DEVELOPING A TECHNIQUE TO SCREEN PEACH STEM CUTTINGS FOR NEMATODE RESISTANCE

by

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Abstract

Research was done on developing a more precise technique to screen peach cultivars for root-knot nematode resistance, utilizing semi-hardwood peach cuttings. 'Nemaguard', 'Rutgers Red Leaf' and 'EarliGrande' stem cuttings were rooted, using Dr. Couvillons new technique for semi-hardwood cuttings. Over 90% of the cuttings rooted successfully. The rooted cuttings were then inoculated with 2 egg concentrations of Meloidogyne incognita nematodes (100 and 5000 eggs/9cm pot). Nemaguard was used to try to determine what concentration of eggs would overwhelm a resistant stock. 'Rutgers Red Leaf' was used to determine what would be the smallest amount to affect a susceptible stock. 'EarliGrande' was selected as an unknown.

Results show that neither 'Rutgers Red Leaf' or 'Nemaguard' are affected by treatments of 5000 eggs or less. While, 'EarliGrande' showed signs of infection at the 5,000 egg level.

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To my parents,

who made college possible.

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[1]DEVELOPING A TECHNIQUE TO SCREEN PEACH STEM CUTTINGS FOR NEMATODE RESISTANCE

Commerically grown peach trees are composed of 2 genetically different parts, a rootstock and a fruiting scion. For over a century peach growers and researchers, in the United States, have concentrated their efforts on scion improvement. More recently, researchers have been trying to improve the rootstock.

One of those improvements is to find a rootstock that is resistant to root-knot nematodes, specifically to Meloidogyne incognita (Kofoid and White 1919) and Meloidogyne javanica (Treub, 1885). This soil borne pest is no more than a millimeter in length and yet it reduces the annual peach crop by an estimated 15% (9). Damage is mainly done to trees grown in the South, where the mild-winter temperatures do not effect the nematode life cycle.

Root-knot nematodes feed and complete their life cycle on the roots of peaches as well as on other plant species. The first stage larva develops in the egg, and after going thru the second molt in the egg, emerges into the soil. The nematode larva then moves in the soil until it finds a susceptible root. After entering the root with its stylet, the nematode releases a chemical that causes nearby root cells to enlarge and at the same time dissolves parts of these cells. The nematode then feeds on this dissolved solution. The nematode undergoes another molt and gives rise to the third stage larva. It continues to feed on the root until the fourth and final molt, when the males leave the root while the female remains.

[1]The format used is from the Journal of The American Society For Horticural Science

The female becomes pear shape and, with or without fertilization by a male, produces eggs which are laid in a gelatinous protective coat. The eggs are usually laid on the outside of the root (Fig. 1).

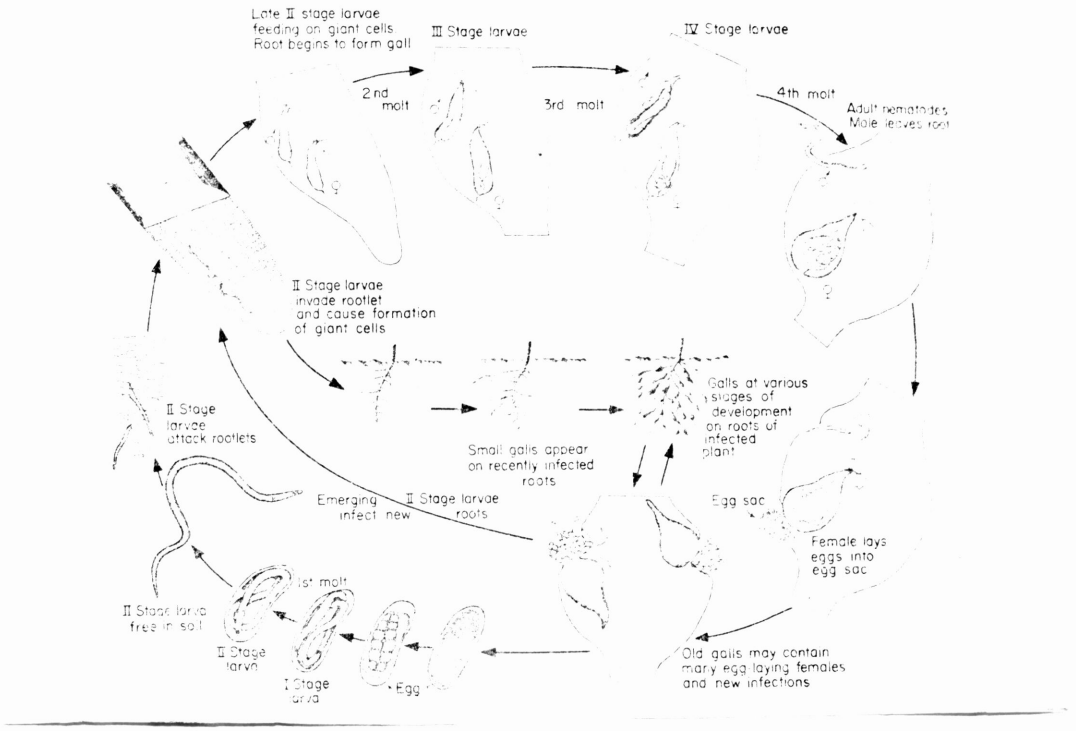

Fig. 1. Disease cycle of root-knot caused by nematodes of the genus Meloidogyne. (From: Argrios: "Plant Pathology" p.240)

The life cycle is usually completed in 45 days.

Nematodes begin their damage during the second stage. The enlarge cells crushes the xylem, blocking th passage of nutrients. In addition to this the weakend root tissues are very susceptible to parastic fungi, eg. Pythium, Fusarium and Rizoctonia. These fungi can grow and reproduce much faster in these areas, thus inducing an earlier breakdown of the root tissues (1).

Currently there is one rootstock, 'Nemaguard', that has a desirable degree of nematode resistance, but it lacks wide climatic adaptability,

such as cold tolerance. Many trees growing on this rootstock have died after a cold winter, up to 90% of the trees in some orchards (6). Also replanting trees, with nemaguard rootstock, on old orchard sites have had poor results. Why this happens is not understood (6).

The current method of screening for potential resistant rootstock involves the following steps. Tomato plants, which are very susceptible to nematodes, are inoculated with nematodes and grown in large tanks. After the nematode population has built up on the tomato roots the tops of the tomatoes are removed and the roots mixed up with the soil. After 5 to 6 months the seedlings are inspected, any that have escaped serious damage are planted out in the field for further testing (7). The major drawback to this test is that it lacks precision. A seedling might be resistant or tolerant to nematodes but the population of the nematodes in the tank may have become so great that the seedling is injured or killed because of the overwhelming numbers of nematodes (2). The opposite situation can also occur, the nematode population may be so few that susceptible plants are not screened out. A more accurate inoculation technique would solve these problems.

Another problem that has nothing to do with the test itself, is the use of seedlings for rootstock. Peaches are heterozygous; that is, a cultivar may be resistant to nematodes but its offspring might not be. Several potential rootstock have been abandoned because of this (6). Other potential stocks have produced non-viable seed, or their seed germination rate is so low that commerical nurseries cannot reproduce them economically. Using clonal stock would solve these problems. It would give the advantages of uniformity and perpetuation of individual rootstock. In the past though, asexual propagation of peaches has been unsatisfactory.

Recently Dr. Couvillon, a professor of Horticulture at the University

of Georgia at Athens, reported a new technique of rooting semi-hardwood cuttings that might be economically feasible for nurserymen to use (3). Couple this with a more precise screening technique, and the chances of finding a resistant rootstock might be improved.

The goals of this research were to repeat Dr. Couvillon's new technique of rooting semi-hardwood cuttings and then to develop a precise method of screening them for nematode resistance.

Materials and Methods

Dr. Couvillons rooting technique

On August 1, 1981 semi-hardwood cuttings, 25 cm long, were taken from the teminal ends of stems from 3 different cultivars (Fig. 2). The cultivars were 'Nemaguard', Rutgers Red Leaf', and 'EarliGrande'. The lower leaves of the stems were stripped leaving 3 to 5 leaves at the top(Fig. 3). The cuttings were labled and bundled in moist towels and stored in an ice chest. They were then transported to a greenhouse where they were wounded twice at the basal end, about an inch on each side. The cuttings were then dipped in a solution of 2500 ppm indolebutryic acid (IBA) and 50% ethanol for 5 seconds and then stuck in vermiculite under a mist system. The mist was run continuously for the first 15 hours then reduced to a 5 sec mist every 3 min.

Rooted cuttings were transplanted to 9 cm pots where they were allowed to grow for 6 weeks before being inoculated with nematodes (Fig. 4). The media used was a 1:1 mixture of sand and peat.

Inoculation of the cuttings.

Three different treatments were applied to each of the 3 cultivars after their sixth week in the pots. One treatment had no eggs(control),

another 100 eggs and the third 5,000 eggs. Each cultivar had 4 reps with 3 plants per rep (Table 1).

The egg inoculum was obtain from tomato plants that had been inoculated with M. incognita nematodes, on June 6, 1981. The roots of the plant were harvested and the soil washed off. The roots were then cut to about 2 cm in length. After being washed again in cold water the roots were placed a jar. Two-hundred ml of 1.5% sodium hypochlorite (NaOCl) was added to the jar, which was then shaken for 4 min. (Fig. 5). The solution in the jar was poured into 2 Tyler sieves, 200-mesh and 500-mesh respectively. The eggs were caught in the 500 mesh screen. The 500-mesh screen was then back washed with cold water into a glass beaker (5).

The number of eggs in the beaker was determined by taking three 1-ml samples (each time the solution was agitated). Each sample was counted

Table 1. Egg treatments used on 'EarliGrande','Rutgers Red Leaf', and 'Nemaguard'.

	Treatments		
cultivar[z]	0 eggs (control)	100 eggs	5,000 eggs
EarliGrande	4 reps[y]	4 reps	4 reps
Rutgers Red Leaf	4 reps	4 reps	4 reps
Nemaguard	4 reps	4 reps	4 reps

[z]Media was a 1:1 mixture of sand and peat.

[y]Each rep had 3 plants.

Fig. 2. Terminal cuttings 25cm long.

Fig. 3. Bottom leaves stripped.

Fig. 4. Four weeks after being transplanted to 9 cm pots.

Fig. 5 Roots cut and place in a jar, two-hundred mls of 1.5% NaOCl added, then the jar was shaken for 4 min.

on a glass grid under a dissecting scope (Fig. 6). The average of the 3 was determined and multiplied by the amount of solution in the beaker. An automatic 10-ml pipetter was used to apply the eggs. The solution of eggs was diluted with water to change the concentration per ml to 100 eggs/ml. Three samples were taken before and after inoculating the peaches to determine if the egg concentration was still close to the desired 100eggs/ml. Each sample taken was within 10 eggs of the 100egg/ml concentration.

Each peach was taken out of their 9cm pots, leaving a 3cm layer of soil on the bottom of the pot. Then the plant was placed on top of this and inoculated with either egg treatment (Fig.7). The egg solution was kept agitated throughout the application.

In addition to inoculating the peaches, tomato plants were inoculated

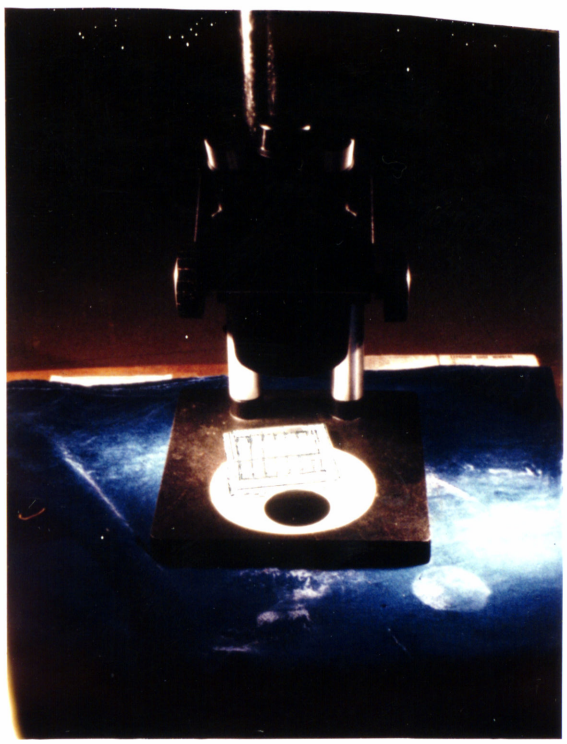

Fig. 6. Each 1-ml sample was counted on a glass grid under a dissecting scope.

intermittantly with the same egg solution used on the peaches. This was done to determine the viability of the eggs. Tomato roots are much more susceptible to nematodes then are peaches (7).

Each plant was to be inspected for galls and eggs after 50 days. Eggs where counted in the same matter they were obtain from the tomato plants.

Results and Discussion

Rooting results.

Over 90% of the cuttings rooted (Table 2). The first signs of rooting occurred after 7 days with the formation of callus tissue which is where root differentiation occurs. Three days later root initials appeared. Four weeks later the cuttings had fully developed root systems (Fig. 8).

Table 2. Rooting percentage for each cultivar.

cultivar	# rooted	rooting (%)	Survival rate after[z] 4 weeks
'Nemaguard'	76	96	56
'EarliGrande'	73	92	55
'Rutgers Red Leaf'	72	91	49

[z]Loss due to damping-off and mite infestion.

Fig. 7.Peach roots being inoculated.

Fig. 8. Root system after 3 weeks

Each step in Dr. Couvillon's technique played an important role in the development of the root system. The stripping of the leaves reduced the transpiration rate. It also allowed better air circulation around each cutting, which in turn reduced the air temperature around them. Reducing the transpiration rate and the temperature kept the cuttings from drying out as fast as they would have.

The basal wounding was beneficial because it helped stimulate cells into division. Auxin and carbohydrates, important factors in new cell formation, accumulated at the wounded area, because of the increase in respiration rate here. Injured tissues from wounding also stimulated ethylene which is known to promote adventitious root formation (8).

The wounding of the cuttings allowed for greater absorption of the applied growth regulator IBA. This is the most commonly used rooting stimulator. It has weak auxin activity and is destroyed relatively slowly by auxin-destroying enzyme found in plants (8). Auxin's that are presistent like IBA are very effective root promotors. It is the balance between auxin and other plant constituents that control organ formation (4).

The use of the mist system also played an important role. The mist maintain a film of water on the leaves which not only raises the relative humidity surrounding the leaf, but also lowers the air and leaf-temperature. This in turn lowers the transpiration rate. Light intensity can also be high when the mist is on, thus promoting full photosynthetic activity (4).

While the primary benefit of the mist is controlling water loss, a secondary benefit of the mist could have been a physiological change in the cutting. Some plants contain chemical compounds that inhibit root formation (8). The mist system may have leached out these substances if they are present in peaches.

After the rooted cuttings were transplanted to 9cm pots, they were allowed to grow for 6 weeks before being inoculating with nematodes. During this time a serious out-break of red-spider mites occurred. Several controls were tried, eg. kelthane and malathion but neither worked. Finally all the plants dropped their leaves. Over 25% of the plants died (Table 2). This lowss caused a reduction in the number of treatments to be tried and also affected the evaluation procedure.

Results of inoculation.

In developing a more uniform screening technique the number of eggs needed to infect susceptible plants without overwhelming resitant plants had to be determined. 'Nemaguard', and 'Rutgers Red Leaf' were choosen for that reason. 'Nemaguard' is known to be resistant so it was chosen to determine what level of eggs would overcome a resistant cutting. 'Rutgers Red Leaf' is susceptible to nematodes and was used to determine what was the least amount of eggs that would not affect a susceptible rootstock 'EarliGrande' was chosen as an unknown and its resistance was to be determined.

The original procedure for evaluating the cuttings was to measure plant vigor and count the number of galls and eggs. The first parameter was dropped because of the mite damage. After 50 days two plants from each treatment was evaluated. There were nematodes on the outside of the roots but no galls had developed. Tomato plants inoculated at the same time under indentical environments and media conditions shown gall development after the 50 days. The remaining plants were inspected every 2 weeks for gall formation. The'EarliGrande'treatment of 5000 eggs develop galls after 75 days. None of the other treatments had galls or eggs after 90 days (Table 3)

Another problem was the cleaning of the roots. The peat moss stuck

to the roots. A media like pure sand might have been easier to clean-off.

Table 3. Results of the inoculation

cultivar	Treatment[z] control # galls	# eggs	100 eggs # galls	# eggs	5,000 eggs # galls	#eggs
Nemaguard	0	0	0	0	0	0
Rutgers Red Leaf	0	0	0	0	0	0
EarliGrande	0	0	0	0	0	0

[z]Several tomato plants were also inoculated to check the viability of the egg solution. Results show that they were.

Since the tomatoes were infected with nematodes, it can be assumed that egg levels of more than 5000 eggs/9cm pot are needed before affecting eithe 'Nemaguard' or 'Rutgers Red Leaf'. EarliGrande is much more susceptible when compared to the other two cultivars.

Conclusion

Dr. Couvillon has developed an excellent technique for rooting semi-hardwood cuttings. It is very feasible and an easy procedure to repeat. 'Nemaguard' was the easiest to root of the 3 cultivars. Control of green-house pest, like red-spider mites, will be important in the survival of the cuttings.

The 100 egg concentration was not sufficient to cause infection on the most senstitive of the 3 plants. The 5,000 egg mass level shown 'EarliGrande' to be even more susceptible to nematodes then the other 2 cultivars.

More refinement is needed for this screening procedure before it

will be ready to use in the selecting of resistant rootstock. The time it takes a nematode to complete its life on a peach root needs to be determined. When compared with tomatoes the life cycle seems to be longer. Another improvement needed is an easier media to clean off the roots. Peat sticks to the roots making it difficult to clean-off. Nematode egg inoculation levels should be more extensively researched to determine what level is required to overwhelm a resistant stock and what level is needed to uniformly infect unknown and susceptible plants.

Once these problems have been worked out, this procedure will aid researchers in the selection of a nematode resistant rootstock.

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