm high plywood block with ~ 10° slope and placed at the center of each plot between the two rows on the raised bed. Leaf wetness and temperature within the canopy were recorded every half hour from 15 May to 3 June, 1998. Leaf wetness was determined by

measuring the resistance across the sensor grid. Values ranged from 0 to 15 ohms.

Based on preliminary misting experiments, leaves were considered wet when resistance across the grid was equal to or greater than 2 ohms. Values were recorded every half hour and total hours of leaf wetness were summed for each day.

Yield. Fruit were harvested weekly from each plot from 21 April to 10 June, 1998 and from 28 April to 2 June, 1999. They were graded into four classes: Marketable (all sound, marketable fruit greater than 2 cm diam); Culls (all misshapen fruit that were less than 2 cm diam, excluding diseased fruit); AFR (all fruit displaying characteristic anthracnose fruit rot symptoms); and Other Rots (all fruit displaying symptoms of other fruit rots including gray mold (*Botrytis cinerea* Pers. ex. Fr.) and leather rot [*Phytophthora cactorum* Leb and Cohn (Schroet.)]). Fruit in each class were weighed. The number of berries with AFR and other rots were counted. All data were converted to a per plant basis.

Data analysis. Yield data were analyzed as a split plot with inoculation as the whole plot and cultivar as the subplot. Means of main effects and interactions significant at $P \leq 0.05$ were separated using Fisher's protected LSD mean separation procedure. A combined analysis of variance was not performed on data from both years because cultivars and their sources changed from 1998 to 1999. Plant source effects (commercial nursery vs. field-grown) for 'Camarosa' and 'Chandler' were tested using single degree of freedom contrasts. In 1998, data (every half hour) from the temperature/humidity recorders, located within the strawberry canopy, were used to calculate total hours of leaf wetness per day and minimum, maximum, and mean air temperature per day during the

harvest season. In 1999, data from the sensor located in a plot of ‘Camarosa’ were not successfully retrieved from the sensor so no comparisons between ‘Camarosa’ and ‘Sweet Charlie’ could be made. Furthermore, no comparisons could be made between the two cultivars for daily rainfall or mean canopy temperature because only one sensor per cultivar was used to take measurements. However, the mean seasonal rainfall was compared between ‘Camarosa’ and ‘Sweet Charlie’ using paired t-tests.

Results

Rainfall and periods of high relative humidity were greater in 1998 than in 1999. Most yield components were also larger in 1998 than in 1999. Genotype had the biggest impact on yield components in both years (Table 1). Inoculation affected the percentage and number of berries with AFR in 1998 and the percentage of berries with other rots in 1999. There was a genotype by inoculation interaction for total yield (g/plant) in 1999. All results are presented on a per plant basis.

Genotype (Marketable). In 1998 ‘Pelican’, ‘Camarosa’ N, and ‘Chandler’ N and F had the highest yield of marketable fruit (Table 2). There was no difference among ‘Pelican’, ‘Chandler’ N and F, ‘Camarosa’ N, or ‘Apollo’. ‘Camarosa’ F yielded the lowest amount of marketable fruit. However, the results for percent marketable fruit were much different. The percent marketable fruit ranged from 49% to 75% in 1998 (Table 2). ‘Sweet Charlie’, ‘Apollo’, and ‘Pelican’ had the highest percent marketable fruit. ‘Camarosa’ and ‘Chandler’ had the lowest percent marketable fruit between 46% to

49%. In 1999 ‘Chandler’ and ‘Camarosa’ had the highest yield (g/plant) of marketable fruit; followed by NCR 95-08, and then ‘Sweet Charlie’ and ‘Pelican’ (Table 3). Again, ranking for percent marketable fruit was different. ‘Sweet Charlie’ had the highest percent marketable fruit. ‘Camarosa’ had significantly higher percent marketable fruit than ‘Chandler’. There was no difference in percent marketable fruit among ‘Camarosa’, NCR 95-08, and ‘Pelican’.

Genotype (AFR). Genotype affected AFR yield: weight (g/plant), by weight (%), and number of berries (berries/plant) in both years (Table 1). Yield of AFR ranged from 16 to 162 g/plant in 1998 (Table 2) and from 0 to 7 g/plant in 1999 (Table 3). The percent by weight and number of berries per plant were also much greater in 1998 than in 1999. In 1998, ‘Chandler’ N had the most total yield (g/plant) of AFR; ‘Camarosa’ had the next highest yield; and ‘Apollo’, ‘Pelican’, and ‘Sweet Charlie’ yielded the lowest AFR. Results for percent by weight of AFR were similar. ‘Pelican’, ‘Sweet Charlie’, and ‘Apollo’ all yielded low percentages of AFR between 6% to 11% that were not significantly different from each other. ‘Camarosa’ N yielded a higher percentage and ‘Chandler’ N, ‘Chandler’ F, and ‘Camarosa’ F yielded similarly high percentages of AFR between 34% to 39%. Results for number of berries per plant with AFR were similar to those for yield (g/plant) of AFR. Incidence and severity of AFR were greatly reduced in 1999. ‘Chandler’ had greater yield, percent by weight, and number of berries per plant of AFR than the other genotypes. All other genotypes did not produce different yield, percentage by weight, or number of berries per plant of AFR.

Genotype (Other rots). Genotype affected the yield (g/plant), percent by weight,

and number of berries exhibiting symptoms characteristic of fruit rots other than AFR (Table 1). In 1998, ‘Camarosa’ N and ‘Pelican’ yielded the highest grams per plant of other rots while ‘Camarosa’ F yielded the lowest. There was no difference among the other genotypes. Percent of fruit rots other than AFR ranged from 26% (‘Pelican’) to 10% (‘Chandler’ N). ‘Pelican’ had a higher percent of other rots than all genotypes except ‘Camarosa’ N. There was no difference among ‘Apollo’, ‘Camarosa’ F, ‘Chandler’ N and F, or ‘Sweet Charlie’. Results were different for 1999. ‘Chandler’ had the highest grams per plant of other rots; ‘Camarosa’ and NCR 95-08 had the next highest yield; and ‘Pelican’ and ‘Sweet Charlie’ had the lowest yield. Results were similar for number of berries per plant. However, the genotypes were not ranked similarly for percent of fruit with other rots. ‘Sweet Charlie’ had the lowest percent by weight of fruit with other rots. There was no difference among the other genotypes.

Plant source. Plant source (commercial nursery vs. field-grown) effected yield components for ‘Camarosa’ and ‘Chandler’ (Table 4). For ‘Chandler’, plant source only affected yield of fruit infected with AFR: ‘Chandler’ (N) yielded 162 g/plant AFR while ‘Chandler’ (F) yielded 110 g/plant (Table 2). Plant source for ‘Camarosa’ affected all yield components except percent marketable fruit (Table 4). Plant source did affect the yield (g/plant) of marketable fruit. For example, percent marketable fruit was identical between Camarosa (N) and Camarosa (F) but the total yield for Camarosa (N) was much higher than for Camarosa (F) (Table 2). Plant source also affected the percent of fruit with AFR. ‘Camarosa’ (N) yielded 21% AFR while ‘Camarosa’ (F) yielded 38% AFR.

Inoculation (1998). Inoculation with *C. acutatum* CA-1 increased the percentage

and number of berries with AFR (Table 1). The inoculated plots had a mean 26% AFR compared to a mean 18% for those that were not inoculated (Table 5). Total yield was not affected. Similarly, the number of berries per plant with AFR was higher (6.1) for the inoculated compared to the uninoculated plots (4.3).

Inoculation (1999). Inoculation did not increase yield or percent AFR but the percentage with other rots was higher in inoculated plots (Table 1). Plots that were inoculated had 13% other rots compared to 9% for uninoculated plots. Environmental conditions were not conducive to development of AFR and little AFR was seen in either inoculated or uninoculated plots.

Cultivar x Inoculation (1998). The cultivar x inoculation interaction was only significant for total yield (Table 1). In general, the total yield of inoculated plots was equal to or less than uninoculated ones (Fig. 1). The total yield of ‘Camarosa (N)’ was lower in inoculated plots (402 g/plant) compared to uninoculated (694 g/plant). However, for ‘Chandler’ (F) the total yield of inoculated plots (495 g/plant) was higher than uninoculated ones (294 g/plant).

Environment. The daily hours of leaf wetness usually appeared to be greater for ‘Camarosa’ than for ‘Sweet Charlie’; although they could not be compared statistically since only one measurement was taken per day (Table 6). Additionally, the seasonal mean hours of leaf wetness for ‘Camarosa’ was greater (10.8 hr/day) than for ‘Sweet Charlie’ (8.2 hr/day). The cumulative hours of leaf wetness from 15 June to 3 July was 215.5 hr for ‘Camarosa’ and 164.5 hr for ‘Sweet Charlie’. The AFR susceptible ‘Camarosa’ consistently had higher hours of leaf wetness per day than the AFR resistant

‘Sweet Charlie’. Daily mean canopy temperatures were usually higher for ‘Sweet Charlie’ than for ‘Camarosa’. The season mean canopy temperature was not different between the two cultivars (paired t-test).

Rainfall was greater in 1998 than in 1999 (Fig. 2). Cumulative rainfall from March to June was 44.2 cm (1998) and 31.7 cm (1999). Cumulative rainfall during the harvest season in 1998 (21 April to 10 June) was 17.8 cm and in 1999 (28 April to 2 June) was 13.3 cm. The total number of days of rainfall was also greater in 1998 from March to June (48 d) and during the harvest season (20 d) compared to 32 d from March to June and 8 d during the harvest season in 1999. The rainfall was also more evenly distributed in 1998 than in 1999; there were fewer prolonged periods of no rainfall. Daily mean temperatures were generally lower in 1999 than in 1998. There were 39 d (1998) and 45 d (1999) where the daily mean temperature was less than 14 °C. There were 34 d (1998) and 21 d (1999) where the daily mean temperature was greater than 22 °C.

Discussion

Genotype (Marketable). The percent marketable fruit was much lower in 1998 than in 1999 across all genotypes. But the yield of marketable fruit was actually lower in 1998 compared to 1999. In essence, environmental conditions were more conducive for both strawberry and AFR formation and development in 1998 compared to 1999. Marketable yields among genotypes also changed between years. ‘Camarosa’ and

'Chandler had the lowest percent marketable fruit in 1998 and the highest in 1999. This was due to the high percent AFR fruit in 1998 but low percent in 1999. In the year where AFR was severe, 'Sweet Charlie', 'Camarosa', and 'Chandler' had the highest marketable yield, even though AFR made up 40% of the total yield. But in 1999 where AFR was not severe, 'Chandler' and 'Camarosa' had the highest marketable yield, followed by NCR 95-08. These results suggest that 'Sweet Charlie' produces more marketable yield (g/plant) and a higher percent by weight than the two industry standards, 'Camarosa' and 'Chandler', during years where AFR is severe and harvest season is extended. But where AFR was not severe and the harvest season was shortened, 'Camarosa' and 'Chandler' clearly outperformed the other genotypes with respect to marketable yield; except for NCR 95-08 which yielded similarly to 'Camarosa'. More evaluation of NCR 95-08 is necessary to determine its performance.

Genotype (AFR). The yield, percent, and number of berries with characteristic AFR symptoms differed among genotypes. 'Chandler was the most susceptible genotype in both years. It had a similar percent by weight of berries with AFR as 'Camarosa' in 1998 but a higher percentage in 1999. 'Chandler' and 'Camarosa' do not differ in years where environmental conditions are conducive for AFR. These results also show that 'Chandler' has a higher marketable yield potential than 'Camarosa'. There were no differences among 'Apollo', 'Pelican', or 'Sweet Charlie'. For the conditions in N.C. (i.e. environmental factors and *C. acutatum* isolates), 'Chandler' was the most susceptible genotype that was tested. 'Camarosa' was the next most susceptible to AFR. 'Apollo', 'Sweet Charlie', and 'Pelican' were the least susceptible to AFR. It is unclear where

NCR 95-08 should be ranked, since it was not included in the 1998 study, but it appears to be no more susceptible than ‘Pelican’ or ‘Sweet Charlie’. NCR 95-08 consistently showed less AFR than ‘Camarosa’ or ‘Chandler’ field evaluations in N.C. (Ballington et al., 1998). Our results confirm that ‘Pelican’ fruit are resistant (Smith et al., 1998), or at least less susceptible than ‘Chandler’ and ‘Camarosa’.

Genotype (Other rots). Reduced susceptibility to AFR does not appear to always be associated with reduced susceptibility to other fruit rots. For example, ‘Pelican’ and ‘Camarosa’ had the highest percent of other rots but ‘Pelican’ had much less percent AFR than ‘Camarosa’. Furthermore, ‘Pelican’ and ‘Sweet Charlie’, two of the least AFR susceptible genotypes, yielded the same percent AFR but ‘Sweet Charlie’ yielded a higher percent of other rots than ‘Pelican’. It is not surprising that a strawberry genotype is much less susceptible to AFR than gray mold and vice versa since plants probably respond differently to attack by these two dissimilar pathogens. For example, gray mold is considered to be more of a problem in cool, humid conditions while AFR is more often a problem in warm, humid conditions. The fruiting structures produced by the two causal organisms are also very different: *B. cinerea* produces ovoid conidia in clusters on tall, slender conidiophores while *C. acutatum* produces ovoid or oblong conidia in disc-shaped, subepidermal acervuli. Plants probably respond differently to the two pathogens. Phenolics and tannins in young fruit have been implicated in resistance to *Botrytis* while phytoalexins have been found in *C. lindemuthianum*-*Phaseolus vulgaris* systems.

Plant Source. Plant source was critical for producing high yields of marketable fruit for ‘Camarosa’ but not for ‘Chandler’. ‘Camarosa’ propagated from nursery grown

runner tips had a lower percentage of AFR than ‘Camarosa’ propagated from field-grown tips or ‘Chandler’ propagated from either source. Plant parts of ‘Camarosa’ may harbor more latent infections of *C. acutatum* than ‘Chandler’. The runners of ‘Camarosa’ are more susceptible to *C. acutatum* than ‘Chandler’ (Gimenez, 1997). Anthracnose may be a problem if ‘Camarosa’ is propagated from on-farm runner tips or anthracnose-infested fields. The propagation source of ‘Camarosa’ is especially critical for reducing levels of AFR, maintaining plant vigor, and increasing marketable yield.

Inoculation. Inoculation with *C. acutatum* isolate CA-1 increased the yield and percentage of AFR. However, the relatively high level of AFR in uninoculated plots indicates that inoculum was present, either in the field harbored as latent infections on plants propagated from field grown runner tips or disseminated from inoculated plots to uninoculated ones. The latter is not likely as AFR was first observed at about the same time, May 6, in both inoculated and uninoculated plots. Inoculation increased the percent by weight of other rots in 1999. There is no simple explanation for these results. It is possible that infection with *C. acutatum* CA-1 may have predisposed the berries to subsequent infection by other fruit-rotting organisms like *Botrytis cinerea* and *Phytophthora cactorum*.

Environment. There was more rainfall and more frequent periods of rainfall in 1998 than in 1999. AFR was much greater in 1998 than in 1999. Rainfall and subsequent, extended periods of high relative humidity have been shown to increase incidence and severity of AFR (King et al., 1997; Madden et al., 1993; Wilson et al., 1990). This rainfall provided the increased relative humidity and longer period of high

RH necessary for conidial formation, germination, and penetration. The increased rainfall probably disseminated conidia further and more frequently. Ambient air temperature may affect incidence and severity of AFR but the weather data was not detailed enough to address this question. Other environmental factors may also play a role. We often observe high incidence and increased severity of AFR in yield trials at the Horticultural Crops Research Station (Castle Hayne, N.C.) (Ballington et al., 1998). The sandy soils typical of the coastal plain, as well as increased RH, may be involved. It is likely that wind-driven sand produces wounds that serve as a point of ingress for *C. acutatum* conidia. This may help to explain why anthracnose can be a problem in Israel (Freeman and Katan, 1997) where RH is generally lower and rainfall is less frequent and of lower volume than in the Southeast U.S.

These results also suggest that strawberry canopy architecture affects the microclimate of strawberry. The daily hours of leaf wetness were generally greater for ‘Camarosa’ than for ‘Sweet Charlie’ while the mean daily temperatures were generally lower. Together, these conditions would enhance pathogen infection, development, and propagule formatio. Canopy architecture has been shown to influence the microclimate and ultimately the incidence of white mold of dry edible beans (Blad et al., 1978) and botrytis bunch rot of grapes (Gubler et al., 1987). The leaf wetness periods were shorter and the temperature was lower in loose, open canopies compared to dense, lush canopies.

A loose, open canopy may be desirable for reducing anthracnose fruit rot. However, sun scald could become a problem if the canopy becomes too open. Furthermore, both ‘Pajaro’ and ‘Oso Grande’ have similar canopy architecture to ‘Sweet

Charlie’ but both are very susceptible to AFR. Therefore, canopy architecture is not the only component of resistance. Future studies should include more cultivars or genotypes and more leaf wetness/temperature sensors per cultivar. Genetic studies could then be done to determine inheritance of canopy architecture. Developing a standardized, quick method of assessing canopy architecture could enhance breeding for AFR resistance.

In summary, our studies show that there are differences in susceptibility to anthracnose fruit rot among genotypes in N.C. field conditions. ‘Chandler’ is the most susceptible. ‘Camarosa’ is slightly less susceptible. ‘Apollo’, ‘Pelican’, and ‘Sweet Charlie’ are the least susceptible. NCR 95-08 appears to respond like ‘Pelican’ and ‘Sweet Charlie’ but more evaluation is necessary during harvest seasons where AFR is severe. When AFR was severe, ‘Sweet Charlie’ produced more marketable yield than the other genotypes while ‘Chandler’ and ‘Camarosa’ produced more marketable yield when AFR was not severe. Nursery source propagation material is critical for ‘Camarosa’ to maintain yield and reduced disease levels while it does not appear to be as critical for ‘Chandler’. Increased rainfall and extended period of rainfall were associated with high levels of AFR in 1998. Canopy architecture may be a component in resistance to AFR and strawberry breeders may want to breed for more open canopies with better ventilation.

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Table 1. Mean squares for strawberry yield components at the Upper Piedmont Research Station (Reidsville, N.C.) for 1998 and 1999

Source	df	Total	Market		AFR			Other Rots		
		g/plant	g/plant	By weight (%)	g/plant	By weight (%)	Berries/plant	g/plant	By weight (%)	Berries/plant
1998										
Inoc (I)	1	10	1740	0.020	6293	0.087*	45.5*	2136	0.019	0.6
Rep x I	3	18731	4561	0.009	2599	0.012	6.9	4338	0.010	10.7
Gen (G)	6	141478***	47282**	0.097***	23726***	0.176***	152.6***	11635**	0.033***	44.8***
G x I	6	48355**	15045	0.010	954	0.011	4.1	6711	0.009	22.1
1999										
I	1	2676	467	0.014	1	0.000	0.0	970	0.022**	7.4
Rep x I	3	4246	2645	0.002	17	0.000	0.1	215	0.000	1.4
G	4	51533**	28127**	0.023**	67**	0.001**	0.4**	1328**	0.008**	8.4**
G x I	4	693	140	0.001	5	0.000	0.0	31	0.001	0.7

^zResults for reps not included because they were not significant. Inoc = inoculated; either with 1×10^6 conidia·ml⁻¹ *Colletotrichum*

acutatum isolate CA-1 or not inoculated. Geno = genotype. In 1998, genotypes were ‘Apollo’, ‘Camarosa’, ‘Chandler’, ‘Pelican’, and ‘Sweet Charlie’. In 1999, genotypes were ‘Camarosa’, ‘Chandler’, ‘Pelican’, ‘Sweet Charlie’, and NCR 95-08.

^y Mean squares are based on yield collected from 10 plants per plot, summed over the harvest season, and converted to a per plant basis. In 1998, harvested weekly from 21 April to 10 June and yield was summed over the 7 harvests. In 1999, harvested weekly from 28 April to 2 June and yield was summed over the 6 harvests. Total = yield of all fruit harvested over the season: including culls, marketable, and rotted fruit. Culls included all misshapen fruit, excluding diseased, that were less than 2 cm diam. Market = all marketable sound fruit > 2 cm diam. AFR = all fruit infected with anthracnose fruit rot. Other Rots = all fruit infected with other fruit rots (gray mold, leather rot, *Aspergillus*).

*, **, *** Significant at P = 0.05, 0.01, and 0.005, respectively.

Table 2. Yield components for strawberry cultivars at the Upper Piedmont Research Station (Reidsville, N.C.) in 1998

Genotype	Market		AFR			Other rots		
	g/plant	By weight (%/plant)	g/plant	By weight (%/plant)	Berries/ plant	g/plant	By weight (%/plant)	Berries/ plant
Apollo	172 c	63 a	30 c	11 cd	2.4 c	44 bc	16 bc	3.6 bc
Camarosa N	276 ab	49 bc	99 b	21 bc	6.0 b	133 a	23 ab	6.0 ab
F	64 d	49 bc	50 c	38 a	3.0 c	15 c	10 c	0.7 c
Chandler N	214 abc	46 c	162 a	39 a	12.2 a	43 bc	9 c	2.9 bc
F	201 abc	48 c	110 b	34 ab	9.9 a	53 bc	12 c	5.1 ab
Pelican	196 bc	62 ab	16 c	6 d	1.3 c	86 ab	26 a	8.1 a
Sweet Charlie	301 a	75 a	25 c	6 d	1.5 c	52 bc	13 c	5.1 ab
LSD =	101	14	35	15	3.0	47	8	3.3

^zStrawberry tips were obtained from two commercial nurseries in Eastern N.C. ('Camarosa', 'Chandler', and 'Sweet Charlie') or previous field trials ('Apollo', 'Camarosa', 'Chandler' and 'Pelican') at the Upper Piedmont Research Station (Reidsville, N.C.);

‘Camarosa’ and ‘Chandler’ were designated N for nursery source or F for field source. Tips were rooted in 40-cell plug flats under mist and planted 15 Sept., 1997.

^yStrawberries were harvested weekly from 21 April to 10 June for a total of 7 harvests. Yields were summed over the season and reported on a per-plant basis. Total = yield of all fruit harvested over the season: culls, marketable, and rotted fruit. Culls included all misshapen fruit, excluding diseased, that were less than 2 cm diam. Market = all marketable, sound fruit > 2 cm diam. AFR = fruit infected with anthracnose fruit rot. Other Rots = fruit infected with all other fruit rots (gray mold, leather rot, *Aspergillus*).

^xMeans (n=8) separation in columns by LSD ($P \leq 0.05$).

Table 3. Yield components for strawberry cultivars at the Upper Piedmont Research Station (Reidsville, N.C.) in 1999.

Genotype	Market		AFR			Other rots		
	g/plant	By weight (%/plant)	g/plant	By weight (%/plant)	Berries/ plant	g/plant	By weight (%/plant)	Berries/ plant
Camarosa	192 ab	82 b	3 b	1 b	0.1 b	26 b	11 a	1.9 b
Chandler	237 a	75 c	7 a	2 a	0.6 a	42 a	13 a	3.2 a
Pelican	87 c	77 bc	<1 b	<1 b	<0.1 b	15 c	12 a	1.9 b
Sweet Charlie	108 c	90 a	>1 b	>1 b	>0.1 b	6 c	5 b	0.5 c
NCR 95-08	171 b	78 bc	<1 b	<1 b	<0.1 b	29 b	12 a	3.1 ab
LSD =	48	7	1	1	0.3	11	4	1.1

^zStrawberry tips were obtained from field trials, researchers, or a commercial nursery, rooted in 40-cell plug flats under mist, and planted on 17 Sept., 1998: ‘Camarosa’, ‘Chandler’, and NCR 95-08 from Mountain Horticultural Crops Research Center (Fletcher, N.C.); ‘Pelican’ from Barbara Smith (USDA, Poplarville, MS); and ‘Sweet Charlie’ from a commercial nursery in Eastern N.C.

^yStrawberries were harvested weekly from 28 April to 2 June for a total of 6 harvests. Yields were summed over the season and

reported on a per-plant basis. Total = yield of all fruit harvested over the season: including culls, marketable, and rotted fruit. Culls included all misshapen fruit, excluding diseased, that were less than 2 cm diam. Market = marketable yield includes all sound fruit > 2 cm diam. AFR = fruit infected with anthracnose fruit rot. Other Rots = fruit infected with all other fruit rots (gray mold, leather rot, *Aspergillus*).

^x Means (n=8) separation in columns by LSD ($P \leq 0.05$).

Table 4. Mean squares contrasting yield components for transplants from the field compared to transplants from a nursery for ‘Camarosa’ and ‘Chandler’ at the Upper Piedmont Research Station (Reidsville, N.C.) in 1998

Contrast	Camarosa	Chandler
	Field vs. Nursery ^z	Field vs. Nursery
<u>Yield (g/plant)</u>		
Total	69224**	10380
Marketable	179670**	690
Anthracnose	9950**	10631**
Other Rots	56335**	1003
<u>% by weight</u>		
Marketable	0.000	0.001
Anthracnose	0.107**	0.009
Other Rots	0.060**	0.002
<u>Infected berries/plant</u>		
Anthracnose	36**	22
Other Rots	111**	20

^zMean squares for each single degree of freedom contrasts were based on 8 replicates of yield collected from 10 plants per plot, summed over the harvest season, and converted to a per plant basis. Field = transplants from strawberry yield trials at Upper Piedmont Research Station (Reidsville, N.C.). Nursery = transplants from a commercial nursery in

Eastern N.C.

**Significant at $P \leq 0.05$.

Table 5. Yield components of strawberry in plots inoculated with *Colletotrichum acutatum* CA-1^z compared to plots that were not inoculated at the Upper Piedmont Research Station (Reidsville, N.C.) in 1998

Source	<u>Yield (g/plant)</u>		<u>By weight (%)</u>	<u>Berries/plant</u>
	Total ^y	AFR	AFR	AFR
Inoculated	359	81	26 a	6.1 a
Not Inoculated	360	60	18 b	4.3 b
LSD =			8	1.6

^zIsolated by R. Milholland (Ballington and Milholland, 1993).

^yTotal = yield of all fruit harvested over the season: including culls, marketable, and rotted fruit. Market = marketable yield includes all sound fruit > 2 cm diam. AFR = fruit infected with anthracnose fruit rot.

^xWithin a column, mean separation (n=28) by LSD (P ≤ 0.05).

Table 6. Daily hours of leaf wetness and mean canopy temperature for ‘Camarosa’ and ‘Sweet Charlie’ strawberry at the Upper Piedmont Research Station (Reidsville, N.C.) in 1998

Date	Hours Leaf Wetness ^z		Mean Canopy Temperature (°C) ^z	
	Camarosa	Sweet Charlie	Camarosa	Sweet Charlie
5/15	7.0	6.0	22.3	24.0
5/16	9.5	7.0	25.7	27.3
5/17	13.0	13.0	25.0	25.9
5/18	10.0	10.0	24.0	24.9
5/19	8.0	3.0	24.5	25.4
5/20	11.0	4.0	26.2	25.4
5/21	11.0	10.5	25.3	25.3
5/22	9.0	9.0	22.5	22.3
5/23	15.0	15.0	17.8	17.6
5/24	8.0	7.0	20.0	20.4
5/25	7.5	7.5	24.7	24.8
5/26	11.5	10.5	24.6	25.4
5/27	17.5	12.0	21.7	22.2
5/28	9.5	8.0	24.3	25.7
5/29	11.0	9.0	26.1	27.4

Table 6, Continued.

5/30	14.5	5.0	26.2	27.1
5/31	9.0	8.0	26.0	26.9
6/1	9.5	1.5	27.2	27.9
6/2	13.5	8.0	24.6	25.1
6/3	10.5	10.5	21.5	21.0
Mean	10.8 a ^y	8.2 b	24.0	24.6
Total	215.5	164.5		

^zLeaf wetness and temperature recorded every half hour using temperature/humidity pens (Spectrum Technologies, Plainfield, IL) (one per cultivar) placed 1 cm above bed surface within the canopy.

^yMeans (n=20) followed by the same letter are not different. Paired t-test, $P \leq 0.05$.

Table 7. Rainfall and mean temperature for each month of the harvest season at the Upper Piedmont Research Station (Reidsville, N.C.) in 1998 and 1999

Month	Rainfall (cm) ^z		Mean Temp (°C)	
	1998	1999	1998	1999
March	9.8	7.2	8.4	7.9
April	14.6	11.4	14.5	15.5
May	11.8	6.0	19.8	18.5
June	<u>7.9</u>	<u>7.1</u>	24.1	21.8
Total	44.2	31.7		

^zRainfall and temperature data from the Rockingham National Climatic Data Center

(NOAA) at Reidsville, N.C. (latitude 36°23' N, longitude 79°42' W, elev. 890').

Rainfall was recorded daily and totaled for each month. Minimum and maximum temperature were recorded each day and a mean calculated. Daily means were used to calculate a monthly mean.

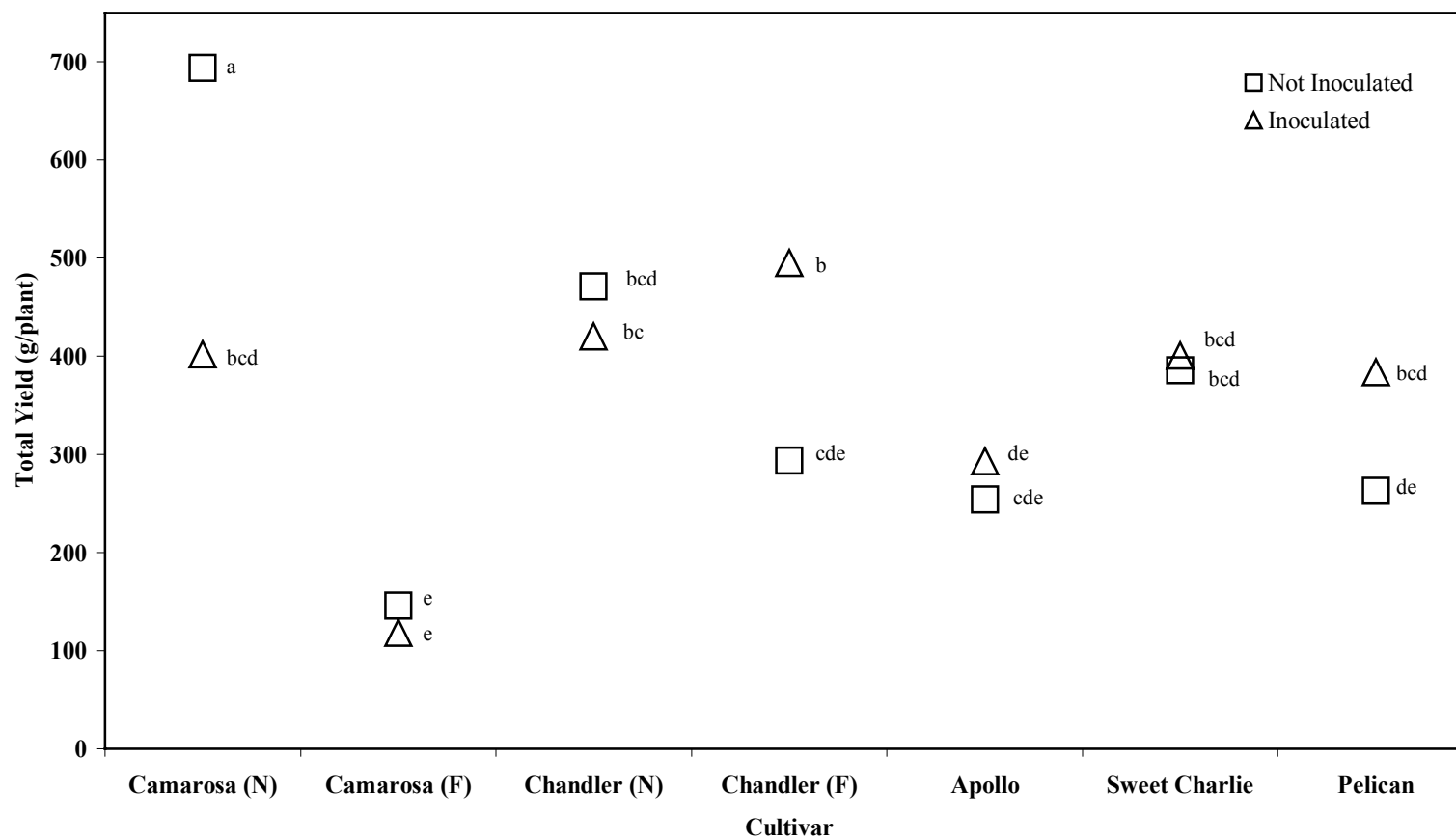


Figure 1. Total yield of strawberry cultivars inoculated with *C. acutatum* isolate CA-1 or not inoculated. Mean separation (n=4) by LSD ($P \leq 0.05$) = 179.

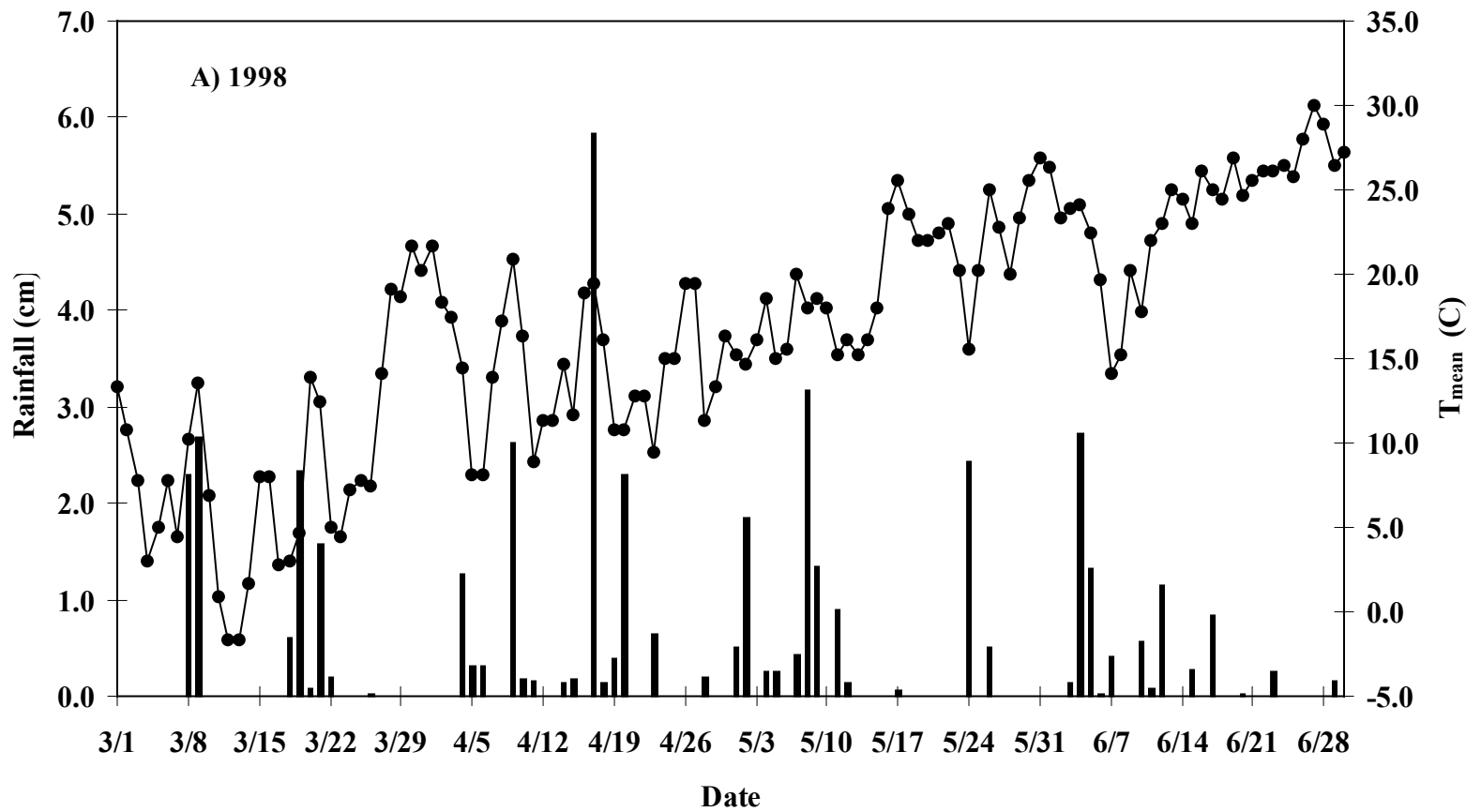


Figure 2, Continued.

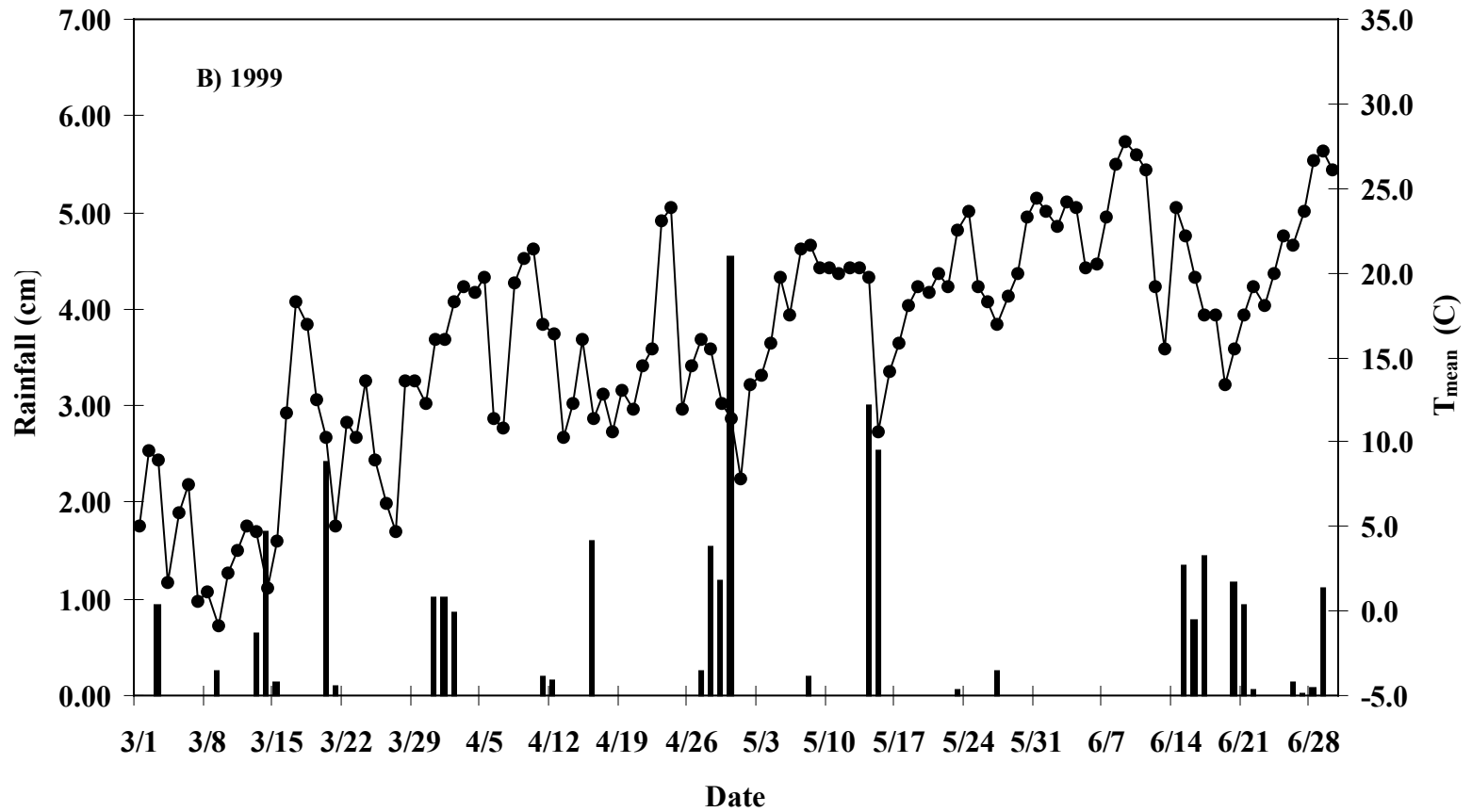


Figure 2. Daily rainfall (columns) and mean ambient air temperature (lines) at the Upper Piedmont Research Station in (Reidsville, N.C.) in A)1998 and B)1999.

Chapter Three

ANTHRACNOSE FRUIT ROT INCIDENCE AND PROGRESS IN STRAWBERRY INOCULATED WITH FIVE INOCULUM CONCENTRATIONS OF *COLLETOTRICHUM ACUTATUM*

(In the format appropriate for submission to Plant Disease)

For: Plant Disease

Plant Pathology

Anthracnose Fruit Rot Incidence and Progress in Strawberry Inoculated With Five Inoculum Concentrations of *Colletotrichum acutatum*

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***Abstract.* Green to fully ripe fruit of seven strawberry genotypes were inoculated with five inoculum concentrations of *Colletotrichum acutatum* Simmonds isolate CA-1 to characterize resistance to anthracnose fruit rot (AFR). The experimental design was a split plot with inoculum concentrations (0, 1×10^3 , 1×10^4 , 1×10^5 , and 1×10^6 conidia·ml⁻¹) as the whole plot. The genotypes (Apollo, Camarosa, Chandler, Pelican, Sweet Charlie, NCH 95-173, and NCR 95-08) were the subplot and were arranged in a randomized complete block with three replicates. The experiment was conducted three times within a growth chamber. Data on percent inoculations that became lesions (percent lesions), lesion length and width, incubation period, and fruit age were collected. Inoculum concentration, genotype, and the inoculum**

concentration x genotype were significant for most response variables studied. Best results were generally observed at 1×10^5 conidia·ml⁻¹. In general, the percent lesions increased as the inoculum concentration increased. Percent lesions were generally lower for genotypes with lower AFR in the field and higher for genotypes with higher AFR in the field. NCR 95-08 had much higher percent lesions than expected and NCH 95-173 was lower than expected. Logistic regression showed that the probability of lesion formation generally is low at young fruit age, increases at median age, and then rapidly decreases at older ages (i.e. over-ripe). This response curve is not the same among genotypes. Chandler had the earliest (~ 13 d) and the largest (~ 34%) peak. Pelican had the latest (~ 30 d) and smallest peak (~ 14%). Lesion diameter increased in a linear manner over time but it did not increase at the same rate for the seven strawberry genotypes for each inoculum concentration: Camarosa, Chandler, and NCH 95-173 generally had the fastest rates; Apollo had moderate rates; Sweet Charlie and NCR 95-08 had lower rates; and Pelican had the lowest rates of lesion expansion. Our results show that genotypes differ in percent lesions, probability of lesion formation due to fruit age, and lesion expansion rate when inoculated by *C. acutatum* isolate CA-1. Our results also show that a lower inoculum concentration, such as 1×10^4 or 1×10^4 conidia·ml⁻¹, may provide a better evaluation of AFR resistance in strawberry.

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Anthrachnose is considered to be the major limiting factor to strawberry production in North Carolina (N.C.) (Ballington and Milholland, 1993). Strawberry anthracnose can occur on all plant parts but the two most economically devastating forms are crown rot and fruit rot. The fungal species causing anthracnose fruit rot (AFR) in N.C. is *Colletotrichum acutatum* Simmonds (Grand et al., 1990). It has also become the dominant species affecting strawberry fruit in other parts of the U.S. such as Ohio (Ellis and Madden, 1991) and Connecticut (Lamondia, 1991).

Hancock et al. (1990) reported that there was no known genetic resistance to AFR. Pelican and Sweet Charlie are two cultivars that were recently released primarily for their resistance to anthracnose (Chandler et al., 1997; Smith et al., 1998). Field observations indicate that genotypes differ in incidence and amount of AFR in Maryland (Maas, 1978), North Carolina (Ballington and Milholland, 1993), and Arkansas (Olcott-Reid and Moore, 1995a). In N.C., mean percent fruit rot ranged from a high of 70% for Chandler to a low of 2% for FL 87-210 and MS/US 478. Results from controlled-environment studies are less definitive. Maas and Howard (1985) suggested that all cultivars were equally susceptible to anthracnose after wound inoculation. Based on pathogenicity tests, Ballington and Milholland (1993) suggested that there is no genetic resistance to fruit infection by *C. acutatum*, particularly on ripe fruit. They found that 100% of ripe fruit (Dover, FL 79-1126, Apollo, FLA 90, and Chandler) became rotted when inoculated with isolate CA-1 or CA-8 but this was not the case for green fruit. It also appeared that CA-8 was less virulent than CA-1. Denoyes-Rothan et al. (1999)

found resistance to AFR in 26 strawberry genotypes. Detached strawberry fruit were inoculated with 2×10^4 conidia·ml⁻¹ of *C. acutatum* isolate 1276b and lesion size and diameter were measured over time. There were differences in mean fruit lesion diameter and disease incidence among genotypes: susceptible genotypes like Pajaro and Elsanta had lesion sizes of 8.2 to 14.4 mm while resistant genotypes like Dover and US438 had lesion sizes of 0.4 to 1.0 mm. They also found that lesion incidence and lesion diameter were highly correlated for a given genotype ($r^2=0.89$ for a quadratic model); although they do not indicate which genotype was used to build this model or if the relationship was the same for all genotypes.

Inoculum concentrations have generally been in the 10^6 conidia·ml⁻¹ range or higher on various plant parts, including fruit (Ballington and Milholland, 1993; Delp and Milholland, 1981; Simpson et al., 1994; Smith and Black, 1987). Inoculum concentrations on fruit have generally been in the 10^4 conidia·ml⁻¹ range (Denoyes-Rothan et al., 1999; Wilson et al., 1990). Fruit age at inoculation also appears to affect amount of AFR observed (Wilson et al., 1990). On Midway strawberry, disease incidence was generally higher for mature fruit (beginning to turn red) compared to immature fruit (beginning to lose chlorophyll and turn whitish) at the same temperature and wetness duration.

Several questions remain unanswered. Does AFR incidence and development remain higher over a range of fruit ages for all genotypes? Are there different rates of disease development among cultivars? The objectives of this research were to: 1)

quantify rates of disease development and 2) determine the effect of fruit age on disease incidence and development by inoculating seven strawberry genotypes with five levels of *C. acutatum*.

Materials and Methods

Experimental design. The experiment was conducted in a controlled environment chamber at the Southeastern Plant Environmental Research Laboratory (phytotron) (NCSU, Raleigh, N.C.) The experimental design was a split plot with five inoculum concentrations (0 , 1×10^3 , 1×10^4 , 1×10^5 , and 1×10^6 conidia·ml⁻¹) as the whole plot. The seven strawberry genotypes were the subplot and were arranged in a randomized complete block with three replicates. The seven genotypes were randomized within each movable cart in the phytotron. Each cart represented one replicate. One additional plant of Sweet Charlie or Chandler was placed within each cart to provide additional pollen. The experiment was repeated three times (runs): from 6 February to 11 April, 1998; from 15 April to 14 May, 1998; and from 12 January to 22 March, 1999.

Plants. Strawberry genotypes included in this study were Apollo, Camarosa, Chandler, Sweet Charlie, and Pelican and the two breeding selections NCH 95-173 (NCH 87-10 x US 438) and NCR 95-08 (FL 87-210 x 'Delmarvel'. Both breeding selections were developed by the strawberry breeding program in the Horticultural Science Department at NCSU. Plants for the first two runs of the experiment came from the

following sources. Runner tips of Camarosa were obtained from a commercial nursery in Eastern N.C. on 1 November, 1997. They were planted in 40-cell plug trays, placed under mist (5 s per 5 min) for 14 d, and transplanted into 127 mm diam. plastic pots filled with Fafard Mix No. 4 (Anderson, S.C.) and placed on the greenhouse bench. Crowns of Apollo, Chandler, Sweet Charlie, Pelican, NCH 95-173, and NCR 95-08 were hand-dug from nursery plots at the Piedmont Research Station (Salisbury, N.C.) on 19 November, 1997 and stored for two d at 3 °C. Leaves were trimmed from crowns until two to three healthy, young leaves remained. Crowns were potted into 127 mm diam. plastic pots filled with Fafard Mix No. 4, and placed on greenhouse benches. On 21 November, 1997 all plants were heeled-in to beds of finely shredded bark in cold frames at the Horticultural Crops Research Station (Raleigh, N.C.). These plants were used in run 1 and 2.

For the third run of the experiment, runner tips were obtained from a number of sources: Camarosa and Chandler from Eric Bish (Horticulture Dept., NCSU); Sweet Charlie from a commercial nursery in Western, N.C.; and Apollo, Pelican, NCH 95-173, and NCR 95-08 from nursery plots at the Upper Piedmont Research Station (UPRS) (Reidsville, N.C.). Runner tips were stored at 3 °C. On 21 August, 1998 runner tips were planted and misted as described above. On 7 September, 1998 plugs were removed from mist and placed on greenhouse benches for 4 d after which they were transplanted to 127 mm diam pots and returned to the benches. On 21 September, 1998 plants were moved outside to a gravel pad at the Horticultural Crops Research Station (Raleigh,

N.C.). On 11 November, 1998 pots were heeled-in to cold frames as above.

Before each run of the experiment, plants were removed from the cold frames. All leaves were cut off except for two to three healthy leaves. Plants were moved into the Southeastern Plant Environmental Research Laboratory (NCSU, Raleigh, N.C.) (Phytotron) and fumigated overnight with a nicotine smoke generator (14 % a.i. expressed as alkaloid in 929 m³ room, Plant Products Corp., Vero Beach, FL). Plants were moved into a 9 m x 9 m x 2.13 m controlled environment chamber within the phytotron on 6 February (run 1), 14 April (run 2) in 1998, and on 12 January (run 3) 1999.

Environmental conditions were chosen to simulate average growing conditions in Reidsville, N.C. during March and April. Day/night temperatures were 22/14 °C. Flowering was induced by exposure to a short photoperiod from 8 AM to 5 PM using incandescent and fluorescent lighting (650 $\mu\text{mol}\cdot\text{m}^{-2}\text{ s}^{-1}$). Relative humidity was maintained at 70% for the growing period (except immediately after inoculation). Each plant received 300 ml/day of reverse osmosis purified water through the automatic watering system. Once a week, each plant received 300 ml of standard phytotron nutrient solution (N=106 ppm) (Thomas and Downs, 1991).

Pollination. Most flowers were self-pollinated by hand using an artist's brush. Flowers lacking pollen were pollinated using pollen from the additional Sweet Charlie or Chandler plant placed within each cart. Each pollinated flower was tagged and date of pollination was recorded on tags. Fruit age was calculated as number of d after

pollination (DAP).

Inoculum. *Colletotrichum acutatum* isolate CA-1, isolated from a lesion on a strawberry runner collected in N. C., was used in all inoculations (Ballington and Milholland, 1993). Cultures were maintained on potato dextrose agar (PDA) at 25 °C under fluorescent lights (12 hr per d). Cultures were transferred approximately 7-10 d before inoculation. To produce inoculum, conidia from approximately 1-wk old cultures were washed from surface of the plate with sterile, distilled water, vortexed for 1.5 min, filtered through cheesecloth, and suspended in 200 ml sterile, distilled water with two drops Tween 20 as a surfactant. The conidial concentration was adjusted with the aid of a hemacytometer to 0, 1×10^3 , 1×10^4 , 1×10^5 , and 1×10^6 conidia·ml⁻¹.

Fruit were monitored until a range of maturities (green to fully ripe) were observed. Using a permanent marker, three dots were equidistantly marked down the center of the outward facing surface of each inoculated fruit; preliminary inoculations with a 10 µl drop of the five inoculum concentrations on marked and unmarked fruit did not result in any differences in lesion development, size, or incidence. Fruit were inoculated by micropipetting a 10 µl drop on each mark. The estimated number of conidia applied in each drop for the inoculum concentrations 0, 1×10^3 , 1×10^4 , 1×10^5 , and 1×10^6 conidia·ml⁻¹ were 0, 10, 100, 1000, and 10000, respectively. Inoculation was completed by 1500 hours. Immediately after inoculation, relative humidity was increased to 100% and then decreased to 70% at 800 hours the next morning. Fruit were inoculated on the following dates: 20 March and 2 April, 1998 (run 1); 5 May, 1998 (run 2); and 19

February, 3 March, and 11 March, 1999 (run 3) for a total of six times.

After inoculation, data were collected about every two days. Incubation period was calculated as: day lesion 1st observed – day inoculated. Lesion length (along the long axis of the fruit) and width (along the short axis of the fruit) were measured using a hand-held caliper. It was assumed that lesions grew in a circular manner and therefore lesion diameter was calculated as: $\frac{\text{lesion length} + \text{lesion width}}{2}$.

Data Analysis. An analysis of variance (ANOVA) was conducted on data as a split plot design using the mixed models (MIXED) procedure of the Statistical Analysis System (SAS Institute, Cary, NC). Data were initially analyzed with run as a factor. If there were no differences among runs, data were pooled and re-analyzed without run as a factor. The relationship between fruit age at inoculation and the probability of lesion formation was investigated using the logistic regression (LOGISTIC) procedure of the SAS system. This was done since values for lesion formation are binary or dichotomous (0 = no lesion, 1 = lesion). Ordinary least squares regression is not an appropriate method since the range of responses is not quantitative. An analysis of covariance was used as a strategy to compare a series of inoculum concentration x genotype combination regression models over time (age of the lesion from inoculation throughout all evaluations), which was used as a covariate. The analysis of covariance was performed using the mixed (MIXED) procedure of the SAS system. The quadratic, cubic, and quartic terms for age of lesion did not significantly add to the model. Therefore, a simpler model with only the linear term was used. Inoculum concentration, genotype,

and the interaction were all significant. Therefore, simple linear regression models were estimated for each treatment combination. The estimated slopes (β 's) were then compared using least squares means (lsmeans, $P \leq 0.05$).

Results

Inoculations that became lesions. The percentage of inoculations that resulted in AFR lesions (percent lesions) was different among inoculum concentration ($P < 0.0001$) and inoculum concentration by genotype interaction ($P < 0.0001$). There were no differences among runs ($P = 0.215$). In general, the percent lesions increased as the inoculum concentration increased for all genotypes (Fig. 1). But the ranking of genotypes was not consistent across inoculum concentrations. A large portion of the interaction is explained by the response of NCR 95-08 which had some of the lowest percent lesions at 1×10^3 and 1×10^4 conidia·ml⁻¹ but some of the higher percentages at 1×10^5 and 1×10^6 conidia·ml⁻¹. Response of Chandler was similar to NCR 95-08. Response of Camarosa was much different. The percent lesions were among the highest at 1×10^3 and 1×10^4 conidia·ml⁻¹, median at 1×10^5 conidia·ml⁻¹, and the lowest at 1×10^6 conidia·ml⁻¹. Compared to the other genotypes, Pelican, Sweet Charlie, and NCH 95-173 had low to moderate percent lesions across inoculum concentrations. For most genotypes, the most dramatic increase in percent lesions was observed from 1×10^4 to 1×10^5 conidia·ml⁻¹. The largest range of mean percent lesions among genotypes was

observed at 1×10^5 conidia·ml⁻¹.

For a given genotype, the number of lesions increased as inoculum concentration increased. All comparisons between inoculum concentrations and genotypes were significant ($P \leq 0.05$) except for the following. There were no differences in the percent lesions between 1×10^3 and 1×10^4 conidia·ml⁻¹ for all genotypes ($P \leq 0.05$) and there were no differences between 1×10^3 and 1×10^5 conidia·ml⁻¹ for Camarosa, Pelican, and Sweet Charlie ($P \leq 0.05$). There were also no differences between 1×10^4 and 1×10^5 conidia·ml⁻¹ for Pelican, Camarosa, and NCH 95-173 ($P \leq 0.05$).

Fruit age at inoculation. Logistic regression revealed that fruit age at inoculation was a significant explanatory variable for predicting probability of lesion formation among the seven strawberry genotypes together (All) and separately (Table 1). Linear ($P < 0.0001$), quadratic ($P < 0.0001$), and cubic ($P < 0.0001$) terms were all significant. Therefore, logistic regression equations with all three terms were built for each genotype. Estimates from the logistic regression were used to calculate and graph the estimated probability of lesion formation due to fruit age at inoculation for each genotype (Fig. 2). The equation is in the form:

$$\text{Prob (Lesion)} = \frac{e^{b_1(\text{AgeInoc}) + b_2(\text{AgeInoc})^2 + b_3(\text{AgeInoc})^3}}{1 + e^{b_1(\text{AgeInoc}) + b_2(\text{AgeInoc})^2 + b_3(\text{AgeInoc})^3}}$$

where: b_1 , b_2 , and b_3 = estimated coefficients,

AgeInoc = fruit age at inoculation (d).

In general, the estimated probability of AFR lesion formation was low when fruit

were inoculated at a young age, increased at median age, and then decreased rapidly at old age. All genotypes showed a decreasing probability of lesion formation around 25-30 d. However, the shape of this response was different among genotypes. Apollo, Pelican, Sweet Charlie, and Camarosa had similar shapes; Pelican had much less probability of lesion formation over all fruit ages. NCR 95-08 and NCH 95-173 also had similar response curves: linearly decreasing probability of lesion formation with increasing fruit age at inoculation. The quadratic and cubic terms were not significant for NCH 95-173 and NCR 95-08 (Table 1). Finally, Chandler had the greatest estimated probability of lesion formation over most fruit ages, especially for younger fruit.

Incubation period (IP). Incubation period was different among inoculum concentrations but was not different among genotypes or the inoculum concentration x genotype interaction (Table 2). Incubation period was longest for the two highest inoculum concentrations (1×10^5 and 1×10^6 conidia·ml⁻¹) and shortest for the lowest inoculum concentration (1×10^3 conidia·ml⁻¹), other than the control. The standard deviations ranged from 2.01 to 2.49.

Lesion diameter. An analysis of covariance, with time (age of lesion) as the covariate, indicated that the quadratic, cubic, and quartic terms did not significantly add to the model (data not shown). Therefore, a regression model with only the linear term was chosen as the simplest model to describe the rate of AFR lesion diameter increase over time. Inoculum concentration (P=0.0095), strawberry genotype (P=0.023), and the inoculum concentration x genotype interaction (P=0.0015) were different.

Therefore, linear regression models were estimated for each inoculum concentration x genotype combination, excluding 0 conidia·ml⁻¹ (Table 3). All genotypes had lesions at all of the inoculum concentrations with the following exceptions: NCH 95-173 at 1x10³ conidia·ml⁻¹, and NCR 95-08 at 1x10³ and 1x10⁴ conidia·ml⁻¹. In general, the rate of lesion growth increased as the inoculum concentration increased across genotypes. However, the slope was not the same among the inoculum concentrations for each genotype. For Chandler, the slopes were different among 1x10³ to 1x10⁵ conidia·ml⁻¹. Results were similar for Camarosa except that slopes were not different between 1x10⁴ and 1x10⁵ conidia·ml⁻¹. Not enough lesions were observed at 1x10³ conidia·ml⁻¹ for NCH 95-173 to estimate a regression line. However, the slope for 1x10⁴ conidia·ml⁻¹ was smaller than the slopes for the two greatest inoculum concentrations, which were not different from each other. Results for the moderately susceptible genotypes were similar. For Apollo, rate of AFR lesion diameter increase was similar between 1x10³ and 1x10⁴ conidia·ml⁻¹ and between 1x10⁵ and 1x10⁶ conidia·ml⁻¹. Slopes for Sweet Charlie, NCR 95-08, and Pelican were similar at each inoculum concentration. However, not enough lesions were observed at the two lower inoculum concentrations for NCR 95-08 to estimate a regression line.

Comparisons were also made among genotypes at 1x10⁴ to 1x10⁶ conidia·ml⁻¹ (Table 3). At 1x10⁴ conidia·ml⁻¹, Pelican and Apollo had the smallest slopes, followed by Sweet Charlie, and then NCH95-173, Camarosa, and Chandler. Results were similar at 1x10⁵ conidia·ml⁻¹: Pelican had the smallest slope, followed by NCR 95-08 and Sweet

Charlie, then Apollo, and finally Chandler, Camarosa, and NCH 95-173. Differences among slopes were less at the highest inoculum concentration. Slopes were not different among Apollo, Sweet Charlie, Pelican, and NCR 95-08 or among Sweet Charlie, Pelican, NCR 95-08, and Chandler. NCH 95-173 and Camarosa had the largest slopes at the highest inoculum concentration.

Discussion

There were differences among inoculum concentrations and strawberry genotypes for the percent lesions, the probability of lesion formation due to fruit age, and rate of lesion diameter increase.

One component of resistance to AFR is the reduced percentage of inoculations that become lesions. Genotypes with the capacity to reduce percent lesions would be useful parents in breeding programs. We expected susceptible cultivars and genotypes would have the highest percent lesions, moderately susceptible would have medium percent lesions, and the least susceptible would have the lowest percent lesions. Most of the results were consistent with these expectations. The biggest differences among genotypes was at 1×10^5 conidia·ml⁻¹ (Fig. 1). As expected, Pelican and Sweet Charlie had some of the lowest percent lesions. Chandler, a susceptible cultivar, had one of the higher percent lesions. Camarosa, also quite susceptible, had a lower percent lesion than Chandler. The two surprising results were for the two breeding selections: NCH 95-173

had percent lesions as low as Pelican's while NCR 95-08 had percent lesions as high as Chandler's. Based upon field observations, we expected NCR 95-08 would have moderate to low percent lesions and NCH 95-173 would have high to moderate percent lesions. However, percent inoculations that became lesions is only one component of resistance. It should also be noted that only one isolate of *C. acutatum* was used in this study.

There are several fruit characteristics that could account for differences in percent lesions: skin toughness and depth of pit surrounding each achene. Some qualitative observations have been made about the correlation of skin toughness and AFR resistance. Apollo has tender-skinned fruit that sometimes show anthracnose symptoms (Smith and Black, 1990). Conversely, Chandler fruit skin is relatively tough but is rated as very susceptible (Gimenez, 1997). Skin toughness was poorly correlated (0.03 in 1991 and 0.22 in 1992) with reduced AFR (*C. gloeosporioides*) in field studies (Olcott-Reid and Moore, 1995b). However, fruit firmness was correlated with reduced AFR (0.26 and 0.30). Pre-harvest calcium treatments have also been shown to reduce the incidence of AFR due to *C. acutatum* (Smith and Gupton, 1993): possibly through delaying onset or by increasing fruit cell wall strength or thickness. Our preliminary studies on skin toughness using a texture analyzer (Instron) or a McCormic Fruit Tester did not indicate any differences in skin toughness among genotypes: variation among fruit was higher than variation among genotypes (data not presented). However, it is possible that fruit age could affect variability in skin toughness. We did not correct for fruit age in these

preliminary studies.

Sunken achenes could also play a role in the incidence and amount of AFR. Deeply sunken achenes could provide a place for conidia to lodge and provide a microclimate with increased relative humidity or moisture. To our knowledge, no studies have evaluated this trait. Depth of the pit around the achenes could be measured or estimated using scanning electron microscopy. However, *F. virginiana* accessions often have deeply sunken achenes and resistance to *C. acutatum* and *C. fragariae* has been identified in accessions collected from Southeastern U.S. states (Ballington et al., In press).

Fruit age at inoculation. Does fruit age at inoculation affect the probability of lesion formation? Our results show that the probability of lesion formation generally is low at young fruit age, increases at median age, and then rapidly decreases at older ages (i.e. over-ripe). It also appears that the shape of this response is not the same among genotypes. For example, the response curve is greater for Chandler than the other genotypes (i.e. Chandler fruit inoculated with *C. acutatum* CA-1 are more likely to develop AFR lesions than fruit of the same age from other genotypes). These results differ from those of King et al. (1997) who reported that ripe fruit were more susceptible than immature fruit since more hours of wetness were required for infection of immature fruit, compared to mature ones, at any given temperature. Wilson et al. (1990) also observed that disease incidence was generally higher for mature fruit (beginning to turn red) compared to immature fruit (beginning to lose chlorophyll and turn whitish) at the

same temperature and wetness duration. However, a range of fruit ages was not inoculated in either study, only immature and mature fruit. Our results also show that immature (10 to 20 d) fruit had less incidence of AFR than mature (20 to 30 d) and that over-ripe (> ~30 d) fruit also had less incidence of AFR than mature fruit. Of course, fruit maturity is somewhat dependent upon genotype.

Incubation period (IP). We postulated that susceptible strawberry cultivars would have shorter IP's. However, the results did not support this hypothesis. There were no differences in IP among the evaluated genotypes. However, a positive relationship was detected between inoculum concentration and IP: strawberries inoculated with higher inoculum concentrations had higher IP's. It was possible that the range of mean IP was greater at lower inoculum concentrations but the standard deviations were only slightly higher for the lower inoculum concentrations. One possible explanation for these results is that higher inoculum concentrations lead to an inhibitory competition among conidia and thus results in longer IP's. To the best of our knowledge, this has not been reported. Incubation period does not appear to play a major role in strawberry resistance to AFR.

Rate of lesion diameter growth. Based on field observations of cultivar susceptibility to AFR, we expected Camarosa and Chandler to have the fastest-growing lesions, moderately susceptible to have moderate-growing lesions, and less susceptible cultivars like Pelican and Sweet Charlie to have the slowest-growing lesions. Results were mostly consistent with these expectations across the levels of inoculum (Table 3). Lesion diameter increased in a linear manner over time but it did not increase at the same

rate for the seven strawberry genotypes for each inoculum concentration. For the susceptible to moderately susceptible genotypes, there were no differences in lesion expansion rate between 1×10^5 and 1×10^6 conidia·ml⁻¹ while there were differences for Sweet Charlie, NCR 95-08, and Pelican. Therefore, lesion expansion rates should probably be compared at 1×10^5 conidia·ml⁻¹. At this inoculum concentration, Camarosa and Chandler generally had the fastest rates of lesion expansion over most inoculum concentrations. NCH 95-173 had rates similar to Camarosa and Chandler. This was somewhat surprising as it often has less AFR in the field than Camarosa and Chandler. Apollo had moderate rates of lesion expansion while Sweet Charlie and NCR 95-08 had lower rates. Pelican had the lowest rates of lesion expansion. Genotypes with slower rates of AFR lesion growth should be useful in breeding for AFR resistance.

Our results also indicate that the “standard high level” of inoculum, 1×10^6 conidia·ml⁻¹ or higher, used by many researchers on other plant parts (Delp and Milholland, 1981; Gupton and Smith, 1991; Simpson et al. 1994), may overwhelm any genetic resistance that could exist in fruit. The most clear-cut differences between genotypes were observed at 1×10^5 conidia·ml⁻¹ for several traits such as percent inoculations that became lesions and rate of lesion growth. These results agree with Denoyes-Rothan et al. (1999) and Wilson et al. (1990) who used 10^4 conidia·ml⁻¹ range. A lower inoculum concentration such as 1×10^4 or 1×10^5 conidia·ml⁻¹, might provide a better evaluation of strawberry resistance to AFR under field and controlled conditions.

In summary, our results indicate that there is genetic resistance to AFR and that it

varies among strawberry genotypes. Two components are rate-limiting resistance and reduced percent inoculations that become lesions. Resistant genotypes had smaller rates of disease progress than susceptible ones and/or reduced percent lesions. However, these two components were not always observed together in the same genotype. For example, NCR 95-08 had high percent lesions but low rates of lesion growth (i.e. many lesions that grew at a small rate). Conversely, Camarosa had reduced percent lesions at 1×10^5 conidia·ml⁻¹ but greater rates of lesion growth (i.e. fewer successful inoculations but growing at a faster rate). Finally, fruit inoculated at a younger age had a higher probability of developing AFR symptoms than older fruit.

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Table 1. Equations and chi-square probabilities from logistic regression for fruit age at inoculation for seven strawberry genotypes together (All) and separately

Genotype	Estimate			Pr > χ^2		
	b_1	b_2	b_3	b_1	b_2	b_3
All	-0.281	0.0156	-0.00028	< 0.0001	< 0.0001	< 0.0001
Apollo	-0.584	0.0387	-0.0007	< 0.0001	< 0.0001	< 0.0001
Camarosa	-0.3628	0.0214	-0.00038	< 0.0001	< 0.0001	< 0.0001
Chandler	-0.121	0.00748	-0.00017	0.02430	0.0470	0.0166
Pelican	-1.339	0.0838	-0.00137	< 0.0001	0.0002	0.0001
Sweet Charlie	-0.319	0.0171	-0.00029	< 0.0001	0.0026	0.0048
NCH 95-173	-0.170	0.00674	-0.00012	0.00570	0.1359	0.1523
NCR 95-08	-0.150	0.00620	-0.00010	0.0128	0.1359	0.1523

^ZEstimates are used to calculate an estimated probability of AFR lesion occurrence with

the equation: $\text{Prob (Lesion)} = \frac{e^{b_1(\text{AgeInoc})+b_2(\text{AgeInoc})^2+b_3(\text{AgeInoc})^3}}{1 + e^{b_1(\text{AgeInoc})+b_2(\text{AgeInoc})^2+b_3(\text{AgeInoc})^3}}$, where: b_1 , b_2 , and b_3 =

estimated coefficients and AgeInoc = fruit age at inoculation (d).

Table 2. Incubation period and ANOVA for seven strawberry genotypes treated with five inoculum concentrations of *Colletotrichum acutatum* CA-1 inoculum

Inoculum concentration (conidia·ml⁻¹)		Mean^z	
1x10 ³		3.71 c	
1x10 ⁴		5.87 b	
1x10 ⁵		6.84 a	
1x10 ⁶		6.91 a	

Source of Variation	df	Sum of Squares	Percent of total
Treatments	24	307	
Inoculum concentration (I)	3	211 ^{***}	69
Genotype (G)	6	23	7
I x G	15	73	24

^zNo AFR was observed at the first level (0 conidia·ml⁻¹) and results were not included in the analysis. Estimated number of conidia for 1x10³, 1x10⁴, 1x10⁵, and 1x10⁶ conidia·ml⁻¹ was 10, 100, 1000, and 10000 conidia per drop, respectively. Genotype means followed by the same letter are not significantly different (LSD=0.83, P=0.05).

^{***} Significant at P ≤ 0.0005.

Table 3. Anthracnose fruit rot lesion diameter regressed over time for seven strawberry genotypes treated with four inoculum concentrations of *Colletotrichum acutatum* isolate CA-1 conidia

Genotype	Inoculum concentration (conidia·ml ⁻¹) ^z			
	1x10 ³	1x10 ⁴	1x10 ⁵	1x10 ⁶
Camarosa	y = 0.50x a, R ² = 0.66 ^y	y = 1.17x b, R ² = 0.74	y = 1.33x c, R ² = 0.92	y = 1.39x c, R ² = 0.88
Chandler	y = 1.11x a, R ² = 0.96	y = 1.23x b, R ² = 0.84	y = 1.32x bc, R ² = 0.91	y = 1.30x c, R ² = 0.89
NCH 95-173	--	y = 1.17x a, R ² = 0.72	y = 1.36x b, R ² = 0.84	y = 1.35x b, R ² = 0.85
Apollo	y = 0.63x a, R ² = 0.76	y = 0.61x a, R ² = 0.75	y = 1.15x b, R ² = 0.77	y = 1.18x b, R ² = 0.82
Sweet Charlie	y = 0.60x a, R ² = 0.96	y = 0.80x b, R ² = 0.68	y = 0.90x b, R ² = 0.92	y = 1.20x c, R ² = 0.86
NCR 95-08	--	--	y = 0.89x a, R ² = 0.88	y = 1.21x b, R ² = 0.86
Pelican	y = 0.54x a, R ² = 0.25	y = 0.59x a, R ² = 0.68	y = 0.70x b, R ² = 0.65	y = 1.24x c, R ² = 0.86

Genotype	Contrasts by inoculum concentration ^x			
	1x10 ^{3h}	1x10 ⁴	1x10 ⁵	1x10 ⁶

Table 3, Continued.

Camarosa	ND	$y = 1.17x \text{ d}$	$Y = 1.33x \text{ d}$	$y = 1.39x \text{ d}$
Chandler	ND	$y = 1.23x \text{ d}$	$Y = 1.32x \text{ d}$	$y = 1.30x \text{ bc}$
NCH 95-173	ND	$y = 1.17x \text{ d}$	$Y = 1.36x \text{ d}$	$y = 1.35 \text{ d}$
Apollo	ND	$y = 0.61x \text{ a}$	$Y = 1.15x \text{ c}$	$y = 1.18x \text{ a}$
Sweet Charlie	ND	$y = 0.80x \text{ b}$	$Y = 0.90x \text{ b}$	$y = 1.20x \text{ ab}$
NCR 95-08	ND	--	$Y = 0.89x \text{ b}$	$y = 1.21x \text{ ab}$
Pelican	ND	$y = 0.59x \text{ a}$	$Y = 0.70x \text{ a}$	$y = 1.24x \text{ abc}$

^zEstimated number of conidia for 1×10^3 , 1×10^4 , 1×10^5 , and 1×10^6 conidia·ml⁻¹ was 0, 10, 100, 1000, and 10000 conidia per drop, respectively.

^y y = AFR lesion diameter (mm), x = AFR lesion age (d). Within a row (genotype), equations followed by the same letter are not different by lsmeans ($P=0.05$). -- = not enough lesions over time to develop an equation.

^xWithin a column (inoculum concentration), equations followed by the same letter, equations followed by the same letter are not different by lsmeans ($P=0.05$). ND = contrasts were not done for 1×10^3 conidia·ml⁻¹.

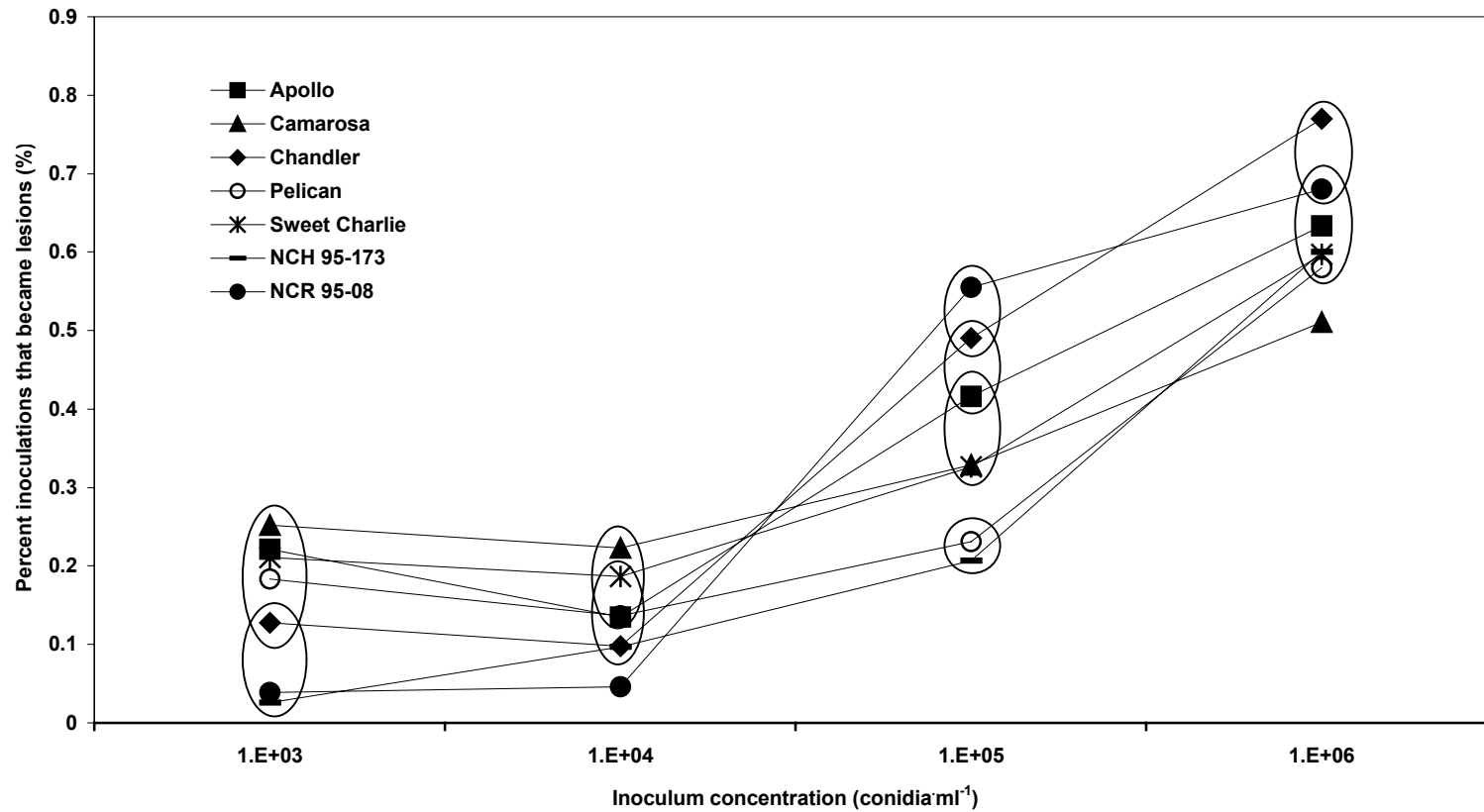


Figure 1. Percent of inoculations that became AFR lesions for seven genotypes inoculated with four concentrations of *C. acutatum* isolate CA-1. Within a concentration, genotypes enclosed by the same circle are not different by lsmeans ($P \leq 0.05$).

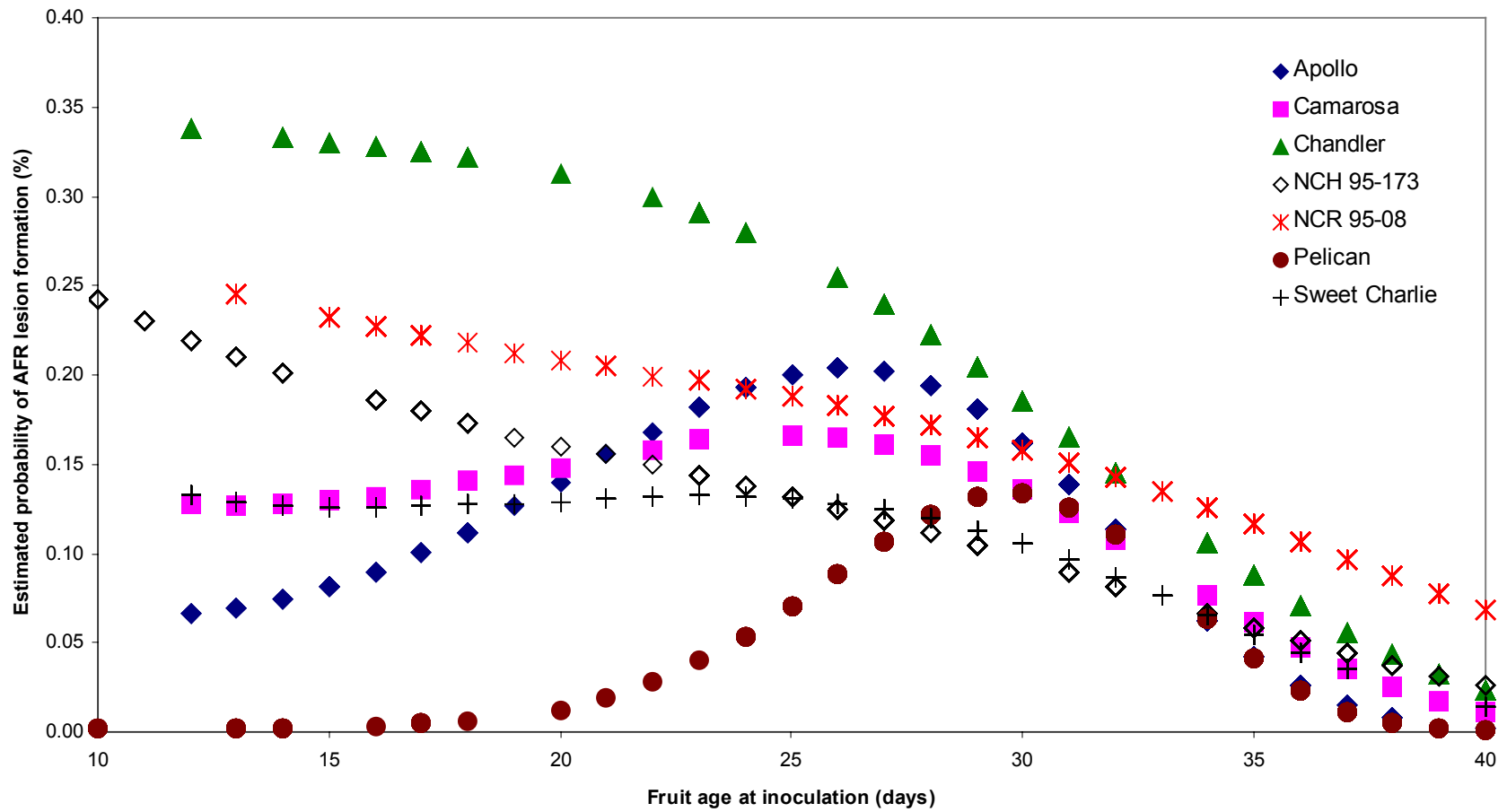


Figure 2. Estimated probability of AFR lesion formation due to fruit age at inoculation for seven strawberry genotypes.

Chapter Four

ANTHRACNOSE FRUIT ROT OF STRAWBERRY INOCULATED WITH THREE ISOLATES OF *COLLETOTRICHUM ACUTATUM*

For: Plant Disease

Plant Pathology

Anthracnose fruit rot of strawberry inoculated with three isolates of *Colletotrichum acutatum*

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***Abstract.* An experiment was conducted in a growth chamber to determine if an interaction exists among strawberry (*Fragaria* × *ananassa* Duch.) genotypes and isolates of *Colletotrichum acutatum* Simmonds with respect to anthracnose fruit rot (AFR). Seven strawberry genotypes, varying from resistant to susceptible, were inoculated with three isolates of *C. acutatum* in a growth chamber at the Southeastern Plant Environmental Laboratory. Green to fully ripe fruit were inoculated on the outward facing surface with three 10- μ l drops at 1×10^6 conidia·ml⁻¹. The incubation period (IP) was determined, lesion length and width were measured, and lesion area was calculated. IP was different among genotypes and isolates. An elite breeding selection, NCR 95-08, from the North Carolina**

strawberry breeding program, had a longer IP than Camarosa, Apollo, Chandler, and Pelican. The resistant cultivar Pelican had the shortest IP. This could indicate a hypersensitive response to AFR in Pelican. The genotype x isolate interaction for mean lesion area (square root transformed) was significant in the early evaluations but not at the final (4th) evaluation. Rates of disease development were different among genotypes and isolates, suggesting that there is resistance to AFR in strawberry.

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Anthraco­nose is considered a major limiting factor to strawberry production in North Carolina (N.C.) (Ballington and Milholland, 1993). Strawberry anthracnose can occur on all plant parts but the two most economically devastating forms are crown rot and fruit rot. The species causing anthracnose fruit rot (AFR) in North Carolina is *Colletotrichum acutatum* Simmonds (Grand et al., 1990). Anthracnose fruit rot is generally considered to be a warm-weather disease restricted primarily to the major production regions in the Southeastern United States and California (Eastburn and Gubler, 1990; Maas, 1984). However, it has also become the dominant species affecting strawberry fruit in other parts of the U.S. as far north as Ohio (Ellis and Madden, 1993) and Connecticut (Lamondia, 1991).

Little is known about resistance of strawberry fruit to *C. acutatum*. Some research has indicated that no has been found in *Fragaria* (Hancock et al., 1990; Maas and Howard, 1985). Other research suggests that it does (Denoyes-Rothan et al., 1999; Ballington and Milholland, 1993; Maas, 1978; Olcott-Reid and Moore, 1995). In vitro inoculation studies using detached strawberry fruit inoculated with one 15- μ l drop containing 2×10^4 conidia·ml⁻¹ of *C. acutatum* 1276b indicated that fruit lesion diameter and disease incidence was different among 26 strawberry genotypes (Denoyes-Rothan et al., 1999). Mean fruit lesion diameter ranged from 0.4 mm for US438 to 14.4 mm for Pajaro. Chandler had a mean lesion diameter of 3.3 mm and disease incidence of 57%. Disease incidence was highly correlated with lesion size ($r^2=0.89$).

Furthermore, no *Fragaria-C. acutatum* system has been definitively identified.

Smith and Black (1990) reported genotype x isolate interactions for *C. fragariae* with respect to stolon and plant resistance. They suggested the presence of a gene-for-gene system. Hancock et al. (1990) summarized data that suggested there is an isolate by cultivar interaction for isolates of *C. acutatum*, *C. fragariae*, and *C. gloeosporioides*. Ballington and Milholland (1993) did not find an interaction with respect to percentage of AFR among five genotypes inoculated with two isolates of *C. acutatum* (CA-1 from fruit and CA-8 from runner in N.C.) The percent AFR was higher for green fruit inoculated with CA-1 compared to CA-8 for all genotypes, indicating that CA-1 is more virulent than CA-8. Isolates from hosts other than strawberry were weakly virulent to almost nonpathogenic on runners.

Our objective was to determine if there is an interaction among strawberry genotypes and *C. acutatum* isolates with respect to AFR.

Materials and Methods

Experimental design. The experimental design was a split plot with three *C. acutatum* isolates (see *Inoculum* below) as the whole plot. The seven strawberry genotypes were the subplots and were arranged in a randomized complete block with five replicates. The seven genotypes were randomized within each movable cart used in the phytotron. Each cart represented one replicate. One extra plant of Sweet Charlie or Chandler was placed within each cart to provide additional pollen.

Plants. Strawberry genotypes included in the study were: Apollo, Camarosa, Chandler, Sweet Charlie, and Pelican and the two breeding selections NCH 95-173 (NCH 87-10 x US 438 and NCR 95-08 (FL 87-210 x ‘Delmarvel’)); both selections were developed by the strawberry breeding program in the Horticultural Science Department at NCSU. Plants were grown in the Horticultural Science greenhouse facilities on the North Carolina State University (NCSU) campus. Runner tips were obtained from a number of sources: Camarosa and Chandler from Eric Bish (Horticulture Dept., NCSU, N.C.); Sweet Charlie from a commercial nursery in eastern N.C.; and Apollo, Pelican, NCH 95-173 and NCR 95-08 from nursery plots at the Upper Piedmont Research Station (UPRS) (Reidsville, N.C.). Runner tips were stored at 3 °C. On 21 August, 1998, runner tips were planted in 40-cell plug trays filled with Fafard Mix No. 4 (Anderson, S.C.) and placed under mist (5 s per 5 min) until 7 September. They were transplanted into 127 mm diam plastic pots filled with Fafard Mix No. 4 and placed on the greenhouse bench. On 21 September plants were moved outside to a gravel pad at the Horticultural Crops Research Station (Raleigh, N.C.). On 12 November pots were heeled-in to beds of finely shredded bark in cold frames at the Horticultural Crops Research Station (Raleigh, N.C.).

Plants were removed from the cold frames before the experiment. All leaves were cut off except for two to three healthy leaves. Plants were moved into the Southeastern Plant Environmental Research Laboratory (NCSU, Raleigh, N.C.) (Phytotron) and fumigated overnight with a nicotine smoke generator (14 % a.i. expressed as alkaloid in 929 m³ room, Plant Products Corp., Vero Beach, FL). Plants were randomly placed into

carts, each genotype once per cart, and moved into a 9 m x 9 m x 2.13 m controlled environment chamber on 25 March, 1999.

Environmental conditions were chosen to simulate average growing conditions in the northern Piedmont of N.C. during March and April. Day/night temperatures were 22/14 °C. Flowers were induced by exposure to a short photoperiod from 800 to 1700 hours using incandescent and fluorescent lighting ($650 \mu\text{mol m}^{-2} \text{s}^{-1}$). Relative humidity was maintained at 70%, except immediately after inoculation when the relative humidity was increased to 100% and then decreased to 70% at 800 hours the next morning. Each plant received $300 \text{ ml}\cdot\text{day}^{-1}$ of reverse osmosis purified water through the automatic watering system. Once a week, each plant received 300 ml of standard phytotron nutrient solution (N=106 ppm) (Thomas and Downs, 1991). Flowers were self-pollinated by hand using an artist's brush. Flowers lacking pollen were pollinated using pollen from the extra Sweet Charlie or Chandler plant placed within each cart. Each pollinated flower was tagged and date of pollination was recorded on each tag. Fruit age was calculated as number of days after pollination (DAP).

Inoculum. Three isolates of *C. acutatum* were used: CA-1, AROM-NCH, and CHAN1-NCH. CA-1 was isolated from a strawberry runner in North Carolina (Ballington and Milholland, 1993). AROM-NCH and CHAN1-NCH were isolated on 27 April, 1999 from strawberry fruit with AFR in yield trials at the Horticultural Crops Research Station (Castle Hayne, N.C.) (collected and isolated by J. Shuman). AROM-NCH was collected from 'Aromas'. Lesions had concentric tan-brown rings, fluffy white

mycelium around the edges, and conidia in acervuli that were orange-black in mass. CHAN1-NCH was collected from large, black, sunken lesions on Chandler with conidia that were orange in mass. Cultures were maintained on potato dextrose agar (PDA) at 25 °C under fluorescent lights 12 hr each day. Cultures were transferred approximately 7-10 d before inoculation. To produce inoculum, conidia from approximately 1-wk-old cultures were washed from surface of the plate with sterile, distilled water, vortexed for 1.5 min, filtered through cheesecloth, and suspended in 200 ml sterile, distilled water with two drops Tween 20 as a surfactant. The concentration was adjusted with the aid of a hemacytometer to 1×10^6 conidia·ml⁻¹. Fruit were monitored until a range of maturities (green to fully ripe) was observed. Three dots were equidistantly marked down the center of the outward facing surface of each fruit using a permanent marker. Fruit were inoculated by micropipetting a 10 µl drop (~10,000 conidia) on each mark. Inoculation was completed by 1500 hours. Fruit were inoculated on 10 May, 1999. The experiment was repeated on 21 May by inoculating a second set of fruit on the same plants.

After inoculation, data were collected about every two days. The incubation period was calculated as: day lesion 1st observed – day inoculated. Lesion length (along the long axis of the fruit) and width (along the short axis of the fruit) were measured using a hand-held caliper. It was assumed that lesions grew in a circular manner and therefore lesion diameter was calculated as: $\frac{\text{lesion length} + \text{lesion width}}{2}$.

Data Analysis. An analysis of variance (ANOVA) was conducted on data as a split plot design using the mixed models (MIXED) procedure of the Statistical Analysis

System (SAS Institute, Cary, NC). The mixed models procedure was used as a strategy to compare a series of isolate x genotype combination regression models over time (age of the lesion from inoculation throughout all evaluations), which was used as a covariate. The quadratic, cubic, and quartic terms for age of lesion did not significantly add to the model. Therefore, a simpler model with only the linear term was used. Isolate, genotype, and the interaction were all significant. Therefore, simple linear regression models were estimated for each treatment combination. The estimated slopes (β 's) were then compared using least squares means (lsmeans, $P \leq 0.05$).

Results

Percent lesions. The percentage of inoculations that resulted in lesions was different among genotypes ($P < 0.0001$) and the isolate x genotype interaction ($P < 0.0001$) but was not different among isolates ($P = 0.885$). Therefore, least squares means were used to compare isolate x genotype combinations (Table 1). Percent lesions ranged from 90% (Pelican) to 47% (NCR 95-08). There were no differences among the isolates for Camarosa and Apollo but there were for the other genotypes. For Pelican, percent lesion was different for all three isolates: CA-1 was highest, AROM-NCH median, and CHAN1-NCH lowest. Results were the reverse for NCH 95-173. Results were similar for Chandler and NCR 95-08. Percent lesion for isolate CHAN1-NCH was lower than isolate CA-1 and AROM-NCH. However, the percent lesions for NCR 95-08 were lower

than for Chandler. For Sweet Charlie, percent lesion for isolate AROM-NCH was lower than the other two isolates.

Incubation Period. The incubation period was different among genotypes and isolates (Table 2). There was no genotype x isolate interaction. The IP for NCR 95-08 was longer than the IP for Apollo, Camarosa, Chandler, and Pelican. The IP did not differ among the other genotypes. There were differences in IP among the three isolates; CA-1 had a lower IP than CHAN1-NCH.

Lesion growth rate. An analysis of covariance with time (age of lesion) as the covariate indicated that the quadratic, cubic, and quartic terms did not significantly add to the model. A model with the linear term for age of lesion at inoculation was used. Isolate ($P=0.0153$), genotype (0.0073), and isolate x genotype (0.0025) were significant. Thus, AFR lesion diameter was regressed over time (linear term only) for seven genotypes inoculated with three isolates (Table 3). Ranking of genotypes was not consistent across isolates. For some genotypes, isolate AROM-NCH had the fastest lesion growth rate while in others isolate CHAN1-NCH had the fastest rate. Growth rates were generally the slowest for CA-1 for most genotypes. Finally, NCR 95-08 had some of the slowest growth rates over isolates.

Discussion

A genotype x isolate interaction has been reported for strawberry-*C. fragariae*

with respect to stolon and plant resistance (Smith and Black, 1990). They suggested the presence of a gene-for-gene system. Hancock et al. (1990) summarized data that suggested there is an isolate by cultivar interaction for isolates of *C. acutatum*, *C. fragariae*, and *C. gloeosporioides*. Ballington and Milholland (1993) did not find an interaction with respect to percentage of AFR among five genotypes inoculated with two isolates of *C. acutatum* (CA-1 from fruit and CA-8 from runner in N.C.). Our results show that there is a genotype x isolate interaction for strawberry-*C. acutatum* with respect to AFR.

Percent lesions. There is a genotype x isolate interaction and a genotype effect (Table 1). There were differences among genotypes with respect to percent lesions. NCR 95-08 had the lowest percent lesions over all isolates. For some genotypes it appears that CA-1 and AROM-NCH had higher percent lesions than CHAN1-NCH. But this was not the case for all genotypes. However, percent lesion may not be the best indicator of resistance. A high percent lesion does not necessarily indicate susceptibility of a genotype to an isolate. For example, Pelican had a higher percent lesion for CA-1 than the field-susceptible Camarosa and Chandler. It is possible that higher percent lesion could be the result of a hypersensitive response. A high percent lesion with a low lesion growth could result in lower AFR than a lower percent lesion but much faster lesion growth. Additionally, the inoculum concentration used, 1×10^6 conidia·ml⁻¹ may have overwhelmed any resistance. Denoyes-Rothan et al. (1999) found that an inoculum concentration of 2×10^4 conidia·ml⁻¹ gave a wider range of disease responses than higher

inoculum concentrations.

Incubation period. There were differences among genotypes and among isolates but there was no interaction (Table 2). This shows that one component of resistance to AFR may be a longer time to sporulation. NCR 95-08 had a longer mean IP than all genotypes except Sweet Charlie and NCH 95-173. A longer IP is important in the field to increase the length of each infection cycle. However, one should probably not plant genotypes with shorter IP's in close proximity to NCR 95-08. The IP was also longer for isolate CHAN1-NCH than CA-1. Isolate CA-1 may be more virulent than the other isolates. Again, a longer IP would lengthen each infection cycle and reduce both the incidence and severity of AFR. These results confirm those of Ballington and Milholland (1993) who also found a difference in incidence of AFR after inoculation with CA-1 and CA-8.

Lesion growth rate. Our results show that genotypes respond differently to *C. acutatum* isolates with respect to disease development over time. Since there was an isolate x genotype interaction, strawberry breeders and pathologists cannot simply use the most virulent isolate in their work. However, it does appear that less susceptible like Pelican, Sweet Charlie, and NCR 95-08 generally had slower rates of lesion growth. Genotypes with slower lesion growth rates should be useful in breeding for AFR resistance. A genotype by isolate interaction could also help to explain differences in results among researchers. A genotype could be labelled susceptible in one study and resistance in another study if different isolates were used. A set of standard isolates and

genotypes would help us begin to develop a better understanding of the strawberry-*C. acutatum* pathosystem throughout the U.S. and the world.

Very little is known about the population structure of *C. acutatum*. No teleomorphs have been reported either *in vivo* or *in vitro* for *C. acutatum* on strawberry and it is probable that the pathogen reproduces asexually for the most part. However, teleomorphs of *C. acutatum* have been produced *in vitro* for the first time for several isolates from apple and one from blueberry (Guerber and Correll, 1997). Production of ascospores would further increase the potential for genetic variability of *C. acutatum* and impact any race-cultivar system or disease resistance breeding progress. More work needs to be done to determine if such a relationship exists for strawberry and *C. acutatum*.

Nevertheless, our results show that there is variability in the *C. acutatum* population because the strawberry cultivars and genotypes did not respond equally to the three *C. acutatum* isolates. This is important in both resistance breeding and epidemiology. AFR resistance breeding should probably be targeted towards horizontal resistance rather than vertical resistance, unless *C. acutatum* populations do not rapidly change over time or within a geographical region. However, we do not believe this to be the case. The potential for pathogen change through asexual and possibly sexual means is quite high. Additionally, plant source is usually not local to the grower.

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Table 1. Percent of inoculations that became lesions for seven strawberry genotypes inoculated with three isolates of *Colletotrichum acutatum*

Genotype	Percent lesions by isolate (%) ^z		
	CA-1	AROM-NCH	CHAN1-NCH
Apollo	-	71a	75 a
Camarosa	74 a	68 a	77 a
Chandler	76 a	76 a	62 b
Pelican	90 a	78 b	67 c
Sweet Charlie	81 a	53 b	75 a
NCH 95-173	49 c	62 b	87 a
NCR 95-08	62 a	69 a	47 b

^zThree 10- μ l drops of 1×10^6 conidia·ml⁻¹ inoculum were equidistantly spaced down the outer surface of each fruit for each isolate. Estimated number of conidia is 10,000 conidia per drop. CA-1 was isolated from a strawberry runner in N.C. by Milholland (Ballington and Milholland, 1993). AROM-NCH and CHAN1-NCH were isolated from Aromas fruit and Chandler fruit, respectively in Castle Hayne, N.C in April, 1999. Within a row, means (harmonic mean n=37) followed by the same letter are not different by lsmeans ($P \leq 0.05$).

Table 2. Incubation period of seven strawberry genotypes inoculated with three isolates of *Colletotrichum acutatum*

Genotypes	Isolate ^z			Mean ^y
	CA-1	AROM-NCH	CHAN1-NCH	
Apollo	4.0	4.7	6.0	5.4 b
Camarosa	4.7	5.8	5.9	5.5 b
Chandler	4.0	6.4	6.3	5.2 b
Pelican	4.9	5.8	4.9	5.1 b
Sweet Charlie	6.0	5.9	7.0	6.3 ab
NCH 95-173	5.8	5.5	5.6	5.7 ab
NCR 95-08	6.6	6.1	7.8	6.9 a
Mean	5.2 b	5.8 ab	6.2 a	

Source of variation	df	Sum of squares	Percent of total
Treatments	24	120	
Genotype	6	60*	50
Isolates	2	29*	24
Genotypes x Isolates	12	31	26

^zCA-1 was isolated from a strawberry runner in N.C. by Milholland (Ballington and Milholland, 1993). AROM-NCH and CHAN1-NCH were isolated from Aromas fruit

and Chandler fruit, respectively in Castle Hayne, N.C in April, 1999.

^yMeans followed by the same letter are not significantly different among isolates (LSD = 1.3, $P \leq 0.05$) or among genotypes (LSD = 0.8, $P \leq 0.05$).

*Significant at $P \leq 0.05$.

Table 3. Anthracnose fruit rot lesion diameter regressed over time for seven strawberry genotypes inoculated with three isolates of *Colletotrichum acutatum*

Genotype	<i>C. acutum</i> isolate ^z		
	CA-1	AROM-NCH	CHAN1-NCH
Apollo	-	$y = 0.84x$ a, $R^2 = 0.56$	$y = 0.79x$ a, $R^2 = 0.55$
Camarosa	$y = 0.75x$ b, $R^2 = 0.57$	$y = 0.82x$ b, $R^2 = 0.55$	$y = 1.06x$ a, $R^2 = 0.72$
Chandler	$y = 0.85x$ b, $R^2 = 0.60$	$y = 1.17x$ a, $R^2 = 0.69$	$y = 0.67x$ c, $R^2 = 0.46$
Pelican	$y = 0.82x$ b, $R^2 = 0.67$	$y = 0.59x$ c, $R^2 = 0.46$	$y = 1.14x$ a, $R^2 = 0.61$
Sweet Charlie	-	$y = 0.67x$ a, $R^2 = 0.45$	$y = 0.75x$ a, $R^2 = 0.49$
NCH 95-173	$y = 0.42x$ c, $R^2 = 0.28$	$y = 0.53x$ b, $R^2 = 0.40$	$y = 0.85x$ a, $R^2 = 0.68$
NCR 95-08	$y = 0.44x$ b, $R^2 = 0.30$	$y = 0.62x$ a, $R^2 = 0.52$	$y = 0.34x$ c, $R^2 = 0.24$

^zRegressions were based on all lesions resulting from inoculating strawberry fruit. Three 10- μ l drops of inoculum were equidistantly placed down the outer surface of green to fully ripe fruit at 1×10^6 conidia·ml⁻¹. Estimated number of conidia was 10,000 conidia per drop. CA-1 was isolated from a strawberry runner in N.C. by Milholland (Ballington and Milholland, 1993). AROM-NCH and CHAN1-NCH were isolated from Aromas fruit and Chandler fruit, respectively in Castle Hayne, N.C in April, 1999.

^y y = AFR lesion diameter (mm), x = days after inoculation (d). Within a row, means (harmonic mean n = 35) followed by the same letter are not different by lsmeans (P \leq 0.05).

APPENDICES

Appendix A. Field Study



Figure 1. Anthracnose fruit rot on fruit from various strawberry cultivars and genotypes during the 1998 harvest season at the Upper Piedmont Research Station (Reidsville, NC).

Appendix B. Inoculum concentration x genotype study



Figure 2. Overview of growth chamber in the phytotron. One plant each of the seven strawberry genotypes were randomized within each movable cart. Fruit were hand-pollinated and tagged. Day of pollination and inoculation was recorded on tag.



Figure 3. Anthracnose fruit rot (middle) on ‘Chandler’ inoculated with 1×10^5 conidia·ml⁻¹. A 10 μ l drop ($\sim 1,000$ conidia) was micropipetted onto each of three dots equidistantly placed down the outer surface of each fruit.