USDA ARS Rootstock Breeding Program

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Introduction:
Root-knot nematodes (*Meloidogyne* species) infest as much as 65% of California's vineyard acreage, with statewide losses up to 20% of production (Nicol et al. 1999). Root-knot nematode feeding results in galled roots, weak vines, shortened vine life, and reduced establishment. The use of nematode resistant rootstock varieties, such as Freedom and Harmony, is limited by the emergence of aggressive virulent root-knot nematode populations that feed on and damage the important resistant rootstocks (Nicol et al. 1999, McKenry 1997, Cain et al. 1984). Apparently the use of today's resistant rootstocks selects for aggressive, resistance-breaking nematode populations. In addition to the diminishing utility of today's rootstocks in nematode control, other management options are decreasing. Methyl bromide is scheduled for phase out and other chemicals are being more strictly regulated. Improved resistant rootstocks that provide durable protection against virulent nematodes are needed.

The USDA Agricultural Research Service grape rootstock improvement program is breeding nematode resistant rootstocks for California and other viticultural regions. This research program works exclusively in grape rootstock breeding, genetics, and evaluation. Introducing rootstocks with nematode resistance superior to the industry standard rootstocks Freedom and Harmony is the primary objective of our program. While Freedom and Harmony have excellent resistance against many nematode populations, the long term phylloxera resistance of these rootstocks has been questioned due to their complex hybrid ancestry that includes *V. vinifera.* In order to breed superior rootstocks, we are crossing phylloxera resistant and tolerant rootstocks and selections with sources of resistance to aggressive root-knot nematodes. Improved varieties will have resistance to nematodes, protect against phylloxera, and be adapted to abiotic stress, soil conditions, and management practices.

Materials and Methods:
We make controlled crosses between parents to produce seedlings with superior nematode resistance and horticultural characteristics. While some commercially available rootstocks have some nematode resistance, resistance sources often are selections or wild species. We cross these with horticulturally acceptable rootstocks to combine resistance with easy rooting and grafting ability, desirable vigor induction, and adaptation to soil conditions. Crossing produces seedling populations from which we choose selections resistant to aggressive root-knot nematodes as potential new rootstocks. We evaluate seedlings in the greenhouse using the technique developed by Cousins and Walker (2001). Grape rootstock seedlings are grown in pasteurized sandy potting mix in individual pots. When the seedlings are at the two true leaf stage (about one month old), we inoculate them with 1500 aggressive root-knot nematode juveniles. We evaluate seedling resistance to root-knot nematodes six weeks after inoculation. Seedlings that show any signs or symptoms of nematode infestation are thrown out. Roots are examined visually and then stained with aqueous eosin-Y (0.25 g/L for one hour). If we see even one gall or stained egg mass on the roots, we discard the seedling. We select the seedlings with no galls and no egg masses—these seedlings completely suppress aggressive root-knot nematode reproduction. Selected seedlings are propagated. By testing the seedlings for resistance before they are planted in the vineyard we save vineyard time and space and accelerate the screening process. For genetics studies, all egg masses are counted on all the seedlings in a population.

We plant the nematode resistant grape rootstock selections in California. We grow the selections in California for several reasons. The project goal is the development of improved nematode resistant grape rootstocks for California, so adaptation to California growing conditions is critical. Our selections grow faster in California than they would in New York. The growing season is longer and warmer. Grape rootstock breeding is not rapid, so accelerating the breeding cycle where possible is beneficial.

Additionally, many of our selections might not be reliably cold hardy in New York, because we use tropical and subtropical grape species and selections as sources of nematode resistance (among other sources).

We evaluate potential parents for rootstock breeding using a screening technique similar to that used for evaluating seedlings. We grow replicated cuttings of rootstock varieties, selections, and wild germplasm accessions and inoculate them with nematodes. We evaluate their resistance by counting all of the egg masses present on the roots six weeks after inoculation. We consider the varieties with no egg masses or galls for use in the rootstock breeding program. We also use progeny testing to determine the breeding value of potential parents.

In order to determine the propagation ability of nematode resistant rootstock selections, we test cuttings for their ability to produce shoots, roots, and callus. We use dormant hardwood cuttings for the test. We collect the cuttings during the dormant season and keep them in cold storage until testing. Before they are planted, the cuttings are soaked in water overnight. The cuttings are planted in moist peat moss in zipper lock bags and kept in the dark in greenhouse conditions (70-90 °F) without bottom heat. We assess propagation ability after three weeks and record the production of shoots, roots, and callus for each cutting.

Results and Discussion:
We have identified and deployed novel sources of resistance to aggressive root-knot nematodes, including *Vitis mustangensis, V. cordifolia*, *V. biformis*, *V. aestivalis*, *V. nesbittiana*, *V. rotundifolia* (muscadine grape), and *V. cinerea* and its hybrids. Nine selections with confirmed nematode resistance and easy propagation have been provided to Foundation Plant Services for virus testing. Rootstock selections grafted to Syrah are under trial in central California and many selections are available to cooperating researchers for further evaluation.

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