Assessing the Resistance of Red Raspberry (Rubus idaeus L.) Genotypes to Phytophthora fragariae var. rubi in Hydroponic Culture

J.A. Pattison1
Department of Horticultural Sciences, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456

W.F. Wilcox2
Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456

C.A. Weber3
Department of Horticultural Sciences, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456

Additional index words. root rot, disease resistance, disease assay

Abstract. A hydroponic method was developed and tested for screening red raspberry genotypes for resistance to Phytophthora fragariae var. rubi, the most common causal agent of Phytophthora root rot in raspberry. Plants of ‘Titan’ and ‘Encore’ exhibited typical disease symptoms, with the latter developing significantly smaller stem lesions and fewer petiole lesions. The resistant cultivar, ‘Latham’, regenerated healthy root tissue from the crown and older-order roots after initial infection and necrosis of young roots and exhibited no other symptoms beyond minor leaf chlorosis. This component of the resistance reaction has not been documented previously. A segregating F1 population from the cross of ‘Latham’ × ‘Titan’ had a survival rate of 56% with 42% classified as resistant, exhibited minimal symptoms, and produced varying amounts of healthy root tissue. This screening method allows multiple observations of all plant tissues, including roots, under repeatable and definable growth chamber conditions. It should be useful for classifying the phenotype of individuals in segregating red raspberry populations to investigate the inheritance of Phytophthora root rot resistance using molecular markers.

Phytophthora root rot is a severe disease of red raspberry (Rubus idaeus L.) in nearly all temperate growing regions of the world and imposes significant limitations on production capacity in the absence of control (Duncan et al., 1987; Wilcox et al., 1993). Although multiple Phytophthora spp. are pathogenic on this crop, P. fragariae var. rubi Wilcox and Duncan is by far the most common cause of serious losses in both Europe and the Americas (Duncan et al., 1987; Wilcox, 1989; Wilcox et al., 1993; Wilcox and Latorre, 2002). The organism thrives in poorly drained soils that tend to remain at or near saturation for extended periods of time, thereby favoring the production and dispersal of asexual, biflagellate zoospores (Wilcox, 1989). Under cool and saturated soil conditions, the polycyclic biology of P. fragariae var. rubi results in a compounded rate of inoculum increase and rapid spread of disease on susceptible hosts (Erwin and Ribeiro, 1996).

Typical disease symptoms include reduced frequency of primocane emergence, unproductive or collapsing floricanes resembling those damaged by low winter temperatures, and stunting of apical growing regions. Foliar symptoms commonly expressed are chlorosis, interveinal and perimeter necrosis, and scorching. In advanced disease stages, the pathogen destroys most or all of the root system and colonizes the crown and lower stem region, producing a characteristic water-soaked lesion that advances acropetally (Wilcox, 1989). Recommended control programs involve an integrated approach using a combination of genetic host resistance, avoidance or amelioration of wet soils, and registered fungicides, although host resistance appears to be the most critical component (Heiberg, 1995; Maloney et al., 1993; Wilcox et al., 1999b). Several cultivars possess high levels of resistance including ‘Latham’, ‘Ascot’ and ‘Newburgh’ (Barritt et al., 1979, 1981; Kennedy and Duncan, 1991; Laun and Zinkernagel, 1997; Wilcox et al., 1999a). Flooding duration can greatly influence the reaction of several red raspberry cultivars to P. fragariae var. rubi (Duncan and Kennedy, 1989). Under an excessive irrigation treatment and without flooding, some cultivars exhibited a response intermediate between symptom free and dead. However, when the same cultivars were exposed to a single 24-h flooding treatment, the intermediate reaction disappeared and these same cultivars died (Wilcox et al., 1999a). Laun and Zinkernagel (1993) suggested using larger plant and pot sizes for assessing intermediate reactions of cultivars. However, when screening thousands of seedlings in a breeding program, greenhouse materials and space are expensive and limited. Thus, there is a need for a cost-effective means for evaluating the resistance of red raspberry genotypes to phytophthora root rot that can differentiate an intermediate reaction and reduce environmental variability. Hydroponic culture offers the ability to regularly observe the root and crown tissues of red raspberry plants challenged with P. fragariae var. rubi and has the potential for providing optimum growth conditions for both pathogen and host. Therefore, the
objective of the current research was to design a phytophthora root rot screening procedure that could 1) reduce environmental variability (i.e., temperature, light, and rhizosphere moisture), 2) identify an intermediate reaction, and 3) classify the phenotype of standard cultivars consistently, compared to previous reports of their resistance levels.

### Methods and Materials

**Hydroponic apparatus.** A plastic storage container (Rubbermaid, Wooster, Ohio) measuring 51 × 36 × 20 cm, with an approximate volume of 38 L served as the hydroponic basin. The fitted lid had 35 evenly distributed 4 cm holes for planting. Presoaked, sterilized rockwool substrate cubes (∼4 cm³) secured the plant crowns into the holes. Roots were grown submerged in 28 L of half-strength Peter’s Professional Hydro-Sol 5-11-26 nutrient solution (W.R. Grace & Co., Fogelsville, Pa.), supplemented with 10 mM Ca(NO₃)₂ and maintained at pH 6.5. Two air stones, placed equidistant from each other and the basin sides, were connected by plastic tubing to a single aquarium-style air pump. Two 1.7-m² growth chambers were programmed with a 16-h day length at a constant 20 °C to accommodate the five experimental basins.

**Plant material.** Small plug plants of three red raspberry cultivars, derived from tissue culture and propagated in a greenhouse to minimize potential contamination with Phytophthora spp., were obtained from a commercial nursery (Nourse Farms, Whatley, Mass.). ‘Latham’ (‘King’ × ‘Loudoun’), ‘Encore’ (‘Canby’ × ‘Cherokee’), and ‘Titan’ (‘Hilton’ × NY598 (‘Newburg’ × ‘St. Walfried’)) were chosen to provide standards for the resistant, intermediate, and susceptible reaction types, respectively, based on observations in replicated field trials in Geneva, N.Y., or previous studies in a commercial nursery (Nourse Farms, Whatley, Mass.). ‘Latham’ (‘King’ × ‘Loudoun’), ‘Encore’ (‘Canby’ × ‘Cherokee’), and ‘Titan’ (‘Hilton’ × NY598 (‘Newburg’ × ‘St. Walfried’)) were chosen to provide standards for the resistant, intermediate, and susceptible reaction types, respectively, based on observations in replicated field trials in Geneva, N.Y., or previous studies in a commercial nursery (Nourse Farms, Whatley, Mass.).

Inoculation. Two pathogenic isolates of Phytophthora fragariae var. rubi, ATCC 16184 (M14) and NY588, were obtained from Peter Bristow at Washington State University and from one of our WFW collections, respectively. Isolates were maintained on solidV-8 agar plates as described by Wilcox et al. (1993). Inoculum was produced by growing the isolates separately in clarified V-8 broth for 14 to 21 d as described by Bristow et al. (1988). 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. Mycelium from each isolate was comminuted separately in a Waring blender to a fine powder and consisted of slight leaf chlorosis with a low incidence of petiole lesions. Crown and stem tissue remained healthy and symptom free. The nutrient solution was sampled weekly and examined under the microscope for the presence of Phytophthora zoospores, which were quantified using a hemocytometer. About 5,000 to 10,000 zoospores/mL of nutrient solution were detected at each sampling date.

Survival rate of the F₁ population based on a plant disease index score of ≤ 3 was 56% (Fig. 1). Both parental phenotypes were recovered with moderate frequencies indicating that a major genetic factor(s) is possibly contributing to the resistant phenotype in this population. Plant disease index scores of 1 and 2 were not observed in the parental or recombinant populations; however, a root regeneration score (0 to 3), where 0 = no new root production and 3 = vigorous production of new root tissue, was 2 = moderate production of new root tissue, and 3 = vigorous production of new root tissue.

<table>
<thead>
<tr>
<th>Plant disease index (0–5)</th>
<th>Y %</th>
<th>Petiole lesion incidence (%)</th>
<th>Root regeneration index (0–3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Latham’</td>
<td>1.5 b</td>
<td>87 c</td>
<td>0 b</td>
</tr>
<tr>
<td>‘Encore’</td>
<td>4.4 b</td>
<td>87 c</td>
<td>0 b</td>
</tr>
<tr>
<td>‘Titan’</td>
<td>4.7 b</td>
<td>87 c</td>
<td>0 b</td>
</tr>
</tbody>
</table>

Values represent the means from three replicate hydroponic basins with six plants of each cultivar per replication. Mean values not followed by a common letter are significantly different (Fisher’s LSD, \( P = 0.05 \)).

### Results

A randomized complete block design was employed with six plants of each cultivar randomly assigned a planting position within each of three hydroponic basins (blocks). Analysis of variance was used to determine significant differences in responses among cultivars and blocks. Cultivars were ranked using Fisher’s LSD based on the different criteria used to evaluate root rot susceptibility.
moderate foliar symptoms, retention of healthy crown and older root tissues, and limited to absent production of new roots. Two-thirds of the seedlings showed no stem lesions with the remaining individuals distributed evenly across a wide range of lesion length categories. Nearly 50% of the F1 population had an incidence of petiole lesions ≤10% (Fig. 1).

In the cultivar test, differences attributable to blocks were nonsignificant for all criteria, indicating that consistent phenotype classification of red raspberry genotypes can be accomplished even when populations, selections, or cultivars need to be tested in multiple basins. Correlation analysis among the four different assessment criteria, using all genotypes tested (including the F1 population), showed a very strong negative relationship between the plant disease index and root regeneration score (Table 3). Stem lesion length and petiole lesion frequency also showed highly significant but less pronounced associations with plant disease index values.

**Discussion**

The hydroponic system for assessing the reactions of red raspberry genotypes to *P. fragariae* var. *rubi* allowed for multiple, nondestructive observations of all tissues, including roots, on plants maintained under repeatable and uniform conditions of temperature, rhizosphere moisture, inoculum, and light. It produced results consistent with previous greenhouse and field tests (Barritt et al., 1979, 1981; Kennedy and Duncan, 1991; Laun and Zinkernagel, 1993; Levesque and Daubeny, 1999; Wilcox et al., 1999a) and allowed us to determine a reaction intermediate between resistant and susceptible. Advantages of this system include repeatability of environmental parameters throughout the year, reduced spatial needs for plant evaluation and the ability to periodically observe the development of disease symptoms on the roots and crown. Thus, it should be useful not only for the identification of resistant genotypes to be used in red raspberry breeding but also in more fundamental studies of this specific host pathogen interaction.

We were able to observe a potentially significant component of the resistant reaction not documented previously. Although roots of ‘Latham’ and resistant F1 genotypes became diseased shortly after exposure to the pathogen, these individuals limited the initial infection, excluded disease from older root tissue and subsequently regenerated healthy roots, which remained symptom free throughout the experiment. This suggests that resistance to *P. fragariae* var. *rubi* may be an induced defense response in red raspberry. Symptoms on the shoots of resistant phenotypes were limited to only minor leaf chlorosis and minimal petiole lesions and may have resulted merely from the initial infection event and subsequent reduction of young feeder roots. Previous reports have associated minor foliar symptoms and root rot, the absence of stem lesions and minimal formation of oospores in root tissue among resistant red raspberry genotypes (Barritt et al., 1979; Heiberg, 1995; Laun and Zinkernagel, 1993, 1997; Kennedy and Duncan, 1991).

‘Encore’ has exhibited an intermediate to susceptible field reaction to phytophthora root rot in extensive field trials at Geneva, N.Y. In the hydroponic system, ‘Encore’ became diseased and the perennial crown of most plants died. However, stem lesion severity and the frequency of petiole lesions were less than on the susceptible standard, ‘Titan’. Another factor potentially influencing the expression of partial field resistance in ‘Encore’ is its propensity to regenerate new primocanes from differentiated root tissue (suckers). ‘Titan’ predominately produces primocanes from existing crown tissue with minimal suckering, whereas ‘Encore’, exhibits a much greater propensity

<table>
<thead>
<tr>
<th>Evaluation parameter</th>
<th>Plant disease index</th>
<th>Root regeneration score</th>
<th>Stem lesion length</th>
<th>Percent petiole lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant disease index</td>
<td>1</td>
<td>-0.94</td>
<td>0.64</td>
<td>0.77</td>
</tr>
<tr>
<td>Root regeneration score</td>
<td>-0.94</td>
<td>1</td>
<td>-0.58</td>
<td>-0.70</td>
</tr>
<tr>
<td>Stem lesion (cm)</td>
<td>0.64</td>
<td>-0.58</td>
<td>1</td>
<td>0.78</td>
</tr>
<tr>
<td>Percent petiole lesions</td>
<td>0.77</td>
<td>-0.70</td>
<td>0.78</td>
<td>1</td>
</tr>
</tbody>
</table>

Fig. 1. Frequency distributions of an F1 population (‘Latham’ × ‘Titan’, n = 51) with respect to the four criteria used to evaluate the susceptibility to *Phytophthora fragariae* var. *rubi* in hydroponic culture.
to produce root derived primocanes (Maloney et al., 1998; Sanford et al., 1985). This may allow such plants to effectively recover from individual infection events in the field when disease development is limited by environmental conditions (e.g., unfavorable soil moisture and/or temperature). However, under the high disease pressure imposed throughout this study, ‘Encore’ was unable to regenerate healthy root tissue and suffered severe crown rot.

Basing the relative susceptibility to Phytophthora root rot on only one symptom of the disease, such as the presence or absence of a stem lesion, may not adequately represent the response to all other symptoms. For example, several F{sub 1} individuals had moderate to severe symptoms occurring on crowns and roots, and leaves, yet failed to manifest stem lesions. Secondary foliar symptoms of root rot including chlorosis, interveinal necrosis, and marginal scorching, likely result from root induced reductions of root surface area, but also are symptomatic of nutrient deficiencies in the absence of disease (Wilder and Strik, 1991). Therefore, a quantitative evaluation of root system necrosis would appear to provide the most direct measure of a raspberry plant’s response to Phytophthora sp. However, quantifying numbers of infected roots among large numbers of individuals may be prohibitively labor intensive, and is not an option in nonhydroponic systems. Given the strong correlation between root regeneration index and plant tissue and suffered severe crown rot.

The repeated, nondestructive examination of this host × pathogen interaction has identified a possible pathogen-induced resistance response, which merits further investigation. Future research will use this method to classify the phenotype of individuals in segregating populations to help aid in understanding the inheritance of root rot resistance through the use of molecular markers.

**Literature Cited**


