Relative Susceptibility of Selected Apple Cultivars to Fruit Rot Caused by Botryosphaeria obtusa

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Abstract. Twenty-three apple (Malus xdomestica) cultivars were tested in the field and laboratory for their relative susceptibility to the black rot pathogen, Botryosphaeria obtusa. Wounded fruit were inoculated in the field at 2 to 3 weeks preharvest with mycelium from 14- to 21-day-old cultures. In the laboratory, detached fruit were inoculated similarly. Fruit were rated for relative susceptibility to the fungus by determining disease severity on the laboratory and field data from two growing seasons, cultivars were classified into three relative susceptibility groups—most susceptible: ‘Orin’, ‘Pristine’, and ‘Sunrise’; moderately susceptible: ‘Suncrisp’, ‘Ginger Gold’, ‘Senshu’, ‘Honeycrisp’, ‘Pioneer Mac’, ‘Fortune’, NY 75414, ‘Arlet’, ‘Golden Supreme’, ‘Shizuka’, ‘Cameo’, ‘Sansa’, and ‘Yataka’; and least susceptible: ‘Creston’, ‘Golden Delicious’, ‘Enterprise’, ‘Gala Supreme’, ‘Braeburn’, ‘GoldRush’, and ‘Fuji’. Compared to previous cultivar rankings, the results of the present study indicate that no new apple cultivars from the first NE-183 planting show greater resistance to Botryosphaeria obtusa than current standard cultivars.

Black rot, caused by the fungus Botrys-

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Table 1. Cultivar, date of inoculation, soluble solids, fruit firmness, and mean temperature for the 5-day period following field inoculation for apple cultivars inoculated with Botryosphaeria obtusa.

| Cultivar       | Inoculation date | Soluble solids (%) | Fruit firmness (Kg) | Mean temp (°C) |
|----------------|------------------|--------------------|---------------------|----------------|
| 2001           |                  |                    |                     |                |
| Pristine       | 11 July          | 10.4               | 8.6                 | 19.9           |
| Sunrise        | 12 July          | 11.3               | 10.0                | 19.9           |
| Sansa          | 25 July          | 12.1               | 7.8                 | 20.8           |
| Ginger Gold    | 2 Aug.           | 12.8               | 8.2                 | 23.7           |
| PioneerMac     | 10 Aug.          | 12.6               | 9.9                 | 23.4           |
| Arlet          | 9 Aug.           | 13.4               | 8.2                 | 24.6           |
| Senshu         | 9 Aug.           | 12.0               | 9.8                 | 24.6           |
| Golden Supreme | 15 Aug.          | 12.5               | 8.2                 | 22.4           |
| Honeycrisp     | 10 Aug.          | 12.7               | 9.6                 | 23.4           |
| Creston        | 31 Aug.          | 14.0               | 7.3                 | 20.4           |
| Fortune        | 5 Sept.          | 14.3               | 7.4                 | 20.0           |
| NY75414        | 30 Aug.          | 12.4               | 8.5                 | 20.6           |
| Golden Delicious| 5 Sept.         | 13.4               | 8.4                 | 20.0           |
| Gala Supreme   | 12 Sept.         | 14.1               | 8.9                 | 15.4           |
| Shizuka        | 6 Sept.          | 14.5               | 7.1                 | 20.7           |
| Orin           | 12 Sept.         | 13.8               | 8.5                 | 15.4           |
| Yataka         | 6 Sept.          | ---                | ---                 | 20.7           |
| Braeburn       | 20 Sept.         | 12.2               | 9.8                 | 18.9           |
| Cameo          | 19 Sept.         | 13.2               | 8.0                 | 19.0           |
| Fuji           | 19 Sept.         | 14.0               | 8.4                 | 19.0           |
| Sunrisp        | 27 Sept.         | 14.9               | 8.3                 | 11.3           |
| Enterprise     | 21 Sept.         | 8.0                | 14.2                | 17.8           |
| GoldRush       | 3 Oct.           | 14.2               | 8.2                 | 16.8           |
| 2002           |                  |                    |                     |                |
| Pristine       | 15 July          | 11.8               | 8.6                 | 19.9           |
| Sunrise        | 22 July          | 13.3               | 6.4                 | 23.6           |
| Sansa          | 29 July          | 12.4               | 8.4                 | 26.9           |
| Ginger Gold    | 5 Aug.           | 12.5               | 9.3                 | 20.7           |
| PioneerMac     | 19 Aug.          | 12.8               | 7.7                 | 24.4           |
| Arlet          | 5 Aug.           | 13.1               | 10.0                | 20.7           |
| Senshu         | 12 Aug.          | 13.5               | 9.0                 | 25.7           |
| Golden Supreme | 15 Aug.          | 12.7               | 8.2                 | 23.4           |
| Honeycrisp     | 12 Aug.          | 11.4               | 8.4                 | 25.7           |
| Creston        | 26 Aug.          | 13.9               | 7.3                 | 19.3           |
| Fortune        | 2 Sept.          | 12.8               | 7.5                 | 20.9           |
| NY75414        | 26 Aug.          | 13.5               | 9.0                 | 19.3           |
| Golden Delicious| 2 Sept.          | 13.5               | 8.4                 | 20.9           |
| Gala Supreme   | 16 Sept.         | 16.8               | 9.2                 | 21.7           |
| Shizuka        | 9 Sept.          | 14.9               | 8.0                 | 19.4           |
| Orin           | ---              | ---                | ---                 | ---            |
| Yataka         | ---              | ---                | ---                 | ---            |
| Braeburn       | 16 Sept.         | 14.6               | 11.8                | 21.7           |
| Cameo          | 23 Sept.         | 15.6               | 7.6                 | 16.7           |
| Fuji           | 30 Sept.         | 17.3               | 8.3                 | 21.2           |
| Sunrisp        | ---              | ---                | ---                 | ---            |
| Enterprise     | 23 Sept.         | 15.5               | 8.7                 | 16.7           |
| GoldRush       | 7 Oct.           | 19.2               | 11.3                | 14.1           |

*Measurements are the means from five fruit arbitrarily selected and measured on the inoculation date.

*Not inoculated; fruit unavailable.

scribed epidermis and placing a 5-mm-diameter agar plug supporting fungus mycelium over the wound. Wounds were wrapped in paraffin to maintain moisture. The paraffin was removed after 4 d. 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. None of the control fruit developed black rot during the course of the study. Re-isolations were conducted periodically to confirm the presence of B. obtusa in inoculated lesions. The study was conducted in 2001 and 2002.

**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 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 B. obtusa in inoculated lesions. Five additional fruit of each cultivar were sampled for determination of fresh weight 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). The study was conducted over two growing seasons, 2001 and 2002.

**Data collection and analysis.** Fruit were rated for relative susceptibility to the fungus using two criteria: disease severity of attached fruit in the field and disease severity of detached fruit in laboratory inoculations of wounded 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 disease severity. In both studies, severity was determined at 5 d 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 parametric Spearman rank correlation analysis was used to determine the relationships among the various measures and with harvest date, fruit firmness, and SSC.

**Results and Discussion**

**Field experiments.** Black rot incidence following inoculations with mycelium in 2001 and 2002 was ≈94% and 87%, respectively. In 2001, severity ranged from 0.33-mm lesion increase/degree-day for ‘Suncrisp’ to 0.04-mm lesion increase/degree-day for ‘Braeburn’ (Table 2). When two B. obtusa isolates were tested in 2002, isolate BoVA-1 was more aggressive than isolate BoVA-3 (P ≤ 0.001); however, the cultivar × isolate interaction was non-significant (P = 0.36), so data from the two isolates were combined to determine the cultivar relative susceptibilities. In 2002, severity ranged from 0.20-mm lesion increase/degree-day for ‘Golden Supreme’ to 0.03-mm lesion increase/degree-day for ‘GoldRush’ (Table 2). ‘Fuji’, ‘GoldRush’, and ‘Braeburn’ had the lowest mean ranks (least susceptible) for field severity, whereas ‘Suncrisp’, ‘Yataka’, and ‘Pristine’ had the highest mean ranks (most susceptible) (Table 3). The cultivar × year interaction for isolate BoVA-1 (used in both years) was significant (P ≤ 0.001), with some cultivars showing differences in their relative susceptibility between years (i.e., most notable, ‘Sunrise’, ‘NY75414-1’, and ‘Fortune’).

Temperatures in the field might have contributed to the variability of the field experiments between years. Mean daily temperatures in 2001 ranged from 11.3 to 24.6 °C, compared to 2002, when temperatures ranged from 14.1 to 26.9 °C (Table 1). During the test period (July through September of each year), 2001 was ≈0.8 °C cooler than 2002, although some cultivars were exposed to differing postinoculation temperatures in each year (i.e., some cultivars were exposed to warmer temperatures in 2001 and others exposed to warmer temperatures in 2002). Most notably, July 2002 was ≈5.6 °C warmer than July 2001. ‘Sansa’ was the most extreme example with a 6.1 °C difference in mean temperature during the 5-d incubation period between years, followed by ‘Gala Supreme’ with a 5.3 °C difference between years (Table 1). Although lesion growth in the field was calculated as a function of temperature, it is possible that preinoculation temperature conditions or other environmental or host variables that weren’t measured may have contributed to the observed cultivar × year variation in susceptibility to B. obtusa.

**Laboratory experiments.** None of the control fruit developed black rot during the observation period although a few (<5%) exhibited signs of infection by Penicillium.
sp. Black rot incidence following inoculations with mycelium in 2001 and 2002 was 93% and 91%, respectively. In 2001, severity ranged from 31.3 mm for ‘Pristine’ to 6.2 mm for ‘Yataka’ (Table 2). When two B. obtusa isolates were tested in 2002, isolate BoVA-1 was more virulent than isolate BoVA-3 (P ≤ 0.001); however, the cultivar × isolate interaction was nonsignificant (P ≤ 0.15), so data from the two isolates were combined to determine cultivar relative susceptibility. In 2002, severity ranged from 23.2 mm for ‘Sunrise’ to 10.6 mm for the apple scab resistant cultivar ‘GoldRush’. ‘Pristine’ and ‘Sunrise’ had the lowest mean ranks (most susceptible) for severity, followed by ‘Senshu’ and ‘PioneerMac’, whereas ‘Yataka’, ‘Gala Supreme’, and ‘Golden Delicious’ appeared most resistant based on the mean ranks of the laboratory tests (Table 3). There was a significant cultivar × year interaction for severity in the laboratory, with some cultivars showing significant differences between years. The cultivars that showed the most variation between years included those mentioned above as showing the most variation between years in the field tests, i.e., ‘NY75414-1’ and ‘Fortune’, as well as ‘Honeycrisp’.

Fruit firmness and percent soluble solids were not correlated in this study, although we have observed both a negative correlation and no correlation in previous studies (Biggs and Miller, 2001, 2003). Softer apples generally possessed higher soluble solids (Biggs and Miller, 2003). Date of harvest was positively correlated with soluble solids (r = 0.66, P = 0.001), but not with firmness (r = 0.21, P =

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![Image](image-url)

**Table 2.** Disease severity on selected apple cultivars inoculated in the field and laboratory with *Botryosphaeria obtusa* in 2001 and 2002.

| Cultivar           | Harvest date rank | Field severity rating (mm lesion increase/degree-day) | Laboratory severity rating (lesion diam (mm) after 5 d) |
|--------------------|-------------------|--------------------------------------------------------|--------------------------------------------------------|
|                    | 2001   | 2002   | 2001 | 2002 | 2001 | 2002 |
| Pristine           | 1      | 1      | 0.24 b–d | 0.20 a | 23.1 a | 12.4 a |
| Sunrise            | 2      | 2      | 0.18 d–h | 0.20 a | 23.5 ab | 23.2 a |
| Orin               | 15     | ---    | 0.25 bc | ---   | ---   | ---   |
| Suncrisp           | 21     | ---    | 0.33 a  | ---   | 17.9 b-f | ---   |
| Ginger Gold        | 4      | 4      | 0.24 b-d | 0.15 cd | 20.5 b-e | ---   |
| Senshu             | 6      | 6      | 0.19 c-f | ---   | 24.7 a-c | 17.9 c |
| Honeycrisp         | 8      | 8      | 0.24 b-d | ---   | 16.8 c-g | 20.2 b |
| Fortune            | 10     | 12     | 0.10 i-l | 0.15 bc | 25.4 ab | 15.5 de |
| NY75414-1          | 11     | 10     | 0.27 ab | 0.11 g | 21.8 b-e | 13.1 f |
| Arlet              | 5      | 5      | 0.13 f-j | 0.14 ef | 19.3 b-f | 16.6 d |
| Golden Supreme     | 9      | ---    | 0.25 bc | ---   | 16.6 d-g | ---   |
| Shizuka            | 13     | 13     | 0.17 e-i | 0.14 c-e | ---   | 14.3 ef |
| Cameo              | 18     | 16     | 0.12 g-k | 0.16 g | 18.8 b-f | 13.3 f |
| Senshu             | 3      | 5      | 0.19 c-g | 0.16 b | 12.4 f-h | 14.2 ef |
| Yataka             | 17     | ---    | 0.26 ab | ---   | 6.2 h   | ---   |
| Creston            | 12     | 11     | 0.16 f-j | 0.11 g | 16.6 d-g | 14.3 ef |
| Golden Delicious   | 10     | 12     | --- | 0.14 d-f | 13.9 e-b | 11.3 g |
| Enterprise         | 22     | 18     | 0.12 h-k | 0.11 g | 14.2 e-g | 15.5 de |
| Gala Supreme       | 14     | 14     | 0.23 b-e | 0.12 g | 9.0 gh | 11.1 g |
| Braeburn           | 21     | 15     | 0.04 l | 0.13 f | 17.3 c-f | 11.2 g |
| GoldRush           | 23     | 19     | 0.09 j-l | 0.03 h | 18.5 b-f | 10.6 g |
| Fuji, B.C. No. 2   | 20     | 17     | 0.06 kl | ---   | ---   | 13.3 f |

*Harvest date rank is from earliest = 1 to latest = 23. Cultivars are arranged from most susceptible to least susceptible based on the combined mean ranks from laboratory and field tests.*

*Field and laboratory severity data are from 5 d post-inoculation for both years.*

*Data are the mean of 24 observations from three replicates of eight fruit per replicate. Different letters denote significant differences among means according to the Waller-Duncan test (P ≤ 0.05).*

*Fruit not available.*
Cultivars that matured later generally tended to possess increased soluble solids. Also, cultivars that matured later tended to have smaller lesions and appeared more resistant to B. obtusa in the laboratory tests ($r_s = 0.56, P = 0.007$) and when assessed with the combined mean rank criterion ($r = 0.58, P = 0.004$). Disease severity in the laboratory was correlated with soluble solids, but not with firmness ($r_s = 0.39, P = 0.08$; and $r = 0.09$, $P = 0.70$, respectively), with fruit possessing lower soluble solids tending to develop larger lesions. Similarly, disease severity in the field was correlated weakly with soluble solids, but not with firmness ($r_s = 0.36, P = 0.10$; and $r_s = 0.35, P = 0.11$, respectively), with fruit possessing lower soluble solids tending to develop larger lesions.

Internal maturity-related changes have been proposed as determining the onset of susceptibility to rot pathogens (Sitterly and Shay, 1960). Therefore, cultivar variation in maturity-related changes could be related to cultivar relative susceptibility to rot pathogens. Increased sugar content has been associated with increased susceptibility of apple to white rot caused by B. dothidea (Kohn and Hendrix, 1983), with “active rot lesions” seldom occurring until soluble solids reach 10% (Biggs 1984). However, in the present study with B. obtusa, it was observed that fruit with lower soluble solids tended to have larger lesions. Brown (1984) provided evidence that linear rot expansion of several apple fruit rotting pathogens was inversely related to levels of endopolygalacturonase inhibitor activity in fruit tissue. With B. dothidea, latent infections occur on apple in the early or middle part of the growing season, with infection occurring whenever environmental conditions are favorable (Biggs, 1995; Parker and Sutton, 1993). Botryosphaeria dothidea has a long incubation period in immature and mature fruit (Parker and Sutton, 1993), 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.

These aspects of the pathology of B. dothidea may be similar to B. obtusa, although more detailed studies are needed to demonstrate the occurrence of latent infection with the latter 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 conidiu 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.

Other factors may influence the relative susceptibility of apple cultivars to the black rot pathogen. For example, differences in amounts of inoculum from mummies, twig and branch cankers, or dead shoots could be an important element of perceived susceptibility in the orchard. Insect feeding preferences among cultivars also could influence perceived susceptibility by creating potential infection courts. Also, early-maturing cultivars could escape the larger amounts of inoculum that occur later in the growing season, although this explanation appears unlikely given the abundance of B. obtusa conidia observed in May and June in North Carolina (Sutton, 1981). Only two isolates of B. obtusa were used for the inoculation tests in the present study. Field populations of B. dothidea have been shown to vary in virulence (Brown-Rytlewski and McManus, 2000; Foster, 1983; Jones and Sutton, 1996; Parker and Sutton, 1993) on leaves, fruit, and woody tissues; however, cultivar specificity has not been reported on apple for either B. dothidea or B. obtusa.

Based on the combined laboratory and field data from 2 years of study, we classified the cultivars 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’. 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. obtusa. Previously published rankings have included ‘Red Delicious’, ‘Empire’, and ‘Cortland’ among the most susceptible cultivars to the black rot pathogen (McKay et al., 1993). Although these cultivars were not included in the present study, McKay 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’. The 23 cultivars were based on their rankings on field observations, and their observations and the results of the present study indicate that only a few new apple cultivars (i.e., ‘Red Delicious’, ‘Empire’, and ‘Cortland’) are more resistant to B. obtusa than current standard apple cultivars.