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Comparative Relative Susceptibility of NE-183 Apple Cultivars to Fruit Rot Pathogens in West Virginia

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Abstract

Twenty-three apple (*Malus* x *domestica* Borkh.) cultivars were tested over a 5-year period with controlled inoculations in the field and laboratory for their relative susceptibility to the pathogens that cause common preharvest ("summer") fruit rot diseases in the mid-Atlantic region of the United States. Pathogens tested were isolates of *Colletotrichum acutatum*, *Botryosphaeria dothidea*, and *B. obtusa*, the causal agents for bitter rot, white rot, and black rot respectively. Wounded (for *Botryosphaeria* spp.) or nonwounded (for *C. acutatum*) fruit were inoculated in the field and laboratory at 2 to 3 weeks preharvest with mycelium (for *Botryosphaeria* spp.) or conidia (for *C. acutatum*) from axenic cultures. Fruit were rated for relative susceptibility to the different fungi by determining disease severity of attached fruit in the field based on lesion growth and detached fruit in laboratory inoculations of wounded fruit. Based on the laboratory and field data from 5 growing seasons, cultivars were classified into three relative susceptibility groups: most susceptible: 'Pristine', 'Fortune', 'Sunrise', 'Orin', and 'Arlet'; moderately susceptible: 'Sansa', 'Ginger Gold', 'Golden Supreme', 'Honeycrisp', 'PioneerMac', 'Suncrisp', 'Cameo', 'Senshu', 'Shizuka', 'Yataka', and NY75414; and least susceptible: 'Enterprise', 'Golden Delicious', 'Creston', 'GoldRush', 'Gala Supreme', 'Braeburn', and 'Fuji'. Results of the present study indicate that new apple cultivars from the first NE-183 planting vary in their resistance to the different rot fungi and none, perhaps with the exceptions of 'Gala Supreme' and 'Fuji,' shows uniform resistance to the spectrum of summer rot pathogens included in these experiments.

Introduction

Regional project NE-183, "Multidisciplinary Evaluation of New Apple Cultivars", was initiated in 1994 to examine the performance of new apple cultivars in replicated trials under a wide range of climatic and edaphic conditions. The project has 26 cooperators in 18 states and two Canadian provinces. A primary objective of the NE-183 project is to evaluate horticultural qualities and pest susceptibility of new apple cultivars, strains, and advanced selections with commercial potential and to determine the limitations and positive attributes of these cultivars. To date, researchers in the mid-Atlantic region and northeast have documented the relative susceptibilities of the NE-183 apple cultivars to apple scab (caused by *Venturia inaequalis*), powdery mildew (caused by *Podosphaera leucotricha*), rusts (caused by *Gymnosporangium juniperi-virginianae*, *G. clavipes* and *G.globosum*), white rot (caused by *Botryosphaeria dothidea*), black rot (caused by *B. obtusa*), and bitter rot (caused by *Colletotrichum acutatum*) (2, 3, 4, 9, 10, 15, 16, 19). The objective of this paper is to compare and contrast the relative susceptibilities of apple cultivars to the fruit rot pathogens, *B. dothidea*, *B. obtusa*, and *Colletotrichum acutatum* from studies conducted over five growing seasons, 1998 through 2002. This paper is a synthesis of information from three previously published articles (2, 3, 4).

Materials and Methods

Test planting. The 23 apple cultivars selected for the NE-183 project were budded on M.9 337 rootstock. Trees were planted in north-south oriented rows in April 1995 at a spacing of 2.5 m x 4.3 m using a mechanical tree planter. The design was a randomized complete block with five single-tree plots per cultivar. Drive middles were planted with Kentucky-31 fescue (*Festuca arundinacea*), and a weed-free strip (1 m wide in 1995; 2 m wide in the remaining years) was maintained in the tree row using paraquat plus oryzalin at recommended rates (14). After planting, a conduit stake secured to a single trellis wire at

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2.1 m height was placed beside each tree and the tree's leader was tied to the stake. Minimal pruning was followed throughout this study allowing trees to assume their natural form. Blossoms were removed in the first season (1995). Trees were allowed to fruit after the first growing season and crop load was adjusted by hand to space fruit about 15 cm apart. Drip irrigation was installed in the summer of 1997. The planting received no pesticide applications in 1995. Insecticides were applied from 1996 through 2002, as were fixed copper (dormant) and streptomycin (bloom) to suppress fire blight. Dodine, myclobutanil, fenarimol, and/or mancozeb were applied at recommended rates (Pfeifer, 1995) beginning mid-April, and continuing through mid-May each year, for early season scab control. No fungicides were applied after mid-May. Temperature, relative humidity, and leaf wetness were monitored with a 7-day recorder (Belfort Instruments, Baltimore, Md.).

Fungal isolates. All fungal isolates used in these studies were selected, and subcultured and maintained on potato dextrose agar in petri dishes. Fungal cultures were subcultured periodically and maintained at 22 °C under continuous fluorescent light during the periods in which experiments were conducted. For *C. acutatum* and *B. dothidea*, one representative isolate was used; and, for *B. obtusa*, two representative isolates were used.

Field experiments. Fruit were inoculated with mycelium for *Botryosphaeria* spp. and conidia for *C. acutatum* in the field at 2 to 3 weeks preharvest, as determined by average ripening date and quality assessments, which included starch index rating, soluble solids concentration (SSC), and flesh firmness. Arbitrarily selected fruit were inoculated by making a 1-mm-deep wound through the fruit epidermis with a sterile 5-mm-diameter cork borer, removing the circumscribed epidermis and placing a 5-mm-diameter agar plug supporting fungus mycelium over the wound. Wounds were wrapped in Parafilm to maintain moisture. Conidia of *C. acutatum* were applied to the fruit in cheesecloth strips and covered with aluminum foil. Thirty-two fruit were inoculated per cultivar per isolate in three replications of eight fruit, and eight fruit were inoculated with sterile agar as a control. Re-isolations were conducted periodically to confirm the presence of inoculated fungi in lesions.

Laboratory experiments. Fruit were picked at 2 to 3 weeks before their normal harvest date, brought to the laboratory, and washed with tap water. Fruit were inoculated as described above, placed in opaque plastic trays with lids, and incubated at 21 to 23 °C in the laboratory. Thirty-two fruit, including three replications of eight fruit, and eight fruit inoculated with sterile agar were used per cultivar per isolate. Re-isolations were conducted periodically to confirm the presence of inoculated fungi in lesions. Five additional fruit of each cultivar were sampled for determination of flesh firmness and SSC. Flesh firmness was measured with a hand-held penetrometer (Effigi Inc., Bologna, Italy) fitted with an 11-mm tip. Soluble solids were measured with a hand-held refractometer (Fisher Scientific, Pittsburgh, Pa.).

Data collection and analysis. The field and laboratory studies were conducted over five growing seasons from 1998 through 2002, with each rot pathogen tested in the laboratory and field over two growing seasons. Fruit were rated for relative susceptibility to the fungi using two criteria: disease severity of attached fruit in the field and disease severity of detached fruit in laboratory inoculations of wounded (for the *B. dothidea* and *B. obtusa*) or nonwounded (for *C. acutatum*) fruit. Severity was obtained from the mean of two measurements (length and width) from each lesion. Only symptomatic fruit were included in the calculation of mean wound severity. In both studies, severity was determined at 5 days postinoculation, however, severity data from the field were adjusted for temperature by calculating lesion diameter increase per degree-day accumulation (base temperature = 0 °C). Mean lesion diameter data were subjected to general linear models analysis and means were separated with the Waller-Duncan *k*-ratio *t*-test (SAS Institute, Cary, N. C.). Data from each year were analyzed separately in some years because of additional isolates and/or missing cultivars. Cultivar ranks from the tests over two growing seasons for each pathogen were used to calculate the mean relative susceptibility rating. Final cultivar ranks were determined by averaging the mean ranks for the four sets (field and laboratory severity from each of the two years' experiments) of observations. The nonparametric Spearman rank correlation analysis was used to determine the relationships among the various measures and with harvest date, fruit firmness, and SSC. Grand mean ranks were calculated for each cultivar by averaging the mean relative susceptibility ranks for the three diseases.

Results and Discussion

Bitter rot. Relative cultivar ranks from field tests were not reproducible in the 2 years studied, whereas laboratory tests showed moderate reproducibility with nonparametric rank correlation tests (2). Based on the laboratory data from 2 years of study, cultivars were classified into four relative susceptibility groups: most susceptible: 'Pristine', 'Honeycrisp', and 'Ginger Gold'; highly susceptible 'Yataka', 'Sansa', 'Arlet', and 'Enterprise'; moderately susceptible: 'Sunrise', 'Golden Supreme', 'PioneerMac', 'GoldRush', 'Golden Delicious', and 'Creston'; and least susceptible: 'Fuji' (Table 1). Previous published rankings have included 'Fuji' and 'Golden Delicious' among the most susceptible cultivars to bitter rot (12). Although McVay et al. based their cultivar rankings on field observations and did not distinguish between the two bitter rot pathogens, their observations and ours indicate that new apple cultivars from the first NE-183 planting show no improvement in resistance to *Colletotrichum acutatum*.

White rot. Based on the laboratory and field data from 2 years of study, cultivars were classified into three relative susceptibility groups: most susceptible: 'Fortune' and 'Pristine'; moderately susceptible: 'Golden Supreme', 'Creston', 'Ginger Gold', 'Sansa', 'Golden Delicious', 'Senshu', 'Orin', 'Sunrise', 'GoldRush', 'Arlet', 'Braeburn', 'Cameo', 'Enterprise', 'Fuji', 'Shizuka', 'Gala Supreme', and NY 75414; and least susceptible: 'Honeycrisp', 'Yataka', 'Suncrisp' and 'PioneerMac' (3) (Table 1). The rankings may be tentative for some of the cultivars showing variation between years in the field and laboratory tests (i.e. 'Ginger Gold', 'Suncrisp', 'Golden Supreme', 'Sansa', and 'Orin'), and additional data may be needed to classify more accurately their relative susceptibility to *B. dothidea*. Previous published rankings have included 'Golden Delicious' among the most susceptible cultivars to the white rot pathogen (12). The observations McVay et al. and the results of our experiments indicate that some new apple cultivars from the first NE-183 planting possess greater resistance to *B. dothidea* than some current standard apple cultivars.

Sitterly and Shay proposed that internal maturity-related changes determine the onset of susceptibility to rot pathogens (17). In this model, cultivar variation in maturity-related changes might be related to cultivar relative susceptibility to rot pathogens. One of the major maturity-related changes in apples is the shift to increased soluble solids from stored starch. Increased sugar content has been associated with increased susceptibility of apple to white rot caused by *B. dothidea* (11), with "active rot lesions" seldom occurring until soluble solids reach 10.5%. Brown (5) provided evidence that linear rot expansion of several apple fruit rotting pathogens was inversely related to levels of endopolygalacturonase inhibitor activity in fruit tissue. More recent findings show that latent infections occur on apple in the early or middle part of the growing season, with infection occurring whenever environmental conditions are favorable (1, 13) rather than being related to physiological status of the fruit. *B. dothidea* has a long incubation period in immature and mature fruit (13), and symptom expression, rather than susceptibility, may be related to the physiological changes in the fruit, one component of which is the increase in soluble solids.

Black rot. Based on the laboratory and field data from 2 growing seasons, cultivars were classified into three relative susceptibility groups: most susceptible: 'Orin', 'Pristine', and 'Sunrise'; moderately susceptible: 'Suncrisp', 'Ginger Gold', 'Senshu', 'Honeycrisp', 'PioneerMac', 'Fortune', NY 75414, 'Arlet', 'Golden Supreme', 'Shizuka', 'Cameo', 'Sansa', and 'Yataka'; and least susceptible: 'Creston', 'Golden Delicious', 'Enterprise', 'Gala Supreme', 'Braeburn', 'GoldRush', and 'Fuji' (4) (Table 1). Previous published rankings have included 'Red Delicious', 'Empire', and 'Cortland' among the most susceptible cultivars to the black rot pathogen (12). Although these cultivars were not

Table 1. Mean ranks and grand mean for field and laboratory disease severity of selected apple cultivars inoculated with fruit rot fungi and listed from most to least susceptible based on the grand mean of the combined mean ranks

Cultivar[z]	*Botryosphaeria obtusa* severity mean rank[y]	*Botryosphaeria dothidea* severity mean rank[x]	*Colletotrichum acutatum* incidence mean rank[w]	Grand mean rank
Pristine	2.8	2.8	2.5	2.7
Fortune	8.5	2.5	9.0	6.7
Sunrise	3.8	11.2	7.5	7.5
Orin	4.5	11.2	--[v]	7.9
Arlet	9.1	11.8	5.0	8.6
Sansa	10.5	10.0	6.5	9.0
Ginger Gold	6.2	10.0	11.5	9.2
Golden Supreme	9.5	9.2	9.0	9.2
Honeycrisp	7.7	16.5	5.0	9.7
PioneerMac	8.4	19.5	1.5	9.8
Suncrisp	6.0	19.5	7.0	10.8
Cameo	10.2	12.2	--	11.2
Senshu	6.5	11.2	16.0	11.2
Shizuka	10.0	13.0	--	11.5
Yataka	11.5	20.0	3.5	11.7
NY75414-1	8.8	14.8	--	11.8
Enterprise	13.4	12.5	10.0	12.0
Golden Delicious	13.2	11.0	12.2	12.2
Creston	12.8	9.5	15.0	12.4
GoldRush	15.8	11.5	11.5	12.9
Gala Supreme	14.0	13.5	13.0	13.5
Braeburn	15.0	12.0	--	13.5
Fuji, B.C. No. 2	16.8	13.0	15.2	15.0

[z] Cultivars are arranged from most susceptible (lower mean rank) to least susceptible (higher mean rank) based on the mean of the combined mean ranks of field and/or laboratory tests with wounded or nonwounded fruit.

[y] Data are the combined mean ranks from field and laboratory experiments conducted in 2001 and 2002. A lower combined mean rank indicates higher levels of disease severity.

[x] Data are the combined mean ranks from field and laboratory experiments conducted in 2000 and 2001. A lower combined mean rank indicates higher levels of disease severity.

[w] Data are mean ranks from laboratory inoculations from nonwounded fruit conducted in 1998 and 1999. A lower mean rank indicates higher levels of disease incidence.

[v] Fruit not available for testing.

included in the present study, McVay lists them as more susceptible than 'Golden Delicious.' Of the 23 cultivars tested in this study, 17 of them were more susceptible to *B. obtusa* than 'Golden Delicious.' Our results indicate that only a few new apple cultivars (i.e. perhaps 'GoldRush', 'Enterprise', and 'Gala Supreme') from the first NE-183 planting possess greater resistance to *B. obtusa* than current standard apple cultivars.

Several aspects of the pathology of *B. obtusa* may be similar to that of *B. dothidea*, as described above, although more detailed studies are needed to demonstrate the occurrence of latent infection with the former fungus. Infections caused by *B. obtusa* are visible as irregular-shaped dark lesions, 1 to 2 cm in diameter, and are often first observed in early to mid-summer. Alternatively, lesions in the field may be caused by ascospores or conidia infecting the sepals and then growing into the fruit as they mature. The present ranking of cultivars based on wound inoculations of the fruit may not reflect the actual perceived susceptibility of the fruit in the field if the infection was initiated in the sepal tissue. However, the data presented here would be reflective of what one might expect if sepal infections grew into the maturing fruit and resulted in a calyx end rot, which is often typical of black rot. Sepal infection is probably more closely related to the susceptibility of the leaves rather than the fruit.

Several factors may influence the real and perceived relative susceptibility of apple cultivars to the rot pathogens, thus altering the relative susceptibility rankings presented here. General tree characteristics could affect relative susceptibility. For example, foliage density could affect the length of wet periods and spray coverage; the tendency to form aborted fruits might affect the amount and species composition of inoculum within the tree; varying effect of different thinning chemicals might influence the tendency to form aborted fruit; tree vigor might affect the amount of woody tissue that must be pruned, thereby affecting the amount of inoculum; variation in the characteristics of the bark and xylem might affect the comparative suitability of host tissues for fungal reproduction; fruit characteristics such as thickness of the epidermis, presence and amount of inhibitory substances in the cuticle, presence or absence and, if present, relative size of open calyx tube area might affect perceived relative susceptibility; disease escape might be related to date of harvest with respect to frequency and duration of wet periods; cultivars might vary in attractiveness to insects (or birds) that cause wounds; and responsiveness of subepidermal tissues following wounding might vary. All or some of the above factors, or others we haven't included, could potentially account for differences between our data and field observations.

Reliance on the grand mean rank values is potentially misleading for some cultivars, i.e. 'PioneerMac' and 'Yataka', both of which showed high susceptibility to the bitter rot fungus, moderate susceptibility to the black rot fungus, and low susceptibility to the white rot fungus, resulting in an overall moderate susceptibility grand mean rank (Table 1). While most cultivars did not demonstrate extreme differences in their relative susceptibility to the different organisms, only a few cultivars exhibited consistent resistant or susceptible reactions to the spectrum of pathogens that were tested in these experiments. A few notable exceptions included the generally high susceptibility of 'Pristine' to all three pathogens, the moderate susceptibility of 'Golden Supreme', and the consistent relative resistance of 'Gala Supreme' and 'Fuji' (Table 1).

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