Efficacy of Resistance to Scab in Transgenic 'McIntosh' Apple Exposed to Populations of *Venturia inaequalis*

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Abstract. McIntosh apples which have been transformed to express an endochitinase gene from *Trichoderma harzianum* have demonstrated substantial resistance to apple scab in previous greenhouse trials. We inoculated transgenic and control McIntosh trees with ascosporic and conidial inoculum, and recorded disease development and plant growth in a greenhouse and orchard study. In greenhouse evaluations, all transgenic lines developed statistically equivalent levels of scab when compared to the McIntosh control with both inoculum sources. Transgenic trees in the orchard generally had fewer leaves per tree, were shorter, smaller in diameter, and had fewer side branches than the nontransformed control McIntosh early in the growing season. However, this was primarily a reflection of different tree sizes among the transgenic lines and control trees when planted in 1999. With only one exception (TM961), current-season shoot length was not significantly different among the transgenic lines and the control McIntosh. In the orchard, the incidence of scab infection (percentage of leaves infected) on all four transgenic lines (including the vector control) was significantly equivalent to that recorded on the untransformed control McIntosh. However, disease severity (percentage of the leaf surface colonized) was significantly lower on all the transgenic lines.

Background and Justification. Apple scab is the most destructive disease of apples worldwide. The disease is routinely controlled in commercial apple production in the northeastern US by the repeated applications of fungicides. Although great improvements have been made in the efficacy and safety of modern fungicides, they remain an economic burden to growers, and a constant cause of concern among consumers.

There has been a substantial effort at Cornell to develop transgenic apples resistant to major diseases. The research has received support from many areas, including the apple industry. Transformation of existing cultivars to augment disease resistance has the advantage of retaining the desirable horticultural characteristics of a known cultivar, as well as the name recognition and established market of such cultivars.

Recently, the cultivar McIntosh, by far the most widely-planted and important cultivar in New York, has been transformed to express an endochitinase gene, an exochitinase gene or both genes, originally isolated from *Trichoderma harzianum*. Expression of one or more of these genes in certain clones of McIntosh has reduced disease severity and sporulation of lesions by up to 90% as compared to the non-transgenic control in greenhouse evaluations. In practical terms, this exceeds

the difference in susceptibility that is generally observed in field evaluations between McIntosh and Golden Delicious. The latter cultivar, while not immune to scab, is regarded as a scab-resistant cultivar in the New York, and is not intensively treated to control the disease.

All primary screening of transgenic apples at Geneva for resistance to apple scab has been performed using a mixture of conidia representing clonal isolates of each of five pathogenic races, plus a small number (1 to 3) of clonal wild-type isolates. Greater variation in sensitivity to transgene products (and hence variation in resistance to scab) may be identified by exposing transgenic and non-transgenic parent trees to ascosporic inoculum. Ascosporic inoculum is a result of sexual crossing and recombination within the pathogen population, and thus even small collections of overwintered leaves contain millions of genetically unique ascospores. It is also important to demonstrate resistance of transgenics under conditions closer to natural infection.

OBJECTIVES

I. Evaluate the degree of resistance to scab in transgenic and non-transgenic McIntosh potted trees inoculated with ascospores and conidia.

II. Evaluate the degree of resistance to scab in transgenic and non-transgenic McIntosh trees exposed to simulated natural infection in an orchard.

PROCEDURES

Objective I. Potted trees raised from in-vitro plants representing a single transgenic line of McIntosh, as well as the non-transformed control were grown in the greenhouse to produce 10-15 leaves. Trees representing transgenic McIntosh lines containing an endochitinase, an exochitinase, or both genes; a transgenic control line of McIntosh without the chitinase gene; and a non-transgenic control of McIntosh, all of which were evaluated in the 1999 trials, were placed in a mist chamber at 25 C for 24 hours. Overwintered scabbed leaves collected beneath unsprayed Rhode Island Greening trees at NYSAES in Geneva shortly before 1 cm green in April of 2000 were incubated until they contained abundant, mature ascospores. Eighteen 30 X 30 cm hardware-cloth cages containing overwintered leaves were be placed on the floor of the mist chamber. A Burkard volumetric spore sampler was placed at the center of the chamber to provide an hourly record of airborne inoculum dose.

After inoculation, the trees were returned to the greenhouse and observed for symptom development 21 days later. Disease incidence and severity were assessed at four leaf positions. The above inoculations were repeated on a second set of trees using a conidial suspension of the isolates used in prior evaluations of the resistance of the candidate lines to apple scab.

Objective II. Field evaluations of transgenic apple were performed in a planting established at NYSAES in April of 1999. The planting consists of 6 rows of 50-55 trees each, with 0.91 m separation between trees and 4.57 m separation between rows. A single 30-cm-square mesh bag containing a layer of overwintered scabbed leaves treated as above was pinned to the ground at the base of each tree one week after bud break. Four transgenic lines of Marshall McIntosh were included in the trial.

Incidence and severity of apple scab was assessed as the number of scabbed leaves per 100 assessed, and the percentage of surface area colonized by scab. Impact of the transgenes upon plant growth was assessed by recording total number of leaves per tree, tree height, tree diameter, and number of side branches.

RESULTS AND DISCUSSION

In greenhouse evaluations, all transgenic lines developed statistically equivalent levels of scab when compared to the McIntosh control with both inoculum sources (Table 1), although overall disease severity was lower and more variable when ascosporic inoculum was used (Table 1). Transgenic trees in the orchard generally had fewer leaves per tree, were shorter, smaller in diameter, and had fewer side branches than the nontransformed control McIntosh (Table 2). However, this was primarily a reflection of different tree sizes among the transgenic lines and control trees when planted in 1999. The four transgenic lines were approximately one-half the size of the control McIntosh at the time of planting. With only one exception (TM961), current-season shoot length in 2000 was not significantly different among the transgenic lines and the control McIntosh (Table 2). Also in the orchard, the incidence of scab infection (percentage of leaves infected) on all four transgenic lines (including the vector control) was significantly equivalent to that recorded on the untransformed control McIntosh (Table 3). However, disease severity (percentage of the leaf surface colonized) was significantly lower on all the transgenic lines (Table 3).

Some, but not all of the reduction of disease severity observed in the orchard study may have been due to lower canopy density and smaller tree size in the transgenic lines. As target size decreases, so does the probability of "hits" when dealing with aerially-dispersed inoculum. Transgenic lines generally bore fewer leaves, and consequently would be expected to intercept fewer asocspores (the reason we expressed disease incidence as percentage of infected leaves rather than infected leaves per tree). This consideration aside, there was still a significant reduction of disease severity (percentage of leaf surface scabbed) once the ascospores were intercepted by transgenic trees in the orchard (Table 3). Why this was not observed in the greenhouse trial (Table 1) is unclear, but may indicate that the method of ascosporic inoculation used in the mist chamber is inherently variable, and thus obscures the treatment differences, or the greenhouse environment (very warm compared to the orchard) affects the differential expression of resistance observed in the orchard. The latter might also explain the greenhouse results obtained with conidial inoculum.

In summary, there does appear to be some resistance to scab conferred by the transgenes used, but it may be variable in its expression between greenhouse and orchard trials. Note that the transgenic lines evaluated in the present study did exhibit significant resistance in earlier greenhouse studies. However, both in 1999 and 2000, we have only been able to detect significant resistance in the orchard trials. This variable effect on scab has been accompanied by a significant and consistent reduction in tree growth in only one of the four transgenic line evaluated in the present orchard investigation: TM961 which was transformed to produce both exo- and endochitinase.

Table 1. Greenhouse study of disease severity measured as percentage of the leaf surface colonized following inoculation of transgenic and control trees of the cultivar McIntosh in 2000.

Line	Introduced gene	Ascosporic inoculum Leaf surface infected (%)	Conidial inoculum Leaf surface infected (%)
McIntosh(control)	none	12.3	85.8
TM286(control)	none	5.3	73.8
TM837	exo	6.3	70.3
TM891	exo	24.8	83.3
TM961	endo+exo	20.5	84.0

Differences within columns are not significantly different from the control (McIntosh) at P = 0.10 according to Student's t-distribution. TM286 was a transgenic McIntosh control containing the vector, but no gene for chitinase. "Exo" indicates that the introduced gene coded for exochitinase and "endo" indicates that the introduced gene coded for endochitinase. Disease was rated 21 days after inoculation of potted trees with either ascospores or conidia. Acosporic inoculum was from an abandoned Rhode Island Greening orchard. Conidial inoculum was harvested from diseased seedings in a greenhouse.

Table 2. Tree height, leaves per tree, tree diameter, and number of side branches on transgenic and control trees of the cultivar McIntosh in 2000 at the Sutton Road Orchard. Note: leaves/tree, tree height, tree diameter, and number of side branches primarily reflect differences in tree size at planting in 1999, not growth during 2000.

Line	Introduced gene	Total leaves per tree	Tree height (cm)	Tree diameter (mm)	Number of side branches	Current season shoot length (cm)
McIntosh(control)	none	1183 a	76.6 a	13.3 a	8.3 a	13.8 a
TM286(control)	none	508 b	73.8 a	10.6 b	3.9 b	13.3 a
TM837	exo	520 b	64.3 b	9.5 b	4.8 b	14.4 a
TM891	exo	473 b	62.6 b	9.4 b	4.1 b	13.5 a
TM961	endo+exo	189 c	44.9 c	6.4 c	0.5 c	12.4 b

Treatment means within columns followed by the same letter do not differ significantly from controls at P = 0.10 according to Student's t distribution. . TM286 was a transgenic McIntosh control containing the vector, but no gene for chitinase. "Exo" indicates that the introduced gene coded for exochitinase and "endo" indicates that the introduced gene coded for endochitinase. Tree diameter was measured at 4 cm above soil level. Tree height, diameter, and number of side-branches were measured on 27 April. Total number of leaves per tree was measured on 19 July. Current season shoot length was measured on 26 May.

Table 3. Percentage of infected leaves (out of 100 examined), and the percentage of the leaf surface on transgenic and control trees of the cultivar McIntosh in 2000 at the Sutton Road Orchard.

Line (introduced gene)	Percentage of leaves infected	Percentage of leaf surface infected
M. McIntosh (none)	30.3 a	29.0 a
TM286 (vector control)	30.8 a	19.2 b
TM837 (exo)	26.8 a	15.0 b
TM891 (exo)	23.7 a	17.0 b
TM961 (endo + exo)	24.3 a	10.2 c

Numbers within columns followed by the same letter are not significantly different at P = 0.10. TM286 was a transgenic McIntosh control containing the vector, but no gene for chitinase. "Exo" indicates that the introduced gene coded for exochitinase and "endo" indicates that the introduced gene coded for endochitinase. The percentage of leaves infected (number infected out of 100/tree assessed), as well as the percentage of the leaf surface infected (on the 4 most-severely diseased leaves encountered) was measured on 19 July.