Resistance of Raspberry Cultivars to Fire Blight

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Abstract. Twenty-five cultivars of red raspberry (*Rubus idaeus* L.) and one purple raspberry (*R. occidentalis* L. × *R. idaeus* L.) were evaluated for their resistance to fire blight caused by *Erwinia amylovora* (Burr.) Winslow et al. Actively growing raspberry cane tips were wound inoculated with three isolates of the pathogen and disease development was assessed over 17 days. Three methods of evaluating resistance were used: area under the disease progress curve (AUDPC), a weighted AUDPC called the area under the disease severity curve (AUDSC), and lesion length. A wide range of resistance levels was observed, but no cultivars were symptomless. Primocane-fruiting cultivars tended to be more resistant than floricanefruiting ones. Of the three *E. amylovora* isolates used in this study, one was significantly more virulent than the other two, but no cultivar × isolate interaction was detected.

Fire blight of apple and pear, caused by *Erwinia amylovora* (Burr.) Winslow et al., has been a significant problem in North America since it was first reported in 1793 (van der Zwet and Keil, 1979). In contrast, fire blight of raspberry, which was first observed in the late 1800s (Ries and Otterbacher, 1977), has not been considered a serious problem. Since, fire blight of raspberry has been relatively uncommon and of little economic importance, no control measures have been developed (Ries, 1997). However, since 1994, several devastating outbreaks of fire blight in raspberry have occurred in Nova Scotia and New Brunswick (Braun and Hildebrand, unpublished observations) and the disease has also been reported from Ohio (Ries, 1997), Wisconsin (Heimann and Jeffers, 1990), Illinois (Ries and Otterbacher, 1977), Maine (Folsom, 1947; Starr et al., 1951), North Carolina (Lehman, 1933), Alberta (Evans, 1996), Michigan (McManus and Jones, 1995), Oklahoma (Oklahoma State University, 2001), Quebec and Ontario (personal communications). Considering the destructive nature of fire blight and its persistence in apple and pear orchards, research on effective disease management strategies for fire blight of raspberry would be prudent.

Management strategies in apple and pear have focused on timely applications of copper, antibiotics such as streptomycin, removal of inoculum sources by pruning and the use of resistant cultivars (Beer, 1990). Of these options, cultivar resistance is the most desirable for its cost effectiveness and long-term stability. Development of resistant cultivars requires advanced planning in breeding programs to incorporate and maintain a diverse range of resistance genes in parental lines, but little is known about raspberry resistance to fire blight.

The purposes of this study were 1) to accurately document fire blight symptom expression for the development of an appropriate disease severity rating system and 2) to identify and quantify resistance in currently available commercial raspberry cultivars to assist growers in making appropriate selections for their disease situation, and to aid breeders in the selection of parental plant material for breeding purposes. An earlier study describing resistance levels of unnamed raspberry selections from the Kentville breeding program has been published (Braun et al., 1999).

Materials and Methods

Twenty-six raspberry cultivars (Table 1) were purchased as bare-root plants from

commercial nurseries. Individual plants were transplanted into 12-L pots with potting medium (8 peatmoss : 3 perlite : 3 sand, by volume) and grown in a greenhouse at 18 to 20 °C and a 14-h photoperiod of natural day length supplemented with sodium vapor lamps.

Experiment 1: Plant inoculation. When the canes, which developed from basal axillary buds below the soil surface, had grown to about 1 m they were transferred to a growth chamber operating at 27 °C with a 14-h photoperiod (350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and >80% relative humidity. Canes were inoculated with one of three isolates of *E. amylovora* obtained from commercial raspberry plantings in New Brunswick. Isolates Ea6-96 and Ea7-96 were isolated from 'Killarney' and Ea8-96 from a raspberry selection, K81-6, from the Agriculture and Agri-Food Canada (AAFC) breeding program in Kentville, NS (Braun et al., 1999). The isolates were maintained at –80 °C in nutrient agar broth (Difco) with 15% glycerol and grown for inoculation on nutrient agar plates overnight at 25 °C. A bacterial suspension ($A_{600\text{nm}} = 0.1$) in sterile distilled water was applied to the growing tips of the canes by wounding with a multi-pin inoculator (Wallin et al., 1979). Most cultivars tested were floricanefruiting while some were primocane-fruiting (Table 2). Canes of primocane-fruiting cultivars were inoculated before terminal flower buds were evident. All inoculated cane tips were immediately covered with a plastic bag for 3 d to maintain high humidity and disease was evaluated 6, 8, 10, 13, and 17 d after inoculation.

Rating scales. Some inoculated canes developed expanding necrotic lesions whereas others showed areas of bacterial ooze, but no necrosis. In both cases, the length of the affected area was measured and regarded as the lesion. These values were then plotted against time and the area under the curve was calculated by trapezoidal integration (Sigma Plot, SPSS Sci-

Table 1. Raspberry cultivars tested for fire blight resistance.

Cultivar	Parentage
Algonquin	Haida x Canby
Autumn Britten	Complex parentage
Avon	Malling Promise x Cuthbert
Boyne	Chief x Indian Summer
Canby	Viking x Lloyd George
Carnival	Ottawa x Rideau
Caroline	(Autumn Bliss x Glen Moy) x Heritage
Comox	Skeena x (Creston x Willamette)
Encore	Canby x Cherokee
Glen Ample	Complex parentage
Glen Magna	Meeker x SCRI7719B11
Glen Moy	Complex parentage
Glen Prosen	Complex parentage
Glen Shee	Complex parentage
Heritage	(Milton x Cuthbert) x Durham
K81-6	(Muskoka (selfed) x Latham) x (Creston x Willamette)
Killarney	Chief x Indian Summer
Latham	King x Loudon
Lauren	Reveille x Titan
Nova	Southland x Boyne
Polana	Heritage x Zeva Herbersterne
Prelude	Titan x NC2967
Reveille	(Indian Summer x Sunrise) x September
Royalty ²	(Cumberland x Newburgh) x (Newburgh x Indian Summer)
Ruby	Heritage x Titan
Taylor	Newman x Lloyd George

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²The Ottawa strain of 'Latham' was used.

³Purple raspberry, *Rubus occidentalis* L. × *R. idaeus* L.

Table 2. Ranking of 26 raspberry cultivars for resistance to three isolates of *Erwinia amylovora* based on the area under the disease severity curve (AUDSC), area under the disease progress curve (AUDPC) and lesion length 17 d after inoculation. Data are reported as square root transformed means.

Cultivar	Fruiting ^a habit	AUDSC	AUDPC	Lesion length (cm)
Royalty (purple)	Florican	7.8	6.0	2.6
Nova	Florican	9.2	6.9	3.2
Ruby	Primocane	10.6	7.7	4.2
Polana	Primocane	12.1	8.8	3.9
Avon	Florican	12.5	10.3	5.2
Caroline	Primocane	13.7	9.9	4.0
Heritage	Primocane	14.5	9.7	5.2
Autumn Britten	Primocane	15.6	11.2	4.9
Taylor	Florican	16.9	12.1	5.5
Glen Prosen (spine-free)	Florican	17.1	12.1	4.8
Reveille	Florican	17.4	12.5	5.8
Comox	Florican	17.7	12.3	5.6
Glen Moy (spine-free)	Florican	17.8	12.6	5.2
Killarney	Florican	18.5	13.2	6.1
Lauren	Florican	18.6	13.4	5.6
Latham	Florican	18.7	13.4	5.8
Canby	Florican	18.8	13.5	5.7
Glen Shee (spine-free)	Florican	20.5	14.4	5.5
Algonquin	Florican	20.9	14.6	6.6
Boyne	Florican	21.5	15.2	6.5
Carnival	Florican	21.9	15.3	6.7
Glen Ample (spine-free)	Florican	22.2	15.1	5.9
Prelude	Florican	22.5	15.3	6.4
K81-6	Florican	24.3	17.1	7.5
Encore	Florican	24.7	17.7	7.5
Glen Magna	Florican	24.9	17.6	7.4
Standard error of difference		2.5	1.7	0.7
Isolate ^b				
Ea6-96		17.2	12.2	5.5
Ea7-96		17.2	12.3	5.4
Ea8-96		18.6	13.3	5.7
Standard error of difference		0.6	0.4	0.2

^aFruiting habit has been classified following Carew et al. (2000) and Jennings (1988) but it is recognized that fruiting habit is not a discrete designation but rather a continuum, i.e., 'Nova', 'Glen Moy', and 'Prelude' bear a small terminal crop of fruit in the fall and a commercial crop on laterals the following summer.

^bAveraged over all cultivars.

ence, Chicago, Ill.) and defined as the area under the disease progress curve (AUDPC). Disease severity of canes was also rated where 0 = no disease, 1 = necrosis but no bacterial ooze, and 2 = bacterial ooze with or without necrosis. A disease severity value was then computed for each cane by multiplying the disease severity rating value by lesion length. The severity values were then plotted against time and integrated as before to obtain the area under the disease severity curve (AUDSC). The experiment was conducted up to six times depending on the availability of cultivars with one to three canes for each cultivar in each replicate. Values for AUDPC, AUDSC and final lesion length at day 17 were transformed to the square root and then analyzed using the Residual Maximum Likelihood (REML) directive in Genstat 5 (Genstat 5 Committee, 1993). This procedure was chosen for the analysis of the data because it is well-suited to analyze unbalanced designs with more than one source of variability.

Experiment 2: Influence of flowering on resistance. Based on the previous experiment, primocane fruiting cultivars appeared to be more resistant than florican fruiting cultivars. To examine this more closely, the resistance of two cane types was compared in the selection K81-6. Bare-root K81-6 plants were transplanted in pots and grown as before. Under greenhouse conditions, this selection produces lateral shoots from axillary buds near the tops of the overwintered, cut-off primocanes (Fig.

1). These atypical lateral shoots grow vigorously often reaching lengths of >1 m with the appearance and flowering typical of primocane fruiting cultivars. Selection K81-6 also produces primocanes from basal axillary buds below the soil surface that remain as primocanes and become floricanes after vernalization. This feature enabled a direct comparison of the two, similarly aged tissue types. Primocanes and lateral shoots were inoculated with isolate Ea8-96 as before when they were about 1 m in length and before flower buds appeared on the lateral shoots. Some laterals in the first replicate developed flower buds after they had been inoculated and so for the second replicate, laterals were inoculated ≈2 weeks earlier than in the first replicate. Lesion length and disease severity ratings were recorded as before and the AUDSC was calculated. The data were square-root transformed and analyzed using the ANOVA directive in Genstat 5 (Genstat 5 Committee, 1993).

Results

In the field, fire blight symptoms on raspberry are similar to those that occur on pome fruits. Infections on succulent canes and leaves are caused primarily by stinging insects (Miridae) that vector *E. amylovora* (Braun and Hildebrand, 2001) while flower infections may arise from contaminated pollinating insects in the current season or by systemic infection of overwintering canes. Symptoms that arise from current

season infections may vary slightly from those that arise from systemic infections. On leaves, symptoms begin with the blackening of veins that spreads rapidly from the petiole and midrib to the leaf margins (Fig. 2). Leaves turn brown

Fig. 1. Under greenhouse conditions, bare root nursery plants of selection K81-6 that have been overwintered develop long and vigorously growing lateral shoots from axillary buds on the cut-off primocane above the soil surface which appear similar to the canes of primocane fruiting cultivars. On the same plants, canes produced from basal axillary buds below the soil surface develop into primocanes typical of florican fruiting cultivars.

Fig. 2. Fire blight symptoms on raspberry selection K81-6: (A) early leaf infection, (B) infected blossom and green fruit with bacterial ooze, (C) bacterial ooze on wound-inoculated primocane, (D) late stage of leaf infection and (E) over-wintered shepherd's-crook symptom.

and wilt and if infected systemically tend to remain attached to the cane until broken off by wind or other activity while those infected directly by insect feeding may drop if the canes do not develop disease symptoms. Symptoms of water soaking appear on succulent primocane tips and laterals of floricanes followed by rapid blackening, wilting and eventual collapse of tissues. Infected flowers also appear water soaked and then blacken. The infection moves basipetally beyond the flower pedicel and may include other flowers of the inflorescence. Many flowers on laterals show symptoms if they become infected from a systemically colonized cane whereas fewer flowers tend to become infected by pollinating insects alone. Green berries may become diseased from a systemically colonized cane or by the feeding of stinging insects. Infected berries turn brown to black, become hard and remain attached to the pedicel. Occasionally, before blackening or wilting is apparent, copious amounts of white-to cream-colored exudate containing bacteria appear on infected canes, petioles, flowers or green berries, on wet or humid mornings. As the humidity decreases, ooze droplets dry to form clear to opaque shiny beads or plaques. Wilted canes assume the characteristic shepherd's crook shape associated with fire blight.

Our inoculation method resulted in typical fire blight symptoms which began to appear 3 to 6 d after inoculation. On some cultivars, blackened areas around the wound sites were the first visible symptoms which subsequently spread to veins of the nearest leaf. As the lesions expanded, droplets of bacterial ooze developed

and the canes wilted. On other cultivars, bacterial ooze was the only initial symptom followed by necrosis and wilting. Lesions stopped expanding after 17 d of incubation.

The REML analysis showed that the cultivars differed ($P < 0.0001$) in their resistance to fire blight, but their ranking from most resistant to susceptible changed only slightly among the three different measurements of disease, i.e., AUDSC, AUDPC and final lesion length (Table 2). Thus, no single method of rating fire blight was obviously better than another. No cultivars were symptomless but the two most resistant cultivars in this evaluation were 'Nova' and 'Royalty', a purple floricanes-fruiting cultivar. Of the five primocane-fruiting cultivars tested, all were at the more resistant end of the spectrum while resistance in the spine-free cultivars ranged from moderate to low.

Isolate Ea8-96 appeared most aggressive when disease was evaluated using the AUDSC and AUDPC methods, but not with lesion length (Table 2). There was no cultivar $\times$ isolate interaction.

The difference in fire blight resistance between primocanes and the atypical lateral shoots of K81-6 was substantial in the first replicate with means of 16.6 and 6.9 for the AUDSC, respectively. Laterals in which flower buds became evident after inoculation were not included in the analysis. In the second replicate, laterals and primocanes were inoculated earlier and no flower buds were evident before the plants were rated for disease. However, the mean AUDSC for the more immature laterals increased to 14.3 but was still less ($P < 0.001$) than the primocane

mean which remained similar to the first replication with a value of 16.7.

Discussion

This study is the first to quantify levels of fire blight resistance in commercial raspberry cultivars (Table 2) and provides a description of disease symptoms (Fig. 2), which expands on previous reports (Folsom, 1947; Heimann and Jeffers, 1990; Lehman, 1933; Ries, 1997; Starr et al., 1951). The cultivars tested showed a wide range of resistance levels to the disease, but none was symptomless. We observed a similar response in unnamed raspberry selections (Braun et al. 1999). Cultivars Latham, Boyne and Fallgold have been previously identified as susceptible but disease levels were not quantified; no symptoms of disease were observed on 'Newburgh' (Heimann and Jeffers, 1990; Starr et al., 1951; van der Zwet and Beer, 1995).

Our observed differences in resistance were related to different rates of lesion development and/or inoculum production. Measuring the final lesion length after 17 d of incubation was the most rapid and simple method to determine relative resistance of raspberry canes. However, from an epidemiological perspective, the rate at which lesions expand is also an important factor in evaluating the relative susceptibility of cultivars. Lesions which develop rapidly become an early source of inoculum for subsequent infections and the rate of disease spread may be greater in cultivars with rapid lesion expansion even if lesion length is less than in cultivars with longer but more slowly developing lesions. Therefore, the AUDPC, which takes into account lesion length and the rate of lesion expansion, would be considered a more suitable measure of cultivar resistance than lesion length alone. Another factor which affects the rate of disease spread is bacterial ooze production on some cultivars before obvious symptoms of necrosis or wilting occur. For example, K81-6 occasionally produced large amounts of bacterial ooze on canes for several days before other symptoms appeared. Under field conditions, this could result in substantial bacterial spread by insects, rain, or fruit harvesters before a grower is aware of the problem. The AUDSC increases the weighting of the AUDPC values for those cultivars with a higher risk of disease spread as a result of inoculum production on otherwise symptomless canes. Therefore, we believe that the AUDSC is the preferred method of evaluating the resistance of raspberry cultivars to fire blight even though it did not markedly alter their ranking compared with the other methods of evaluation in this study. There was considerable variability in the data and the AUDSC method may have had a more pronounced difference in the ranking of cultivars in an experiment with tighter controls on sources of variability. Much of the variability observed in this study was likely related to the age of canes at the time of inoculation. Due to the large number of cultivars and constraints in available space, it was difficult to precisely synchronize their physiological development to the same state within and between replications of the experiment.

Fire blight symptoms on raspberry primocanes are restricted to tender growing tissues, the amount of which is a function of both the growing environment and the cultivar. Resistant cultivars like 'Royalty' and 'Nova' have very short lengths of tender apical growth while highly susceptible cultivars have relatively long, tender canes. All of the primocane-fruited cultivars evaluated were positioned at the resistant end of the spectrum (Table 2) and they, like 'Nova' and 'Royalty' have relatively short, tender cane tips as flower bud development approaches and vegetative growth slows. This was observed in greater detail when the resistance of lateral shoots and primocanes of selection K81-6 was compared. Laterals were more resistant than primocanes, especially when inoculated closer to flower bud development. Since primocane-fruited cultivars were inoculated at about one meter in length they were approaching flowering and this may explain, in part, their relative resistance to fire blight observed in this study. Although all of the spine-free cultivars had more tender canes than those with spines, this trait did not appear to affect their resistance level which ranged from moderate to low.

Three isolates of *E. amylovora* were chosen to screen raspberry cultivars for fire blight resistance to determine if cultivar $\times$ isolate interactions exist. Norelli et al. (2003) observed interactions between isolates and rootstocks in a fire blight resistance trial of apple. In our study, one isolate, Ea8-96 appeared more virulent in the AUDPC and AUDSC assessments (Table 2). However, no cultivar $\times$ isolate interaction was observed. The use of a larger number of

isolates may increase the probability of detecting an interaction.

No cultivar was symptomless, but rather a continuum of resistance levels was observed, indicating that resistance is likely governed by multiple genes. This type of resistance tends to be durable and is desirable in a cultivar. Although this study examined the resistance of only vegetative tissues of raspberry cultivars and further work is required to evaluate their resistance to flower infections, this report should serve as an initial guide for growers and breeders in making appropriate raspberry selections for their needs.

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