Pathogenicity testing of *Agrobacterium tumefaciens* and *Rhodococcus fascians* isolates on micropropagated plants

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Introduction

Both *A. tumefaciens* and *R. fascians* have a wide host range that includes woody and herbaceous plants. Virulent and avirulent forms of the bacteria exist in nature, and isolation and identification of the bacteria from infected plants must be followed by inoculation of isolates to susceptible plants. The bacteria infect many nursery crops such as fruit trees, berries, grapes, vegetables and ornamentals. The symptoms observed for *A. tumefaciens* infections are tumors (Fig. 1a) and those for *R. fascians* are leafy galls (Fig. 1b) and shoot proliferation (Fig. 1c). In some cases, symptoms of leafy gall have been confused with crown gall.

Figure 1a. Tumor on rose, 1b. Leafy gall on *Scabiosa* and 1c. Shoot proliferation and leafy galls on *Physocarpus.*

Testing for pathogenicity of isolates from infected plants requires a ready supply of susceptible plants and ample greenhouse space. Results may be compromised by unintentional spread of the bacterial isolates by water splash. Symptom development on test plants may require as long as four weeks, or often longer. An alternative to plant inoculations in the greenhouse is the use of micropropagated plantlets *in vitro*. Advantages of this system are a) reduced space requirements, b) plants produced are gnotobiotic, c) rapid multiplication of host plants, and d) more rapid symptom development. The objective of this research was to establish plants in tissue culture for inoculation that could easily be maintained and if possible, find individual plant hosts that are susceptible to both bacterial species.

Materials and Methods

Shoot tips of test plants were surface sterilized and placed into tubes of liquid growth medium with 0.300 g of the antibiotic cefataxime. The tubes were placed in a rack on a rotary shaker at 1,500 rpm for 48 h. Those plants showing no contamination were then transferred to a growth medium solidified with agar. The plantlets were transferred to multiplication and rooting media as needed. Plants established in tissue culture are listed in Table 1.

Plants used for inoculation were grown on rooting medium and inoculated with bacteria grown for 24 – 48 h prior to testing. *A. tumefaciens* isolates were inoculated to wounded plant leaves and stems or the apex of a cut stem. Either a sterile 27 gauge needle or a sterile toothpick was used to apply bacteria directly from the Petri dish to the wounded plant. *R. fascians* isolates were applied to leaf axils using a sterile toothpick. Controls included plants inoculated with a) a known virulent strain and b) an avirulant strain, c) wounded plants and d) unwounded and un-inoculated plants. Plants were maintained in a growth chamber at 23 C with a 16 h photoperiod.

Initial inoculations of *A. tumefaciens* strains were made to cherry, apple and Himalayan blackberry *in vitro* with isolates from these same plant hosts. Subsequent inoculations to blackberry included virulent and avirulent bacteria from culture collections, isolated from a wide variety of hosts. *R. fascians* isolates were tested for pathogenicity on *Oenothera speciosa,* as it had produced the most consistent results when compared to other plants in greenhouse tests.

Plant host	Susceptibility	Plant host	Susceptibility
Apple	*A. tumefaciens*	*Erysimum*	*A. tumefaciens* *R. fascians*
Argyranthemum	*A. tumefaciens* *R. fascians*	Grape	*A. vitis* *A. tumefaciens*
Blackberry	*A. tumefaciens* *A. vitis*	*Heuchera*	*A. tumefaciens* *R. fascians*
Bryophyllum	*A. tumefaciens*	*Oenothera*	*R. fascians*
Cherry	*A. tumefaciens*	*Petunia*	*A. tumefaciens* *R. fascians*
Echinacea	unknown	Raspberry	*A. tumefaciens*

Table 1. Plant hosts established in tissue culture

Results

Blackberry was clearly superior to apple or cherry as a host in pathogenicity testing of *A. tumefaciens* strains from our culture collection. Symptoms were observed after one week, and after two weeks, 82% of the wound sites had tumors as compared to 39% for cherry and 4% for apple. Of the 69 known virulent strains tested, 58 (84%) produced symptoms on blackberry, and none of the 18 avirulent strains tested produced tumors.

Figure 2a. Blackberry plantlets inoculated with *A. tumefaciens* strain B206B and Fig. 2b. Apple plantlets inoculated with *A. tumefaciens* strain B209.

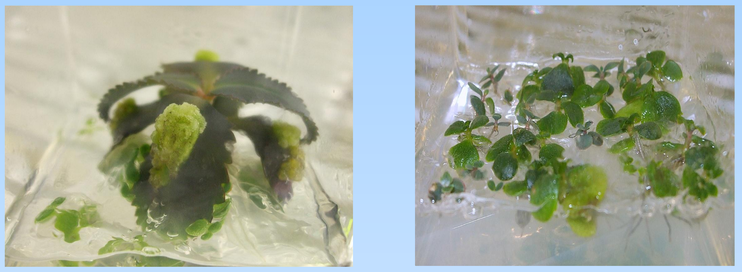

Figure 3a. Tumors on micropropagated *Bryophyllum* in tissue culture one month post- inoculation with *A. tumefaciens* strain AF52/95 . 3b. Propagation of *Bryophyllym* from new plants arising on leaf margins.

Symptoms on *Bryophyllum in vitro* inoculated with *A. tumefaciens* strains could be observed after five days, as compared to two weeks in the greenhouse. Symptoms at three weeks post-inoculation are shown in Fig. 4 for *in vitro* vs. greenhouse plants. Two pathogenic strains, EU8 from Euonymus and P1/75 from dahlia produced no symptoms on blackberry but were positive on *Bryophyllum*. There were other pathogenic strains that did not produce symptoms on blackberry or *Bryophyllum*, three from grape and two from pecan.

Fig. 4a. Tumors on *Bryophyllum* in tissue culture three weeks post-inoculation and with A. tumefaciens strain B49c/83. 4b. The same strain inoculated to *Bryophyllum* in the greenhouse after three weeks.

Figure 5a. Inoculations of blueberry isolate L21/94 onto red raspberry and 5b. Grape isolate 05-2 onto Syrah grape The tumors that develop on grape plantlets become necrotic over time, as shown on the plantlet at the left.

Red raspberry *in vitro* produced symptoms from a blueberry strain as shown in Fig. 4a, as did isolate 05-2, a grape strain inoculated to grape plants. One of the most commonly infected hosts with crown gall submitted for diagnosis to the OSU Plant Clinic is *Argyranthemum*. This plant produces enormous tumors when inoculated in the greenhouse, and is easily infected *in vitro.* The plant appears to be systemically infected, as tumors frequently appear at leaf margins as seen in Fig. 6a. Both *Argyranthemum* and *Heuchera* are susceptible to infection by *A. tumefaciens* (Fig. 6) and *R. fascians*, (Fig. 7).

Fig. 6a. *Argyranthemum* inoculated with *A. tumefaciens* strain A51 two weeks post-inoculation. Fig. 6b. *Heuchera* inoculated with strain A47a showing small tumors at several wound sites two weeks post-inoculation.

Pathogenicity of isolates from OSU Plant Clinic submissions with leafy gall have been confirmed from *Campanula, Coreopsis, Erodium, Lamium, Leucanthemum, Oenothera,* and *Sorbaria* by inoculations to *Oenothera in vitro* this past year.

Fig. 7a. *Argyranthemum* inoculated with *R. fascians* strain 3b on the left and wounded control plant on the right and 7b. *Heuchera* inoculated with virulent *R. fascians* strains 05-2255-3c on the left, A25f on the right and avirulent strain 04-516 in the center.

Fig.8a. Uninoculated *Oenothera* plantlet growing on stage three rooting medium. 8b. *Oenothera i*noculated with *R. fascians* strain A3b.

Fig. 9a. *Sorbaria* with natural infection showing leafy galls and shoot proliferation. 9b. Tissue culture grown *Oenothera* inoculated with avirulent *R. fascians* isolate 04-516 (plant on left) and virulent isolate 08-446-1(3)a from *Sorbaria* (plant on the right).

Discussion

The variability that exists in populations of *A. tumefaciens* and *R. fascians* makes it difficult to find one universal host for testing pathogenicity of individual bacterial isolates. Blackberry was a very good indicator host for *A. tumefaciens* from many woody plant isolates, but not all pathogens tested from our collection produced symptoms on this host. Some of these pathogens may have a limited host range or be host specific (1). It is difficult to inoculate hosts from which *R. fascians* was originally isolated and reproduce symptoms. Germinating pea seedlings have been used successfully to detect pathogenicity and have been used as an indicator host plant (2). The most reliable host representing plants affected in the greenhouse in our experience has been *Oenothera speciosa*. Since there is sometimes confusion about the origin of galls on some plants, finding host plants that will respond positively to both *A. tumefaciens* and *R. fascians* with clearly differentiated symptoms is very desirable. *Argyranthemum* and *Heuchera* have produced symptoms from both species. Greenhouse inoculations of *Erysimum* and *Petunia* also display symptoms from both bacteria and have just recently been established in tissue culture. Micropropagated plants are gnotobiotic, take up little space, are easy to propagate and inoculate, develop symptoms rapidly and offer an underutilized alternative to greenhouse testing.

References

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