Methods and Results of Screening for Disease- and Insect-Resistant Apple Rootstocks

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The apple rootstock breeding and evaluation program at Geneva, NY, a joint effort of the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) and Cornell University, has been a leader in the development of new rootstock genotypes of apple. In comparison to rootstock development programs in other parts of the world, a greater emphasis has been placed on disease and pest resistance. Geneva series rootstocks undergo rigorous screening procedures to verify tolerance to fire blight (*Erwinia amylovora*), crown/collar rot (*Phytophthora* spp.), and woolly apple aphid (*Eriosoma lanigerum*). In addition, these genotypes are selected for high levels of productivity in replicated orchard trials.

The apple rootstock breeding program uses a protocol of approximately 30 years that leads to the development and identification of superior rootstock genotypes. The program is subdivided into ten stages of approximately 3 years each, during which the number of genotypes is steadily reduced, but the number of replications observed for retained genotypes steadily increases. New seedlings enter the program annually, and less promising genotypes are discarded from the program annually, resulting in an overall steady state in the number of genotypes under evaluation. Figure 1 is a schematic diagram describing the process.

The general method employed by plant breeders is to generate large populations of novel genotypes and then to progressively decrease the population size by removing the genotypes that are not commercially desirable. The complication that makes apple rootstock breeding more challenging is that the final evaluations of the genotypes are as a part of a compound system with both a rootstock and a scion genotype. Because the most expensive phase of the breeding program is orchard trials, we prefer to eliminate disease- and insect-susceptible genotypes in the seedling stage, stage 1 (Fig. 1), whenever possible. Rigorous screening increases the proportion of new rootstock genotypes in orchard trials that are already disease and insect resistant.

For promising genotypes from the Geneva program, as well as for genotypes from other programs, confirmation of disease and insect resistance is required, and this occurs in stages 4-10 (Fig. 1). Seedling screens are not perfectly accurate, and susceptible individuals may continue beyond the seedling stage. In addition, new rootstock genotypes from other breeding programs, which may or may not have included seedling screens for insect and disease resistance, are continuously introduced to the marketplace, sometimes without adequate levels of testing.

In contrast with seedling screens, these elite genotype screens provide a relative ranking of stress tolerance among the genotypes rather than a pass/fail test that determines the survival of each genotype. A major goal of the Geneva apple rootstock breeding and evaluation program is to provide the results of these elite screening procedures to the public, allowing growers to make informed decisions when choosing among rootstock genotypes for specific applications.

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Six specific disease and insect targets warrant concerted effort through breeding. These are, ranked in order of economic importance to North American agriculture, fire blight, crown rot/root rot, woolly apple aphid, southern blight, white root rot and Texas root rot.

- Fire blight (*Erwinia amylovora)*
- Crown rot/root rot (*Phytophthora* spp.)
- Woolly apple aphid (*Eriosoma lanigerum*)
- Southern blight (*Sclerotium rolfsii*)
- White root rot (*Rosellinia necatrix*)
- Texas root rot (*Phymatotrichum omnivora*)

FIRE BLIGHT—*ERWINIA AMYLOVORA*

Fire blight is economically the most important disease affecting apple rootstocks in North America. This bacterial disease exhibits multiple strains with differential pathogenicity to apple genotypes. While fire blight is most commonly considered with respect to scion damage, the most severe losses due to the disease occur when scion infections are translocated through the trunk and into hypersensitive rootstock cultivars, such as the popular M.9 and M.26 genotypes. Fortunately there are apple genotypes that show effective resistance to known strains of fire blight, and these have been incorporated as parents into rootstock breeding programs.

At Geneva, fire blight screening begins on seedlings in stage 1 of the breeding program (Fig. 1). Screening is repeated in stage 2 on individual stool trees prior to evaluation in orchard trials. In stage 1 and stage 2 screening, direct, manual inoculation with mixtures of fire blight strains is used to reduce the chances of selecting genotypes that are resistant to only some strains of the bacteria. Successful genotypes that survive this initial seedling screen, as well as elite genotypes from other programs, are included in more in-depth evaluations: strain-specific tolerance tests and orchard rootstock blight tests.

Rootstock genotypes that show commercial promise following orchard trials in stage 3 (Fig. 1) or that are approaching commercialization from any source are collected for an evaluation of possible differential resistance to strains of fire blight. Beginning at stage 6 (Fig. 1), multiple plants per genotype are directly, manually inoculated with one of each of several strains of fire blight. The progression of disease symptoms in the rootstock tissue is measured as an indication of the relative tolerance of each genotype to each strain of fire blight.

Final evaluations of fire blight resistance are conducted for the most elite genotypes in orchard trials. Young orchard trees, typically third leaf in their first year of production, are directly sprayed on open flowers with a high concentration of fire blight inoculum. This method of inoculation results in fire blight infections at every flower cluster on the young trees. The severity and speed of infection from direct floral inoculation are more intense than any naturally occurring outbreak of fire blight and result in rapid translocation of the bacteria into the rootstock. Systemic necrosis of hypersensitive rootstocks, such as M.26 and M.9, results on most trees before the end of the growing season, followed rapidly by tree death. Resistant genotypes may exhibit some temporary symptoms of rootstock infection, but most or all trees recover rapidly and resume normal growth.

CROWN ROT/ROOT ROT—*PHYTOPHTHORA* SPP.

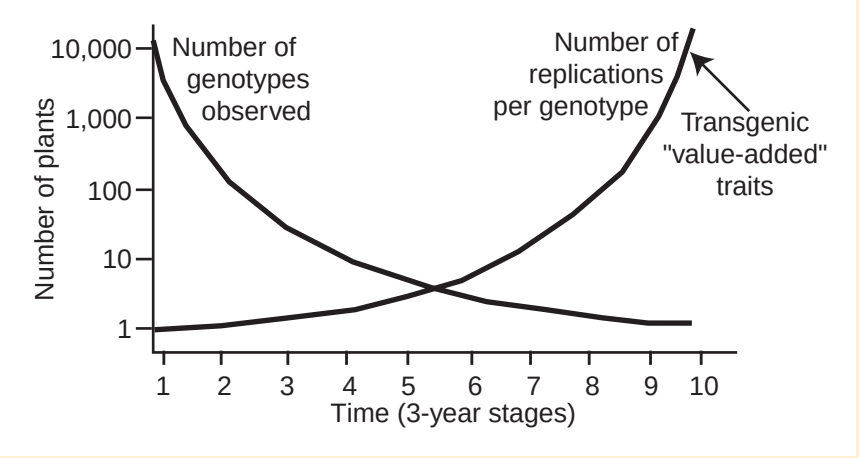
Phytophthora induced root rot or crown rot diseases are common in all major apple regions of the world, and these fungal pathogens affect many species of crop plants. In addition, there are multiple species of the fungus, and perhaps multiple strains within species, that have differential abilities to cause disease on apples. *Phytophthora* is a relatively difficult pathogen to manipulate experimentally because it requires very exacting conditions to initiate infection. The pathogen has a relatively narrow range of temperatures at which infection can occur and can cause severe disease only when soils are waterlogged. Some rootstock genotypes are said to be resistant (M.9) or susceptible (MM.106) to infection by *Phytophthora*, but these are generalizations. Under severe disease pressure, even so-called resistant genotypes can exhibit disease. Management of *Phytophthora* induced root rots requires a combination of genetic resistance, good site choice and proper orchard management.

Seedlings are particularly susceptible to infection by *Phytophthora*, and this provides an excellent opportunity for screening in the Geneva apple rootstock program. Seeds of new potential rootstock genotypes are planted in specially designed boxes that allow the soil to be flooded and drained. Five to ten days after seedling emergence, an inoculum of *Phytophthora cactorum* zoospores is applied to the flooded boxes at a rate of approximately $1x10^{9}•m^{-3}$. The boxes are drained after 24 hours, and disease symptoms are visible beginning in 48 hours. Typically 80% or more seedlings are infected and killed by the fungus at this stage, resulting in populations that have a high frequency of resistance to the pathogen.

Beginning at stage 6 (Fig. 1) in the program, elite genotypes are subjected to replicated tests with multiple strains of *Phytophthora* spp. isolates. Although protocols for this screening procedure are still under development, the general method is to expose 15 rapidly growing, potted liners to one of each of several species and isolates of *Phytophthora* fungus. Both treatment (inoculated) and control (non-inoculated) plants are grown on special tables designed to allow flooding of the plants for a period of 72 hours per week during the infection stage. We hope to be able to determine if different types of *Phytophthora* produce different patterns of infection across genotypes. We also hope to verify that the relatively tolerant genotypes are in fact tolerant to all of the most common types of the pathogen. Susceptible Geneva series rootstocks are discarded if they exhibit susceptibility to common strains of the pathogen.

FIGURE 1

Schematic diagram of the apple rootstock breeding and evaluation program at Cornell University. The horizontal axis represents time, in 3-year increments, during which specific operations are performed on each genotype under evaluation. The vertical axis represents the approximate average number of genotypes or plants under evaluation at any given time in the breeding program. The down-sloping line represents the decrease in number of genotypes observed as less promising genotypes are discarded over time. The up-sloping line represents the increase in the number of individual plants of each genotype propagated asexually for evaluation as genotypes approach commercialization. Transgenic methods of genotype enhancement are considered for commercially accepted genotypes from both the Cornell program and from other programs.



WOOLLY APPLE APHID—*ERIOSOMA LANIGERUM*

Woolly apple aphid (WAA) is the major insect pest affecting apple rootstocks, and it is the primary limitation on production in many regions of the southern hemisphere. WAA severity is increased in mild winter areas and in areas where the natural predators (primarily *Aphelinus mali)* are less active. The MM (Malling-Merton) series of rootstocks, including MM.106 and MM.111, were developed in England specifically for resistance to woolly apple aphid. Unfortunately there are presently no commercially available apple rootstocks smaller than MM.106 (about 70% standard seedling size) with WAA resistance. There also appears to be differential tolerance of some apple rootstocks to different strains of WAA.

In the Geneva program, seedlings of apple rootstock genotypes are exposed to WAA while still in pots in the greenhouse. This stage 1 (Fig. 1) screening follows screens for fire blight and *Phytophthora* resistance and is the final screen prior to field planting (stage 2). Colonies of WAA are selected in the field and distributed throughout the potted plants, and those that exhibit WAA colony formation in the greenhouse are discarded. WAA infestation in rootstock field plantings is noted when it occurs, and susceptible genotypes are typically discarded. In the future, we intend to develop screening protocols for replicated WAA tests that will verify the WAA resistance status of elite genotypes.

SOUTHERN BLIGHT—*SCLEROTINIA ROLFSII*

Southern blight is a fungal disease with a broad host range that attacks apples in a few important production regions. Typically southern blight is economically significant only in regions with mild winters and high levels of soil organic matter, such as is found in some orchards in California, New Zealand and the southeastern USA. Severe outbreaks of southern blight on susceptible varieties can result in complete orchard loss, and disease severity is higher on young trees. Malling 9 has the highest level of natural tolerance to *Sclerotinia rolfsii* among apple rootstocks, and rootstock choice can help to prevent major outbreaks. Screening for tolerance of this pathogen is not performed on seedling populations because the disease is less widespread. Many elite genotypes have inherited tolerance through their M.9 ancestry because most elite genotypes from all rootstock breeding programs have M.9 at some point in their pedigree. Until recently, resistance to this pathogen has not been a major objective of breeding programs for apple rootstocks. We intend to develop screening protocols for southern blight in the future that will evaluate elite genotypes (stages 7-10, Fig. 1) for resistance. We are presently seeking collaborators and sites with high levels of infestation.

WHITE ROOT ROT—*ROSELLINIA NECATRIX*, AKA *DEMATOPHTHORA*

White root rot caused by *Rosellinia necatrix*, not to be confused with white root rot caused by *Scytinostroma galactinum*, is a broad host range fungal pathogen that is widely distributed. This pathogen is usually most severe in subtemperate regions (due to higher soil temperatures), on heavy soils and in moist conditions. White root rot is a major constraint to production in regions such as southern Brazil, where conditions favor the disease. No commercially available rootstocks show resistance to this fungus. However, preliminary studies have shown that some wild relatives of cultivated apples may tolerate the fungus. Resistant rootstocks from traditional breeding methods will not be available for many years. However, concerted effort toward the development of transgenic *Rosellinia* resistant rootstocks could be productive. Screening for *Rosellinia* resistance is needed for seedlings in a future traditional breeding strategy at stage 1 and for future transgenic genotypes at stage 10 (Fig. 1).

TEXAS ROOT ROT—*PHYMATOTRICHUM OMNIVORA*

Texas root rot is a fungal pathogen that is the primary constraint to apple production in many parts of the southwestern US and northwestern Mexico. This pathogen is known as one of the most destructive and wide-ranging diseases of plants and is adapted to alkaline soils low in organic matter. There are no known sources of resistance to this pathogen. Grasses, however, are not affected by the disease. Because of the lack of resistance sources, transgenic approaches are probably the only viable techniques for obtaining resistance to this pathogen. We are seeking collaborators in the USA and Mexico to address this disease.

REPLANT DISEASE

Replant disease is characterized by diminished growth when apple orchards are planted in sites that held apple (or other orchard trees) in the past. Although replant disease is perhaps the most significant disease orchardists face economically, there is not a simple genetic solution to the problem. There is not a single cause of this syndrome, but instead a group of bacteria, fungi, nematodes and chemicals that diminish growth. Researchers have found that replant disease is site specific and not easily predicted. Because of the multiple causes, screening for replant disease is not a simple process, and seedling screens (stage 1, Fig. 1) are useless.

It does appear that there are differences among rootstocks in their tolerance of replant conditions. These differences are difficult to measure, but we are conducting pilot studies at Geneva in an attempt to develop screening protocols that might be effective in identifying replant-tolerant genotypes. In the pilot studies, soils are collected from sites with severe replant disease symptoms, soils are mixed and half of the soil is steam pasteurized to eliminate all disease-causing organisms. Equal numbers of rooted, unbudded rootstock plants are grown in the treatment soils (unpasteurized) and control soils (pasteurized).

Following 1 year of growth, the plants are removed and weighed. We then look for rootstock genotypes that have relatively little difference in growth between the treatment and the control. In the first year of the pilot study, we observed no clear patterns across genotypes using New York replant soils other than that the pasteurized soils resulted in 20% more overall growth. In the second year we will look at relative growth rates in Washington replant soils. If we identify genotypes that appear to be relatively unaffected by replant soils, we will follow the pot study with orchard trials.

Plant pathologists are working to identify the specific pests and pathogens that cause replant disease in various regions. Successful screening techniques for the specific pests and pathogens underlying replant disease are much more likely once they have been identified conclusively. Until that time, screening procedures for replant disease will remain crude.

CONCLUSIONS

New rootstock genotypes with resistance to many of the major pests and pathogens are gradually becoming available through the efforts of breeders worldwide. The primary challenge is identifying the genotypes that have both the desired disease and pest resistance but that also have the excellent nursery and orchard characters of the best commercially available rootstocks.

- Fire blight resistant rootstocks are now available.

- *Phytophthora* root rot resistance of new rootstocks is improving.
- Woolly apple aphid resistant rootstocks in dwarf size classes are approaching commercialization.
- Southern blight resistant rootstocks are probably common, their identification requires only effective screening.
- White rot resistance breeding is in the earliest stages.
- Texas root rot resistance requires strong efforts.
- Replant disease tolerance might not be addressed easily through breeding at this time.

Productive, size-controlling and disease-resistant rootstocks are gradually being developed and released, and growers can look forward to steady improvement in the quality of rootstocks that will be available in the future.

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Replant disease: Ian Merwin (Cornell), Mark Mazzola (USDA-ARS, Wenatchee, WA)

Woolly apple aphid: Harvey Reissig (Cornell)

White root rot: Herb Aldwinckle (Cornell)

Southern blight, Texas root rot: Interested parties may apply

CONVERSION FACTORS ENGLISH VS. METRIC

To convert Column 1 into Column 2, multiply by:	Column 1	Column 2	To convert Column 2 into Column 1 multiply by:
	Length		
.621	kilometer, km	mile	1.609
1.094	meter, m	yard	.914
3.281	meter, m	foot, ft	.3048
39.4	meter, m	inch	.0254
.03281	centimeter, cm	foot, ft	30.47
.394	centimeter, cm	inch	2.54
.0394	millimeters, mm	inches	25.40
metric:	1 km = 1000 m; 1 meter = 100 cm; 1 meter = 1000 mm		
English:	1 mile = 5280 ft; 1 mile = 1760 yards; 1 yard = 3 ft; 1 ft = 12 inches		
	Area		
247.1	kilometers^2, km^2	acre	.004047
2.471	hectare, ha	acre	.4047
.4047	trees/hectare	trees/acre	2.471
metric:	1 ha = 10,000 m^2 = .01 km^2		
English:	1 acre = 43,560 ft^2		
	Volume		
1.057	liter	quart (US)	.946
English:	1 US gallon = 4 quarts		
	Mass—Weight		
1.102	ton (metric), MT	ton (English)	.9072
2.205	kilogram (kg)	pound, lb	.454
52.5	ton (metric) of apples	apple packed box, *carton	.01905
metric:	1 metric ton = 1000 kg		
English:	1 ton = 2000 lb; 1 packed box or carton* of apples = 42 lb		
	Yield or Rate		
0.446	ton (metric)/hectare, MT/ha	ton (English)/acre	2.242
.892	kilogram/hectare, kg/ha	pound/acre	1.121
.991	ton (metric) of apples/hectare, MT/ha	bins* of apples/acre	1.009
.4047	trees/hectare	trees/acre	2.471
0.107	liter/hectare	gallon (US)/acre	9.354
metric:	1 metric ton = 1000 kg; 1 hectare = 10,000 m^2		
English:	1 ton = 2000 lb; apple bin* = 900 lb; 1 acre = 43,560 ft^2		
	Temperature		
1.8 C + 32	Celsius, C	Fahrenheit, F	.555 (F-32)

**Commercial cartons (packed boxes) of fruit and field/storage bins of fruit do not have universal weights. The weight of fruit in a packed box or carton varies around the world and with the type of fruit, but is here taken for apples as 42 lbs (19.05 kg); the weight of fruit in a bin also varies but is here taken for apples as 900 lbs (408.2 kg).*