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Evaluation of Red Raspberry Cultivars for Resistance to Phytophthora Rot Root

J.A. PATTISON[1] AND COURTNEY A. WEBER[1*]

Abstract

Eighteen red raspberry genotypes originating from breeding programs around the world were tested for resistance to *Phytophthora fragariae* var. *rubi* in hydroponic culture under growth chamber conditions. All plants were inoculated with two isolates of *P. fragariae* var. *rubi* and evaluated for resistance using qualitative and quantitative measurements of root and shoot symptoms. 'Prelude', 'Anne', 'Caroline', 'Nova', 'Josephine' and NY258 were identified as having high to moderate levels of disease resistance to the pathogen. All of the resistant genotypes tested in this study can be categorized as likely deriving their resistance to *P. fragariae* var. *rubi* from either 'Latham' or *Rubus strigosus* Michx. germplasm.

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Introduction

Phytophthora root rot (PRR) of red raspberry (*Rubus idaeus* L.) is a persistent, soil borne disease regarded as a major cause of decline in red raspberry plantations in the Americas and Europe (8, 24). The disease was first described by Waterston in 1937, who associated zoospores resembling *P. citricola* Sawada with severe root rot and plant die-back on red raspberry plants in Scotland (20). Converse and Schwartze later observed the disease in North America in 1965, and in 1968 classified the red raspberry pathogen as *Phytophthora erythroseptica* Pethybr (4, 5). Several *Phytophthora* spp. have been reported as pathogens causing root rot, but Wilcox et al. (24) concluded that the most prevalent and pathogenic species affecting red raspberries share morphological and biochemical similarities with *P. fragariae* Hickman, and is host specific to members of *Rubus*. Therefore, the pathogen was named *P. fragariae* var. *rubi* Wilcox and Duncan and is presently the species used by most North American and European breeding programs for screening populations, selections and cultivars for root rot resistance (12, 13, 14, 15, 17, 18, 19, 24)

PRR is often most problematic on finer textured soils that drain poorly. Recommended control programs integrate avoidance or amelioration of wet soils and use of registered fungicides, in combination with host resistance (23). Site modifications such as installing drainage tile and planting on raised beds are helpful in reducing the impacts of PRR for those cultivars having moderate to high levels of resistance (10, 16, 26). The use of resistant cultivars appears to be the most effective means for control, however, market demands for the highest fruit quality tends to negate the use of resistant cultivars favoring the more commercially acceptable but root rot susceptible plant material.

Greenhouse screening methods have proven to be a reliable tool for determining the relative susceptibility of red raspberry cultivars and seedlings under controlled environmental conditions thus reducing the inherent variability encountered under field

[1] Department of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456
* Corresponding author: C.A. Weber, Email: caw34@cornell.edu Phone: 315-787-2395

conditions (12, 14). Pattison et al. (18) developed a hydroponic screening procedure that allows for repeated, non-destructive whole plant sampling capable of producing results consistent with previous reports of the resistance levels of several red raspberry cultivars. Following greenhouse testing, field experimentation remains a necessary component for assessing the performance of selected genotypes under environmental conditions found in commercial production settings.

Resistance to *P. fragariae* var. *rubi* has been identified in *R. idaeus strigosus* Michx., the native North American red raspberry, *R. spectabilis* Pursh. (salmonberry) and less so in germplasm derived from the native European red raspberry, *R. idaeus vulgatus* Arrhem (3, 6, 13, 15). Red raspberry cultivars, such as 'Latham' and 'Newburgh', possess high levels of resistance to the pathogen but are used primarily as resistance sources in breeding programs and not for commercial fruit production (1, 2, 12, 15, 19). 'Autumn Bliss', released in 1984 from the East Malling Research Station in England, possesses field and greenhouse resistance against *P. fragariae* var. *rubi* equal to that of 'Latham', the resistant standard (12, 14, 15). Since its release, many new cultivars have become available, although there are no scientific reports on the relative susceptibility of these cultivars, and hence, usefulness for growers with contaminated soils or for breeders trying to create new resistant genotypes. Thus, the objective of this research was to evaluate the relative susceptibility to *P. fragariae* var. *rubi* of cultivars that have not been characterized previously. A second objective was to use the results from this experiment to compare pedigrees for identifying the likely source(s) of resistance to *P. fragariae* var. *rubi* within the germplasm of commercial red raspberry cultivars.

Methods and Materials

Plant Material

Tissue culture plug plants of 18 red raspberry genotypes were obtained from various nurseries in either a dormant or an actively growing condition and tested for PRR resistance as described by Pattison et al. (18) (Table 1). Resistant ('Latham', 'Killarney', and 'Boyne') and susceptible ('Titan') control cultivars were included in all replications.

Root systems of plants were cleaned of soil and soaked in antibacterial soap solution for 15 minutes. After cleaning, the roots were pruned to between 4 and 8 cm prior to transplanting into the hydroponic basin as described by Pattison et al. (18). Roots were grown submerged in 28L of half strength Peter's Professional Hydro-Sol 5-11-26 nutrient solution (W.R. Grace & Co., Fogelsville, PA), supplemented with 10 mM $Ca(NO_3)_2$ and maintained at pH 6.5. Dormant plant material was cleaned as described above and transplanted first into the hydroponic system and allowed to begin growing approximately two weeks prior to the planting of those genotypes that were actively growing. A total of ten plants of each genotype were originally planted from which eight plants of each genotype of uniform size were selected to be screened for PRR resistance. The experiment was set up as a randomized complete block design with two plants of each genotype randomly assigned a planting position in each block. Each hydroponic basin (block) was replicated four times for a total of eight observations for each genotype. A 1.7 m^2 growth chamber was programmed with a 16-hour day length at a constant 20°C to accommodate the four blocks. Analysis of variance was used to determine significant differences in genotype response and among blocks. Cultivars and selections were ranked using Fisher's LSD based on the different criteria used to evaluate PRR susceptibility.

Inoculation

Two pathogenic isolates of *Phytophthora fragariae* var. *rubi*, ATCC 16184 (M14) and NY588 were obtained from Dr. Peter Bristow of Washington State University and Dr. Wayne F. Wilcox of Cornell University, respectively. Isolates were maintained on solid V-8 juice agar plates as described by Wilcox et al (22). Inoculum was produced by growing the isolates separately in clarified V-8 juice broth for 14 to 21 days as described by Bristow et al. (3). Mycelial mats were collected from the two to three week old liquid cultures into a Buchner funnel, washed with tap water, blotted dry and weighed. Two grams of mycelium from each isolate were comminuted in a Waring blender for two consecutive five second pulses in approximately 500 ml of filter sterlized

Table 1. Ancestry and nursery source of the red raspberry cultivars and advanced selections tested for Phytophthora root rot resistance in hydroponic culture.

Cultivar	Source[z]	Parentage[y]
Anne	Nourse Farms (a)	Amity x Glen Gerry
Autumn Byrd	Sakuma Bros. (b)	Autumn Bliss x EM 532611[w]
Esquimalt	Sakuma Bros. (b)	Comox x Glen Ample
Boyne	Nourse Farms (b)	Chief x Indian Summer
Caroline	Nourse Farms (a)	(Autumn Bliss x Glen Moy) x Heritage
Cowichan	Sakuma Bros. (b)	Newburgh x Qualicum
Dinkum	Sakuma Bros. (b)	Autumn Bliss x Glen Moy
Encore	Nourse Farms (a)	Canby x Cherokee
Josephine	Nourse Farms (a)	Amity x Glen Gerry
Killarney	Nourse Farms (a)	Chief x Indian Summer
Latham	Nourse Farms (b)	King x Loudon
Lauren	Nourse Farms (a)	Southland x Titan
Nova	Nourse Farms (a)	Southland x Boyne
NY 258[x]	Sakuma Bros. (b)	Canby x (Royalty x Skeena)
NY 283[x]	Sakuma Bros. (b)	Encore x (Titan x Cherokee)
Polana	Nourse Farms (a)	Heritage x Zeva Herbsternte
Prelude	Nourse Farms (a)	[Hilton x (Durham x September)] x Hilton
Titan	Nourse Farms (a)	Hilton x (Newburgh x St. Walfried)

[z] All plant material was propagated using tissue culture from the designated nurseries and was obtained as either (a) dormant or (b) actively growing plug plants.
[y] Pedigree information was procured from either The Brooks and Olmo Register of Fruit and Nut Varieties (7) or breeder notes.
[x] New York State Agricultural Experiment Station raspberry breeding program selection.
[w] Complex pedigree including: *R. arcticus, odoratus, strigosus, crataegifolius* and *spectabilis*

deionized water. Each hydroponic basin was inoculated with a mycelial suspension containing a total of four grams (2 grams of each isolate) of *P. fragariae* var. *rubi* when plants were between 25 and 30 cm in height. Aeration was withheld for 48 hours after inoculation.

Disease Assessment

Plants were observed regularly and evidence of disease was noted. Disease symptoms were assessed on each plant 40 days post inoculation using four different criteria: (1) a qualitative plant disease index score (0-5) assigned on the basis of both root and shoot symptoms (Table 2); (2) stem lesion length (cm) (measured from the intersection of the stem and crown to the highest level of necrotic stem tissue); (3) incidence of petiole lesions (%) (petiole lesion = presence of black/ brown basal petiole tissue with necrotic vascular tissue originating at the stem and extending into the petiole); and (4) a root regeneration score (0-3), where 0= no new root production and original roots and crown necrotic, 1= no to few new roots produced but original root and crown tissue healthy, 2= moderate production of new root tissue, and 3= vigorous production of new root tissue.

Results

Five to seven days following inoculation, all genotypes exhibited necrosis on young feeder roots. Shoot symptoms such as foliar chlorosis and wilting were evident on 'Titan' approximately 10 to 15 days post inoculation. Resistant check cultivars, 'Latham' and 'Boyne', displayed healthy

Table 2. Plant disease index for assessing susceptibility of red raspberry genotypes following inoculation with *Phytophthora fragariae* var. *rubi*.

Score	Symptoms
0	No root rot, no shoot symptoms
1	Slight root rot, no shoot symptoms
2	Slight root rot, slight shoot symptoms
3	Moderate root rot, moderate shoot symptoms
4	Severe root rot, severe shoot symptoms with the presence of living crown tissue
5	Perennial crown dead

new root production immediately following the initial limited necrosis of young feeder roots. 'Killarney' showed a reduced ability to regenerate new root tissue (Table 3) yet maintained symptomless stem, crown and older root tissue. As the screen progressed, new root tissue of the resistant check cultivars remained healthy while continuing to be submerged within the infested nutrient solution. These results were consistent with previous experiments that have used red raspberry seedlings and cultivars that were derived from tissue culture (18).

To assess the susceptibility of the test genotypes, values obtained for all evaluation criteria were compared to 'Titan' at 40 days post inoculation. Eight genotypes including 'Dinkum', 'Cowichan', 'Esquimalt', 'Autumn Byrd', 'Encore', 'Lauren', 'Polana' and NY 283 were ranked as susceptible as 'Titan' based on disease index scores (Table 3). Stem lesion lengths among these same eight genotypes were either of the same magnitude or of significantly larger length compared to 'Titan'. Observed percent petiole lesions provided the most significant separation among all of the genotypes with the susceptible genotypes having 50 percent or more affected petioles. Root systems of all the susceptible genotypes were either completely necrotic or possessed limited crown and older root tissue free of symptoms. The mean root regeneration index for 'Killarney' and 'Dinkum' were not significantly different, however, all plants of 'Killarney' possessed crown and older root tissue free from necrosis. Meanwhile, all plants of 'Dinkum' possessed root systems with unlimited necrosis and expressed severe shoot symptoms.

Genotypes possessing high to moderate levels of resistance were 'Prelude', 'Anne', 'Nova', 'Caroline', 'Josephine' and NY 258. Ranking of these was consistent over the different criteria measuring resistance (Table 3). Percent petiole lesions and mean root regeneration index provided the greatest separation among the resistant cultivars (Table 3). According to the plant disease index, 'Killarney' was not as resistant as 'Latham', however, all other scored criteria ranked this cultivar as highly resistant. Stem lesions were absent in all resistant cultivars except for one plant of 'Josephine' that developed a small lesion (< 1 cm). Attempts to isolate the pathogen from this symptomatic tissue failed, indicating that the lesion may not have been from *P. fragariae* var. *rubi*.

No significant differences were identified between the blocks, indicating that variability within the test basins was low ($P = 0.986$). Correlation analysis among the four assessment criteria identified strong relationships between the plant disease index with root regeneration index and percent petiole lesions (Table 4). Root regeneration index also was found to be strongly correlated to percent petiole lesions while the remaining trait comparisons possessed highly significant but less pronounced associations (Table 4).

Discussion

'Prelude', 'Anne', 'Nova', 'Caroline',

Table 3. Response of 18 red raspberry genotypes with respect to the different criteria used to evaluate the relative susceptibility to *Phytophthora fragariae* var. *rubi.*

Cultivar	Plant disease index[z]	Root regeneration index[z]	Stem lesion size (cm)[z]	Incidence of petiole lesion (%)[z]
Prelude	1.0	2.80	0.00	15
Anne	1.5	2.80	0.00	13
Latham	1.6	2.40	0.00	20
Nova	1.8	2.10	0.00	33
Josephine	2.0	2.00	0.75	25
Boyne	2.3	2.00	0.00	19
Caroline	2.3	2.00	0.00	19
NY258	2.3	1.50	0.00	23
Killarney	2.6	1.40	0.00	20
Dinkum	4.3	0.75	9.63	64
Cowichan	4.3	0.25	6.00	55
Esquimalt	4.3	0.25	3.80	58
Autumn Byrd	4.3	0.00	3.50	67
Encore	4.4	0.50	6.00	59
Titan	4.4	0.00	3.90	78
Lauren	4.6	0.38	4.60	79
Polana	4.6	0.25	17.00	79
NY283	4.8	0.00	8.00	83
LSD	0.87	0.65	2.31	22

[z] Values represent means from four replicate hydroponic basins with two plants of each cultivar per replicate. Comparisons that exceed the Fisher's LSD are significantly different at P = 0.05.

Table 4. Correlation coefficients (r^z) among the different evaluation criteria used to determine the relative susceptibility of the 18 red raspberry genotypes to *Phytophthora fragariae* var. *rubi* (df = 142).

Evaluation parameter	Plant disease index	Root regeneration score	Stem lesion (cm)	Percent petiole lesions
Plant disease index	1.00			
Root regeneration score	-0.91	1.00		
Stem lesion (cm)	0.68	-0.60	1.00	
Percent petiole lesions	0.85	-0.82	0.72	1.00

[z] For each correlation, $P \leq 0.001$ when $r > 0.28$.

'Josephine' and NY 258 were identified as possessing high to moderate levels of PRR resistance. Symptom expression on 'Titan' was delayed relative to a previous experiment (18) possibly due to the increased size of the plants at inoculation (25 to 30 cm vs. 15 to 20 cm). Maximum separation of the genotypes tested was accomplished with percent petiole lesions followed by root regeneration index, whereas plant disease index and stem lesion length identified considerably fewer differences. Using the resistant and susceptible check cultivars as baselines, cut-off values for disease resistance were identified. All resistant genotypes scored < 3, > 1, and < 50 % for plant disease index, root regeneration score, and percent petiole lesions, respectively. With the exception of one 'Josephine' plant, resistant genotypes could be classified based on the absence of stem lesions with significant differences present among the susceptible genotypes only. The quantitative criteria used in this study (percent petiole lesions and stem lesion size) appear to permit the identification of more differences in the resistance levels of the tested genotypes and may be useful for classifying plants of intermediate resistance. Therefore, selection of highly resistant genotypes may be best accomplished by evaluating the quantitative and qualitative aspects of the whole plant response to *P. fragariae* var. *rubi*.

Although no previous results are published for the test cultivars used in this study, 'Nova' has been described as susceptible to PRR in nursery catalogs. However, under this study 'Nova' possessed resistance equivalent to the resistant checks as well as other cultivars including 'Prelude' and 'Caroline', which have been observed to possess field resistance to PRR in a variety trial at Geneva, New York. It is possible that other species of *Phytophthora* are able to colonize and cause disease on 'Nova' resulting in this apparent contradiction. This has been shown previously by Wilcox et al. (25) where 'Latham' displayed differential susceptibility to various species of *Phytophthora*. However, this is untested and is speculative as to the reaction of 'Nova' to other *Phytophthora* species that are less prevalent and virulent.

Examining the pedigrees of the resistant cultivars described in this study revealed two potential sources of resistance to PRR; one originating from 'Latham' and the other from *Rubus strigosus*. 'Latham' ('King' x 'Loudon') has been considered the industry's resistant standard for many years (7). 'King' is described in The Small Fruits of New York (9) as thriving on clay soils and is believed to be the original source of resistance in 'Latham'. Cultivars that trace their resistance directly to 'Latham' include 'Boyne' and 'Killarney', which are siblings and have 'Chief' ('Latham' x 'Newburgh'?) as their resistant parent (7). 'Nova' ('Southland x 'Boyne'), also illustrates a direct link to a 'Latham' derived source of resistance (7). NY 258 and 'Prelude' contain 'Newburgh', a moderately resistant cultivar that originated from 'Newman' x 'Herbert', where the former was selected from a mixed population derived from open pollinated seed of 'Herbert', 'King', 'Loudon', 'Cuthbert', and 'Eaton' (9). Therefore, a likely common source of resistance in 'Newburgh' ('King') indirectly associates NY 258 and 'Prelude' with 'Latham' and may represent the same resistance gene(s).

Siblings 'Anne' and 'Josephine' have 'Amity' as their female parent, which is noted for possessing moderate to high levels of resistance (7). 'Amity' contains 'Newman', as well as *Rubus strigosus* Michx. germplasm making it difficult to decipher the donor of resistance (7). 'Caroline's resistant parent, 'Autumn Bliss', is described as a complex hybrid made up of several *Rubus* spp. and red raspberry cultivars (7) and remains the only resistant cultivar that cannot be reasonably associated with deriving its resistance from 'Latham'. However, the male parent of 'Autumn Bliss' was derived strictly from *Rubus strigosus* germplasm providing some additional evidence for this species' value in breeding for root rot resistance (11). It has also been suggested that the original source of resistance found in 'Latham' was derived from *R. strigosus* germplasm and could therefore possibly be the only exploited resistance source found among commercial red raspberry germplasm (15).

This study has illustrated that over the past 20 to 30 years breeders have successfully introgressed high levels of PRR resistance into elite red raspberry genotypes. Most notable for New York State and similar climates

are 'Prelude' and 'Caroline', which have consistently produced excellent fruit quality and exceptional yields compared to other industry standards (21). These two cultivars have diverse pedigrees with two or more generations of recombination from the original resistant parent(s). Undesirable traits such as small fruit size and unacceptable quality may possess tight linkages to the resistance gene(s) and have been common outcomes among resistant F_1 hybrids developed from 'Latham'. Therefore, a possible breeding strategy would be to create complex populations derived from several parents followed by the intermating of several selected F_1 individuals to create resistant recombinants with acceptable fruit and cultural characteristics. Furthermore, exploiting other *Rubus* sp. having resistance to *P. fragariae* var. *rubi* in breeding may show promise for creating such desirable recombinants while also diversifying the genetic interactions of the pathogen population with the host and slowing the development of new pathogenic races.

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