Early and Late-Season Susceptibility of Peach Fruits to *Monilinia fructicola*

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ABSTRACT

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Peach fruits of the cultivars Redhaven and Loring were harvested weekly beginning in early June and continuing to full ripeness, and were inoculated under controlled conditions with *Monilinia fructicola* at a range of 10^3–10^6 conidia/ml. In both years of the study, fruits were susceptible to infection at all inoculum concentrations for 2 to 3 wk in June. At pit hardening they became resistant to infection at all the inoculum concentrations. Fruits again became increasingly susceptible to infection approximately 2 wk before full ripeness.

Brown rot caused by *Monilinia fructicola* (Wint.) Honey is one of the most important diseases of peach (*Prunus persica* (L.) Batsch) in the temperate fruit-growing regions of North America (2). In Ontario and elsewhere, the disease is controlled by use of sanitation practices to reduce fungal inoculum in combination with protective fungicide programs (1,15). In recent years, losses from brown rot have been minimized by the use of benzimidazole and dicarboximide fungicides. However, the intensive use of these fungicides could result in the selection of resistant pathogens and the loss of fungicide efficacy. A knowledge of the phenological stages of least susceptibility of fruit to infection by *M. fructicola* would permit a reduction of fungicide use, with associated reductions in the risk of pathogen resistance and in the cost of fruit production.

The resistance of young, green stone fruits to infection by *Monilinia* spp. has been reported (3–5,9,11,12). However, the timing of the shift from susceptibility at the blossom phase to resistance in the green fruit phase, and the shift back to susceptibility as the fruit ripen, has not been examined for peaches. The objective of the present study was to elucidate the effect of fruit maturity on the susceptibility to brown rot of Redhaven and Loring, two important peach cultivars in southern Ontario.

MATERIALS AND METHODS

Fruits. Samples of fruits were harvested at weekly intervals and taken to the laboratory for inoculation beginning the week after "June drop" (the period of early abscission of nonpollinated or aborted fruits) and continuing until full

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ripeness (about 1 wk after commercial harvest). The orchards from which the fruits were harvested received no fungicide applications during the growing season, and weather conditions were seasonal. A minimum of 56 fruits per cultivar at each inoculum concentration was used at each sampling date. Fruit were placed on 1.3-cm mesh screens within 23 × 30 × 8 cm aluminum baking trays in preparation for inoculation. Later in the growing season, larger fruit were supported on 4-cm-diameter juice jar lids in wooden boxes. Green fruit with red or purple pigmentation were placed green side up. Fruits at each sampling date were characterized by length and width, weight, volume, surface color, pulp color, stage of pit hardening (determined by cutting fruit with a pocket knife), and content of soluble solids measured with a hand-held refractometer.

Inoculum. A suspension of conidia was prepared from cultures of *M. fructicola* isolate S.4 (benomyl-sensitive) growing on potato-dextrose agar (PDA) in 9-cm polystyrene petri dishes incubated for 10–14 days in the dark at 20 C. The isolate was maintained on PDA and passed through fruit annually to verify pathogenicity. Inoculum was prepared by slicing each agar culture into 10 pieces and shaking with 40 ml of sterile distilled water in a 500-ml sterile flask. The flask was shaken vigorously for about 30 sec and the resulting suspension of conidia and mycelial fragments was passed through coarse filter paper to remove the hyphal fragments. Two further 50-ml aliquots of sterile water were used to rinse the remaining conidia from the agar pieces. The suspension was shaken vigorously to break up chains of conidia and then was passed through 0.22-μm Millipore filters. Conidia were rinsed with 5 ml of sterile water while still under vacuum and then rinsed from the filter disks using an atomizer with sterile water and compressed air. The suspension was adjusted with sterile distilled water to twice the desired inoculum concentration. These suspensions were then mixed with an equal volume of Miller's solution (8) containing 0.1% Tween 20 to give final suspensions containing 0.05% Tween 20 and 10^3, 10^4, 10^5, and 10^6 conidia/ml. Conidial suspensions were tested routinely for percent germination on PDA to verify a high (≥95%) germination.

Nonwounded fruits were inoculated with 30 μl (one drop) of spore suspension and incubated in the dark for 22 hr at 20 C in a stainless steel inoculation room with a relative humidity greater than 95% (measured with an aspirated psychrometer), then moved to a controlled environment room (20 C, 60% RH) where the inoculum droplets were allowed to dry over a 2-hr period. Fruits were incubated at 20 C and 95% RH and observed daily for 6 days for the presence of necrosis and sporulation. Disease severity was assessed for individual fruits on a scale from 0 to 3 (0 = infection; 1 = necrosis equal in diameter to the inoculum droplet, no sporulation; 2 = necrosis larger in diameter than the inoculum droplet, no sporulation; and 3 = sporulating necrotic lesion). Control fruit, used to detect infections that had occurred in the field, were treated with a drop of Miller's solution and 0.05% Tween 20 only. Data on percentage of fruit infection were analyzed by the GLM procedure (SAS Institute, Cary, NC) with least squares analysis of variance and Type III sums of squares for unbalanced linear models with a randomized design using years as blocks (10). Data reported in the figures represent the percentage of fruit rated 2 or 3 on the sixth day after inoculation.

RESULTS AND DISCUSSION

Analysis of data for percentage of fruit infection obtained by inoculating fruit with varying concentrations of conidia

Table 1. Least squares method analysis of variance for percentage of fruit infection for peach cultivars Redhaven and Loring inoculated weekly with varying conidial concentrations of *Monilinia fructicola*[y]

	Redhaven		Loring	
Source	df	Mean squares	df	Mean squares
Inoculum concentration	3	13,488.7 **[z]	3	31,480.2 **
Sample date	10	11,373.5 **	11	15,227.6 **
Replication	3	45.7	3	76.9
Block (year)	1	7,778.2 **	1	9,300.9 **
Inoculum concentration × sample date	28	1,140.1 **	28	1,418.1 **
Residual	238	241.1	242	231.8

[y] Fruit were inoculated with 10^3, 10^4, 10^5, or 10^6 conidia/ml, incubated, and the percentage of fruit infected was determined. Mean squares were derived from Type III sums of squares for unbalanced linear models and a randomized block design over time.
[z] ** Indicates significance at $P \leqslant 0.001$.

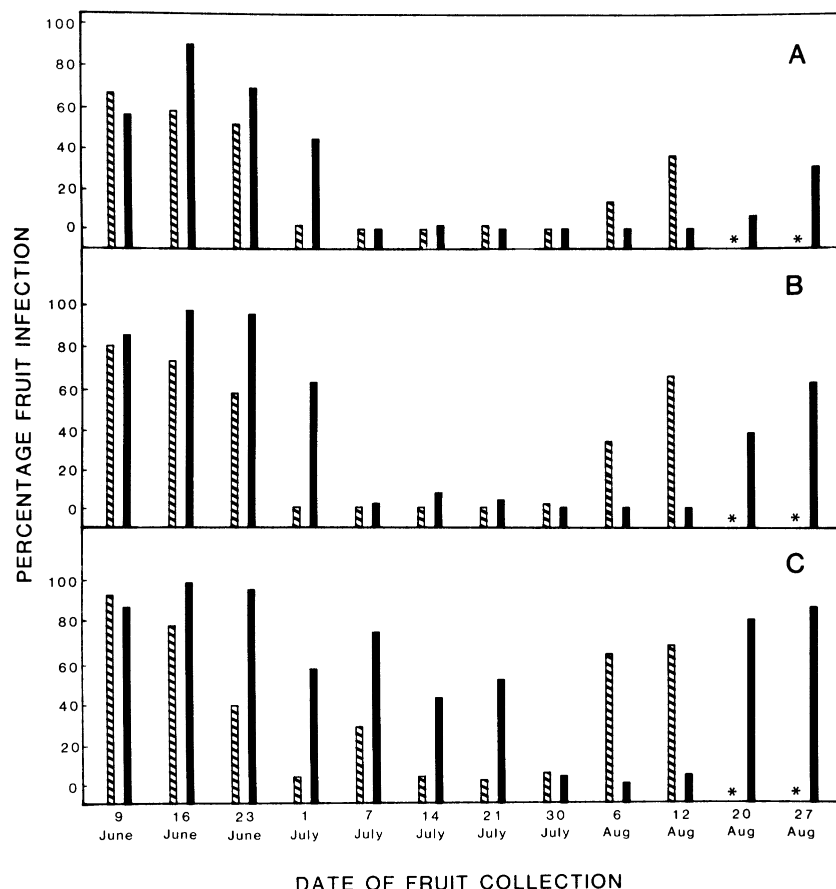

Fig. 1. Percentage of peach fruit infection from the week following June abscission to full ripeness in 1986, inoculated with **(A)** 10^4, **(B)** 10^5, or **(C)** 10^6 *Monilinia fructicola* conidia/ml. Solid and hatched lines represent cultivars Loring and Redhaven, respectively. Asterisks indicate that fruit were unavailable at certain dates due to harvest maturity or limited quantities for experimentation.

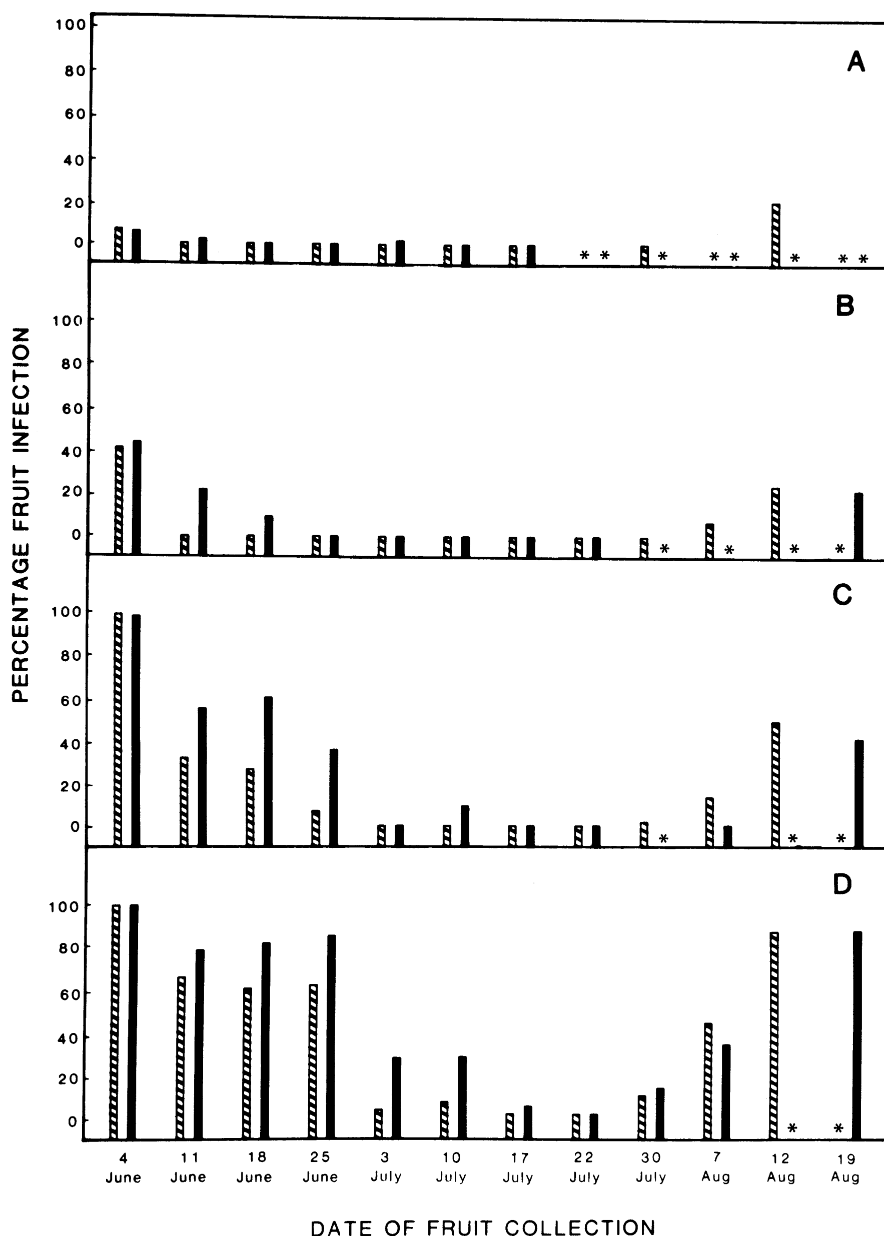

Fig. 2. Percentage of peach fruit infection from the week following June abscission to full ripeness, in 1987, inoculated with **(A)** 10^3, **(B)** 10^4, **(C)** 10^5, or **(D)** 10^6 *Monilinia fructicola* conidia/ml. Solid and hatched lines represent cultivars Loring and Redhaven, respectively. Asterisks indicate that fruit were unavailable at certain dates due to harvest maturity or limited quantities for experimentation.

revealed a significant interaction of inoculum concentration with sampling date for the two cultivars (Table 1). Figures 1 and 2 show the incidence of fruit infection following inoculation in 1986 and 1987, respectively. In both years, fruits from each cultivar were susceptible to infection by *M. fructicola* for a period of about 3 wk in June, followed by decreased susceptibility during July. Increased susceptibility to infection recurred about 2–3 wk before full ripeness. Percentage of infection increased as inoculum concentration increased, although most fruit were resistant to even the highest inoculum concentration during July.

This seasonal pattern of susceptibility of peach fruits to brown rot was similar in both years regardless of the inoculum concentration used. At the lower inoculum concentrations (10^3 and 10^4 conidia/ml), fruits became resistant earlier in the season and were susceptible later in the season than those inoculated at the highest inoculum concentration (10^6 conidia/ml). In 1987, fruits inoculated with 10^3 conidia/ml remained relatively symptomless except at the final sampling of Redhaven taken on 12 August (Fig. 2).

Growth of peach fruit (Tables 2 and 3) could be divided into three stages, coinciding with those described by Conners (3). Stage I was characterized by rapid development of the fruit due mainly to an increase in size of the ovule. Stage II was a rest period during which the ovule was completely formed and the pit (pericarp) hardened. Stage III was a period of rapid growth of the flesh to maturity and usually began 4 to 5 wk before full ripeness. According to Conners (3), stage II is variable in length depending upon the ripening date of a particular cultivar. The early and late-season susceptibility of fruit to *M. fructicola* observed in this study was associated with stages I and III of fruit development (Tables 2 and 3), although their relationship could be influenced by inoculum concentration (Figs. 1 and 2). Fruits exhibited resistance to inoculation

Table 2. Phenological characteristics and percentage of fruit infection for Redhaven peach fruit during the 1987 growing season[z]

Date	Growth stage	Percentage of fruit infection	Length (mm)/ width (mm)	Density (g/cm^3)	Surface color	Pulp color	Pit hardening (%)	Soluble solids
4 June	I	61.9 a	25.3/19.3	0.82	G	G	0	5.0
11 June	I	24.6 c	32.4/26.1	1.0	G	G	0	4.5
18 June	I	21.9 cd	39.7/33.7	0.98	G	G	60	6.5
25 June	I	17.3 d	43.0/37.7	1.0	G	G	20	6.5
3 July	II	0.8 e	43.2/38.1	0.99	G	G	100	6.6
10 July	II	1.8 e	45.0/41.0	0.99	G	G	100	6.8
17 July	II	0.4 e	46.1/42.0	0.98	G/R	G	100	8.0
22 July	II	0.6 e	48.3/45.8	1.01	G/R	G	100	8.0
30 July	III	3.1 e	57.5/55.8	1.35	G/R	G/R	100	7.4
7 August	III	21.6 cd	60.0/60.0	1.01	G/R/Y	Y	100	9.5
12 August	III	45.5 b	57.2/57.4	1.01	R/Y	Y	100	9.1

[z] All observations except percentage of fruit infection represent means from 10 fruits. For color characteristics, G = green, Y = yellow, and R = red. Percentage of fruit infection means are from 56 fruit per sample date. Data are the main effect means for sample date and letters denote significant differences according to Duncan's multiple range test ($P \leqslant 0.05$).

Table 3. Phenological characteristics and percentage of fruit infection for Loring peach fruit during the 1987 growing season[z]

Date	Growth stage	Percentage of fruit infection	Length (mm)/ width (mm)	Density (g/cm^3)	Surface color	Pulp color	Pit hardening (%)	Soluble solids
4 June	I	62.1 a	24.7/20.9	0.85	G	G	0	4.6
11 June	I	39.3 c	33.0/28.2	1.0	G	G	0	7.0
18 June	I	37.9 c	39.4/33.9	0.98	G	G	50	6.5
25 June	I	30.3 d	40.3/35.7	0.96	G	G	30	6.4
3 July	II	7.4 gh	44.0/38.0	0.98	G	G	100	6.8
10 July	II	9.4 fg	45.5/39.8	1.01	G	G	100	7.0
17 July	II	1.3 hi	49.3/44.9	1.03	G	G	100	7.0
22 July	II	0.6 i	45.1/40.4	1.01	G	G	100	6.8
30 July	III	14.3 ef	54.1/48.2	1.05	G	G	100	7.2
7 August	III	17.5 e	57.0/53.3	0.98	G	G	100	6.9
12 August	III	...	58.0/56.5	0.98	G/R	G/Y	100	8.4
19 August	III	50.4 b	73.1/73.2	0.99	Y/R	Y	100	10.2

[z]All observations except percentage of fruit infection represent means from 10 fruits. For color characteristics, G = green, Y = yellow, and R = red. Percentage of fruit infection means are from 56 fruit per sample date (except 12 August when fruit availability became a limiting factor). Data are the main effect means for sample date and letters denote significant differences according to Duncan's multiple range test ($P < 0.05$).

Table 4. Weekly maximum and minimum temperatures and precipitation for the period before peach fruit sampling for controlled inoculations with varying conidial concentrations of *Monilinia fructicola* in 1986 and 1987

1986				1987			
	Temperature (C)				Temperature (C)		
Sample date	Max.	Min.	Precipitation (mm)	Sample date	Max.	Min.	Precipitation (mm)
9 June	19.9	10.1	16.4	4 June	26.9	17.8	0
16 June	22.7	12.8	22.6	11 June	22.2	11.2	12.8
23 June	23.3	12.6	7.6	18 June	27.1	14.7	6.4
1 July	23.2	13.7	13.2	25 June	25.8	15.9	44.6
7 July	26.4	16.9	37.0	3 July	23.9	16.2	21.6
14 July	23.0	15.4	26.8	10 July	27.9	19.4	4.8
21 July	26.5	18.2	47.4	17 July	26.9	17.9	29.6
30 July	26.9	18.4	8.0	22 July	30.5	20.6	24.2
6 August	25.3	16.7	32.6	30 July	28.9	19.0	13.4
12 August	24.2	16.1	15.6	7 August	25.2	16.8	41.2
20 August	25.5	17.5	0	12 August	22.9	15.7	56.4
27 August	23.4	14.9	10.6	19 August	29.5	20.3	6.4

with 10^4 conidia/ml at the end of stage I and remained generally resistant to all but the highest conidia concentration 1 wk after pit hardening and during the remainder of stage II. Increased susceptibility to infection at an inoculum concentration of 10^6 conidia/ml occurred between 6 and 20 August in 1986 and on 30 July in 1987, and coincided with the initiation of stage III.

The weather conditions during July, when the peach fruit were most resistant to *M. fructicola*, were characterized by temperature maxima and minima and rainfall patterns that did not differ greatly (Table 4) from the data collected in June and August when the fruit were more susceptible. No other phenological or environmental factors were associated with the observed pattern of fruit susceptibility to *M. fructicola*.

Whereas previous researchers have demonstrated the susceptibility of green peach (6), plum (11), and apricot (12) fruits to infection by *M. fructicola*, precise information regarding the relationship between disease susceptibility, inoculum concentration, and the phenological stage of fruit development has been lacking. As control of peach brown rot is achieved through a combination of sanitation and chemicals (15), the benefit of reducing inoculum density by sanitation is supported by our data that show that the duration and the degree of fruit susceptibility increased as inoculum concentration increased.

Chemical control strategies have been advocated that time spray applications with periods of known tissue susceptibility, i.e., bloom, shuck fall, and preharvest, and may include midseason cover sprays applied near the onset of pit hardening. Our data suggest that the latter spray may not be required and that existing spray schedules may have overlooked the significance of the relatively high degree of susceptibility of green peaches before pit hardening. One cautionary observation would be that in Ontario (1) and South Carolina (7), nonabscised, aborted fruits are a significant source of brown rot inoculum. Although it is not known precisely when these fruit become infected, earlier work has shown that *M. fructicola* can be isolated from them as early as stage I (1). Lack of adequate protection during stage I could, therefore, contribute to a buildup of summer inoculum originating from nonabscised, aborted fruit (1,7).

Fungicide cover sprays for early and midseason cultivars, timed approximately for the onset of pit hardening, may be unnecessary except in orchards where disease pressure is extremely high or where fruit thinning has been delayed past the pit hardening stage (1). An optional postshuck fall spray would be more appropriate than a spray at pit hardening for limiting the increase in summer inoculum. The postshuck fall spray would protect fruit that abort because of lack of pollination as well as pollinated fruit that are relatively susceptible during this period. Fungicides with eradicant properties might be useful at this stage, although additional information on the temperature and moisture conditions that favor infection of immature fruits is required.

The possibility that the early season susceptibility of peach fruits observed in the present study could be related to the formation of latent or quiescent infection, as observed in Australia (5,6,13) and California (14), is currently under investigation in our laboratory.

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LITERATURE CITED

1. Biggs, A. R., and Northover, J. 1985. Inoculum sources for *Monilinia fructicola* in Ontario peach orchards. Can. J. Plant Pathol. 7:302-307.
2. Byrde, R. J. W., and Willets, H. J. 1977. The Brown Rot Fungi of Fruit. Pergamon Press, New York. 171 pp.
3. Conners, C. H. 1920. Measurement of peaches. NJ Agric. Exp. Stn. Annu. Rep. 40:82-88.
4. Curtis, K. M. 1928. The morphological aspect of resistance to brown rot in stone fruits. Ann. Bot. 42:39-68.
5. Jenkins, P. T., and Reinganum, C. 1965. The occurrence of a quiescent infection of stone fruits caused by *Sclerotinia fructicola* (Wint.) Rehm. Aust. J. Agric. Res. 16:131-140.
6. Jerome, S. M. R. 1958. Brown rot of stone fruits: Latent contamination in relation to spread of disease. J. Aust. Inst. Agric. Sci. 24:132-140.
7. Landgraf, F. A., and Zehr, E. I. 1982. Inoculum sources for *Monilinia fructicola* in South Carolina peach orchards. Phytopathology 72:185-190.
8. Miller, H. J. 1944. The use of *Venturia inaequalis* and *Sclerotinia fructicola* with pure chemical stimulants in slide-germination tests of fungicides. (Abstr.) Phytopathology 34:1009.
9. Roberts, J. W., and Dunegan, J. C. 1932. Peach brown rot. USDA Tech. Bull. 328.
10. Steel, R. G. D., and Torrie, J. N. 1980. Principles and Procedures of Statistics. 2nd ed. McGraw-Hill, New York.
11. Valleau, W. D. 1915. Varietal resistance of plums to brown rot. J. Agric. Res. 5:365-396.
12. Wade, G. C. 1951. Progress report of brown rot of apricot investigations. Tasmanian J. Agric. 22:258-266.
13. Wade, G. C. 1956. Investigations on brown rot of apricots caused by *Sclerotinia fructicola* (Wint.) Rehm. I. The occurrence of latent infection in fruit. Aust. J. Agric. Res. 7:504-515.
14. Wilson, E. E., and Ogawa, J. M. 1979. Fungal, bacterial, and certain nonparasitic diseases of fruit and nut crops in California. Agric. Sci. Publ. University of California, Berkeley. 190 pp.
15. Zehr, E. I. 1982. Control of brown rot in peach orchards. Plant Dis. 66:1101-1105.