The Use of Citrus Rootstocks to Control the Burrowing Nematode

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Introduction

The "spreading decline" disease of citrus trees in Florida is caused primarily by the burrowing nematode, *Radopholus similis* (Cobb) Thorne. Infected trees deteriorate in appearance and productivity but do not die from the disease. Symptoms spread from tree-to-tree at an annual rate of 1 to 3 trees per year, hence, the common name of spreading decline. The root system of infected trees was found to be devoid of feeder roots below a depth of 1 meter. The need for a resistant rootstock was recognized soon after research on controlling spreading decline had begun.

As used in this paper, citrus cultivars studied for burrowing nematode tolerance, resistance or immunity were rated as: 1) immune, if no burrowing nematodes could be detected at any time on roots of the citrus cultivar; 2) resistant, if *R. similis* could not be detected after a period of time; 3) tolerant, if *R. similis* populations continued for indefinite periods, even if at very low levels, with minimal effects on plant growth.

Rootstock Studies

The search for, and development of resistant and tolerant rootstocks has encompassed 4 areas of study: 1) The evaluation of healthy looking trees located in spreading decline areas; 2) The mass screening of all kinds of citrus secured from citrus collections throughout the world; 3) Improving resistance by selection within seedling populations of promising candidates; 4) Hybridizing promising candidates with citrus varieties containing desirable horticultural attributes.

1. Healthy appearing trees in spreading decline areas.--Between 1951 and 1973, a total of 107 trees were found by a number of individuals, that appeared healthy although surrounded by declining trees. Ninety-four of these trees were eliminated as possible burrowing nematode resistant candidates after a preliminary inspection and root distribution study. The feeder roots of each tree recommended for study were sampled for the presence of burrowing nematode.

A feeder root distribution pattern was prepared and only those that compared favorably with that of a healthy non-infected tree were accepted. Thirteen trees were entered in a testing program.

In order to obtain test material, roots of the desired trees were severed and both cut ends of each root were lifted above the surface of the ground and tied to stakes. Five to 40% of the severed roots of rough lemon and 5 to 12% of sweet orange stocks produced sprouts. Sprouts with 8 to 15 expanded leaves were removed and cut into leaf bud cuttings and rooted under intermittent mist.

A candidate rootstock clone was first evaluated by comparing growth rates of 6 cuttings planted in subsoil fine sand infested with burrowing nematodes with 6 cuttings planted in noninfested citrus grove subsoil. The cuttings were permitted to grow for periods up to 1 year in order to evaluate shoot growth, root damage, and burrowing nematode populations.

Promising candidates tested as cuttings were topworked on older citrus trees to obtain seeds for testing for nematode tolerance. Two candidates have been under field testing. They are 'Estes' rough lemon and 'Milam' lemon.

'Estes' rough lemon (originally called Rough Lemon B) was first observed in 1952 as the rootstock of a healthy looking 13-year-old 'Valencia' orange tree in a grove in Alturas, Florida. It had been transplanted into burrowing nematode infested soil in 1941. 'Estes' rough lemon also was classified tolerant because, even though burrowing nematode populations were maintained at relatively high levels in greenhouse tests of cuttings and seedlings, shoot growth was not reduced more than 20%. Seedlings of 'Estes' rough lemon cannot be distinguished from ordinary rough lemon in Florida. The nucellar rate was found to be 96% by controlled pollination with *Poncirus trifoliata* (L.) Raf.

The parent tree has been in an area of spreading decline for 31 years. Fruit quality of the 'Valencia' sweet orange scion has been the same as comparable 'Valencias' in nondecline areas. In 1967, the tree was 6 meters in height and had dense, normal size foliage, and consistently yielded 3 to 5 times more fruit than surrounding infected trees. Fruit quality measurements and other observations of the 'Valencia' top of the parent tree at that time suggested that, except for nematode tolerance, horticultural response of 'Estes' was the same as ordinary rough lemon. Root distribution measurements on the 'Estes' parent tree between 19-56 and 1967 indicated that feeder roots penetrated to depths of 3.6 meters. There have been fluctuations in root densities presumably occurring from feeder root damage by burrowing nematodes. Occasionally, live feeder roots were found only to depths of 2.3 to 3 meters. The deepest root penetrations of adjacent trees with visible symptoms of spreading decline have been only 1.6 meters.

In 1967, the parent tree was subjected to a severe drought and did not receive supplemental water because of a breakdown in the irrigation system. The tree was severely defoliated. It never recovered. With each succeeding year the tree showed increasing symptoms of spreading decline so that in 1973 the root system was only 1 meter deep, and the tree was showing typical symptoms of spreading decline. The observation would indicate that 'Estes' once infested with the burrowing nematode should not be permitted to remain under severe stress conditions.

In laboratory and glasshouse studies, the nematodes penetrated, reproduced, and damaged 'Estes' similarly to that of ordinary rough lemon. Feeder roots of 'Estes' were more vigorous than ordinary rough lemon and 3 new feeder roots developed for 1 on ordinary rough lemon during tests designed to promote vigorous growth.

'Estes' has been found to be tolerant to tristeza. The parent tree of 'Estes' carried exocortis and xyloporosis viruses but is symptom-free indicating that the tree is tolerant to these diseases. 'Estes' was released to the citrus industry in 1964. 'Estes' is susceptible to the young tree decline disease which virtually eliminates it for use in commercial groves.

'Milam' lemon is a citrus of unknown parentage that has been found to be resistant to the burrowing nematode. Seedlings, cuttings, and budded trees of 'Milam' have consistently eliminated burrowing nematode populations from containers of infested soil held in the greenhouse for periods of 9 to 12 months. 'Milam' was found as the rootstock of a 'Parson Brown' sweet orange tree in a burrowing nematode-infested grove in Polk County in 1954. The fruit had about 1° Brix more solids than comparable 'Parson Brown' on rough lemon rootstock. The unknown rootstock had a dense root system to a depth of 2.3 meters. No roots were found infected with burrowing nematodes over an 18-month sampling period although roots on adjacent trees were heavily infected. The tree was moved to the Lake Alfred Agricultural Research and Education Center (LAREC) in 1956 because the grove was bulldozed.

'Milam' is susceptible to attack by the citrus nematode, *Tylenchulus semipenetrans* Cobb as indicated by the presence of the nematode on the roots of the parent tree and on root systems on budded trees in large tanks in greenhouses. 'Milam' is not resistant to the meadow nematode, *Pratylenchus* sp., when greenhouse growth tests were performed in pots. 'Milam' appears to be tolerant to the tristeza virus based upon strain inoculation tests. Its degree of susceptibility to phytophthora foot rot is still being observed. Its reaction to the young tree decline disease is still in question. Trees are not old enough for a reliable evaluation.

A few 'Milam' variant seedlings have been discovered. One mutation occurred on a 'Milam' "seed" tree producing a fruit resembling ordinary rough lemon. The seedlings were similar to ordinary rough lemon and, when grown in infested soil, supported populations of burrowing nematodes.

'Milam' is distinct and different from rough lemon in several characteristics. The leaves have smooth to slightly depressed veins on the under surface and long petioles with small wings. Mature leaves do not have the lemon aroma usually noted with crushed leaves from rough lemon. The flowers are white, whereas, rough lemon flowers have a purple tinge. The immature, developing fruits of 'Milam' are rough and bumpy. Mature yellow fruits have a relatively smooth surface and are ovate pyriform in shape. The seeds are more than 98% nucellar based upon a population of 15,000 seedlings secured from controlled pollination studies using *P. trifoliata* as the pollen source.

Cuttings of 'Milam' were budded with 6 different citrus varieties and planted in the field in 1957. The varieties were 'Marsh' and 'Ruby Red' grapefruit; 'Hamlin,' 'Parson Brown', 'Pineapple', and 'Valencia' sweet orange. The trees were all bearing fruit before they were damaged in the freeze of 1962. More elaborate field and rootstock trials were initiated. 'Milam' was released to the industry in 1964.

2. Mass screening of all types of citrus.--A cooperative project between LAREC and the Horticultural Field Station of the U.S. Department of Agriculture, at Orlando, Florida was conducted between 1956 and 1966 to screen all available citrus species, varieties, and relatives for resistance to attack and damage by burrowing nematodes. About 90% of the seeds for these studies were obtained from Florida and California collections. About 10% were received from foreign countries. At the present time, 1,385 different citrus species, varieties, hybrids, and relatives have been screened.

The procedure consisted of growing the seedlings to a height of about 15 cm in steamed soil and then transplanting to large soil tanks filled with nematode-infested citrus grove subsoil. Each replicated selection was allowed to grow for 3 to 6 months before being evaluated on a scale of excellent to poor for vigor of tops and roots, the appearance and number of nematode lesions on the roots, and the number of burrowing nematodes that could be incubated from the root systems. Candidates that received a satisfactory rating or higher for all factors were considered promising to be evaluated in growth comparison tests in pots in shaded greenhouses. Most of the promising candidates received growth comparison tests which consisted of growing 10 seedlings in burrowing nematode-infested Lakeland find sand subsoil and comparing their growth with 10 seedlings growing in steamed soil of the same type. The seedlings were permitted to grow for a period of 9 months to 1 year. Candidates that eliminated burrowing nematode populations with less than 10% reductions in growth were considered resistant to burrowing nematodes. Candidates that supported burrowing nematode populations but suffered no more than 20% reduction in growth were rated tolerant to burrowing nematodes.

'Carrizo' citrange was one of the first promising candidates found in the mass screening program. 'Carrizo' was classified tolerant to the burrowing nematode after initial growth tests. Variations in growth were found between different sources of 'Carrizo' seed.

'Carrizo' citrange received numerous growth tests between 1957 and 1972. Although scion growth may be reduced up to 25% in greenhouse tests, burrowing nematode populations gradually diminish and seem to disappear after about 1 year. Improved testing procedures after 1968 involving tests of more than 2 years duration indicate that 'Carrizo' can only be considered tolerant to burrowing nematode since populations actually increased slightly after 2 years and did not disappear from the root systems. Populations of burrowing nematode could be transferred from one seedling to another and from 'Carrizo' to nightshade *(Solanum nigrum* L.*)*, a weed host that can support populations of burrowing nematode.

The vigor of 'Carrizo' in the nursery, its tolerance to citrus nematodes, and better fruit quality of scion varieties have spurred interest in this rootstock in Florida. Experience with 'Carrizo' in Florida does not extend beyond 15 years. Scions on 'Carrizo' were damaged by cold to the same extent as scions on rough lemon in rootstock trials in 1962 demonstrating that trees on 'Carrizo' are not necessarily cold hardy, at least during the early years. 'Carrizo' has exhibited better than average tolerance to flooding in laboratory studies, a factor of considerable importance in wetland citrus areas.

'Ridge Pineapple'–In 1957, seeds from several different 'Pineapple' orange selections were collected from trees in the citrus collection of the U.S. Department of Agriculture at Orlovista, Florida. Seedlings from the 'Ridge Pineapple' tree had vigorous, normal-looking feeder roots when grown in burrowing nematode-infested soil. The roots of other 'Pineapple' trees were injured and showed visible root damage from attack by the burrowing nematode. Growth tests with 'Ridge Pineapple' in the greenhouse indicated that populations of burrowing nematodes decreased rapidly within 4 months and disappeared within 9 months. 'Ridge Pineapple' grew as well in nematode-infested soil as in steam sterilized soil. The seedlings have shown excellent growth with satisfactory root systems when compared to other susceptible kinds of citrus.

'Ridge Pineapple' cannot be readily distinguished from the midseason 'Pineapple' sweet orange variety commonly used throughout Florida. In all other factors besides burrowing nematode resistance, 'Ridge Pineapple' has responded similarly to regular sweet seedling rootstock. 'Ridge Pineapple' was released to the citrus industry in 1964.

'Algerian' navel sweet orange was evaluated by using seeds collected from a tree in the horticultural collection at Orlovista, Florida. 'Algerian' is a seedy navel, the root system of which is not visibly damaged by burrowing nematodes. Growth of plants in nematode-infested soil has been equal to growth in noninfested soil. 'Algerian' navel has been considered resistant to burrowing nematodes because populations disappear during growth tests. 'Algerian' navel has not been publicized to the industry at the present time because it has not been considered a distinct improvement over 'Ridge Pineapple'.

3. Improving resistance by selection within seedling populations of promising candidates.–Tests have been in progress for 8 years to develop an improved selection of 'Carrizo' citrange resistant to burrowing nematodes. In 1962, approximately 2,000 seeds of a California source were received from the Citrus Research Center, Riverside, California. The seeds were germinated and the seedlings grown for 9 months in a large tank of soil infested with *R. similis*. The average population of *R. similis* in

'Carrizo' roots at the end of the test was only 2.8 females per gram of root. Five of the largest seedlings with the smallest populations of burrowing nematodes were selected from the more than 2,000 plants growing in the soil tank. Each selected seedling was multiplied by vegetative propagation and then subjected to a 9-month nematode survival and growth comparison test in the greenhouse.

One candidate was retained after the test because the plants were more vigorous than the other seedlings, and no burrowing nematodes were found in the root systems.

Extensive testing of the candidate for periods of more than 2 years duration indicated that populations of the nematode were being maintained. The 'Carrizo' selection apparently is no more tolerant to nematodes than the other sources of 'Carrizo'.

Attempts to improve the nematode resistance of 'Milam' by selection have also not been successful. A program similar to that used for 'Carrizo' yielded 3 seedlings that appeared to eliminate the burrowing nematode in 6 months rather than 9 to 12 months in greenhouse studies. Extensive testing of the candidates indicated that the resistance of the seedlings could not be considered superior to other sources of 'Milam'.

4. Hybridizing promising candidates.--Crosses of 'Estes' rough lemon X *P. trifoliata*, when tested for tolerance and resistance to burrowing nematodes, were not superior to 'Estes' rough lemon. The cross of 'Milam' lemon X *Citropsis gilletiana* Swing. and M. Kell. was not successful. Crosses involving 'Milam' lemon X *P. trifoliata* have not yielded a hybrid during the past 5 years involving more than 25,000 seedlings. The reciprocal cross of *P. trifoliata* X 'Milam' lemon over the past 5 years since 1967 has yielded 210 plants with leaf shape differing significantly from normal variation within populations of *P. trifoliata*. About 50 plants have eliminated burrowing nematodes within 1 year. One candidate with a more vigorous and denser root system than the majority of *P. trifoliata* seedlings has usually eliminated burrowing nematodes within 9 months in nematode survival tests.

Distinct phenotypes with trifoliate leaves amounting to 5% of seedling populations have been obtained from crosses involving *P. trifoliata* on 'Ridge Pineapple'. The hybrids, more than 400 in number, are currently being subjected to nematode survival tests.

The nature of resistance

'Milam' and 'Estes' were tested by a laboratory procedure wherein the ability of burrowing nematodes to penetrate roots, lay eggs, and multiply populations was determined after 5 and 35 days. Seedlings of the candidates were planted in petri or plastic dishes filled with sterile fine sand. Root tips of small seedlings were laid on moist rectangles of filter paper placed in a depression in the sand. Twenty-five female burrowing nematodes were placed on each root by using a micropipette. Filter paper and retarded downward migration of the nematodes, and served to identify the area of inoculation when the root segments were eventually removed. The small root pieces were removed from the soil, washed, and stained in aceto-osium. 'Estes' roots stained with aceto-osmium showed lesions, nematode eggs, and larvae restricted predominately to cortical tissues. The situation in 'Milam' was unique. Adult nematodes were found in the cortex but no larvae were observed. The only nematode eggs observed were located close to bodies of females. Only the light outline of eggs at increasing distance from the female could be seen since they did not stain dark with osmic acid. About 10% of the nematodes were found in the stele. They laid eggs, and the eggs developed into larvae. It has, therefore, been suggested that the resistant factor in 'Milam' is associated with the destruction of the eggs of the burrowing nematode laid in the root cortex. The chemical mechanism has not been evaluated.

Field rootstock trials

Long-term field trials with 'Milam', 'Ridge Pineapple', 'Carrizo', and 'Algerian' navel have been disappointing because of cold injury, lack of care in commercial grove sites, variations in maintenance procedures, etc. The mild strain of exocortis affected the growth of 'Pineapple' and 'Valencia' scions on 'Carrizo'. Good grove care offset differences between rootstocks in many locations.

At the present time, observations are being confined principally to blocks of trees in commercial groves to determine whether the trees meet minimum criteria for commercial acceptance. In general, 'Ridge Pineapple' has been similar in response to sweet orange seedling rootstock with the usual problems of drought susceptibility and damage from phytophthora foot rot. 'Milam' has been a vigorous grower under permanent irrigation systems. 'Hamlin' and 'Pineapple' have looked particularly good on 'Milam'.

Its record for phytophthora is still a question because of the practice of budding trees low in Florida. 'Algerian' navel is not in commercial use at the present time.

Present uses of burrowing nematode resistant rootstocks in Florida

Uses have been for: 1) biological barriers against the spread of burrowing nematode; 2) replants after pulling and fumigating infested groves; and 3) replacement trees in groves where only occasionally infested trees were pulled.

Biological barriers

Orange and grapefruit scions on 'Milam' rootstock have been evaluated for 9 years for use in biological barriers. Budded trees on 'Milam' spaced 60 cm apart were planted in large soil tanks 6.8 m in length. Burrowing nematodes were established on the roots of a susceptible host planted in one end of each tank. To date, the nematodes have not migrated across the first row of 'Milam' (a distance of 1 m) abutting the susceptible host. The barrier concept was successful in tank tests only when the roots were cut between the host source of *R. similis* (burrowing nematode) and the 'Milam' barrier area.

Numerous commercial groves have utilized 'Milam' rootstock as biological barriers where *R. similis* (burrowing nematodes) exist in abutting groves. By 1973, more than 25 km of biological barriers have been installed. In 1 grove, burrowing nematodes were found in the first and second row of 'Milam' in the barrier zone. A detailed sampling indicated that nematodes had entered the barrier zone on roots of rough lemon from an abutting burrowing nematode-infested grove, a distance of 27 m. A root-killing chemical and weed free barrier (usually DD soil fumigant plus a herbicide) was placed between the infested grove and the first row of 'Milam'. All susceptible weed hosts and sprouts from rough lemon roots were controlled by hand hoeing at bimonthly intervals. After 6 months and up to 2 years later in 1972, no burrowing nematodes were found on 'Milam' at the sites where burrowing nematodes were present prior to installing the chemical barrier. Apparently the infestation was predominantly on the roots of rough lemon that had grown into the barrier zone or 'Milam' eliminated the remnant of the burrowing nematode infestation after the rough lemon substrate had been destroyed.

Burrowing nematodes were found on border 'Milam' trees in another grove abutting trees on rough lemon. Five adjacent rows of trees on infested rough lemon were removed and the pulled area treated with DD soil fumigant. Removing the infested trees on rough lemon created a wider border between the 'Milam' planting and the infested trees. Within 1 year, 'Milam' trees which previously harbored burrowing nematodes were found to be free of the nematode. Two years later, the trees were still free of burrowing nematodes.

The biological barrier concept of planting a minimum of 4 rows of trees on 'Milam' plus the use of a root-killing chemical barrier between trees harboring an infestation of burrowing nematodes and an area of young trees to be protected against invasion, has encountered a problem of nematode biotypes. In 1972, burrowing nematodes were discovered on roots of 'Milam' abutting a block of infested 20-year-old 'Pineapple' sweet orange budded on 'Cleopatra' mandarin rootstock. The 'Milam' rooted trees had been free of burrowing nematodes for the 5 years since they had been planted. The source of the infestation on 'Milam came from the 'Cleopatra' rootstock. Burrowing nematodes were found on the roots of nightshade growing in the chemical root killing barrier area. Nightshade is a common weed host capable of supporting high populations of burrowing nematodes. All of the weed cover was removed from around 'Milam' and the chemical root-killing barrier in anticipation that burrowing nematode would be eliminated by 'Milam'. When examined 6 months later, burrowing nematode on 'Milam' was actually higher (10 females per gram of root) than had been previously recorded on this rootstock in the laboratory or the field. This represented the first evidence for the possible occurrence of a biotype of burrowing nematode. Studies, to compare this isolate with other isolates of burrowing nematode inoculated on 'Ridge Pineapple' and 'Milam', are currently in progress.

The trees on 'Cleopatra' rootstock supplying the nematode infestation that crossed the chemical barrier on the roots of nightshade have not shown typical symptoms of spreading decline. For the past 10 years, there has never been a highly visible margin of symptoms between the infested and noninfested 'Cleopatra,' although the margin had been delineated by sampling for burrowing nematode. The trees have been producing acceptable crops of fruit to the extent that the owner does not desire to eliminate the grove. Therefore, it is possible that the burrowing nematode biotype may not induce typical decline symptoms. The 5 infested 'Milam' trees have not shown visible symptoms of decline.

The 'Milam' trees are also adjacent to 35-year-old grapefruit on rough lemon that are infested with burrowing nematode and show typical symptoms of spreading decline. Burrowing nematode has not become established on 'Milam' roots adjacent to these trees.

The transport of burrowing nematodes by weed hosts plus the specter of the development of resistant biotypes raises serious reservations concerning the future of biological barrier programs without adequate safeguards such as the maintenance of weed and root free zones between the sources of infestation and the resistant rootstock.

Rootstocks for pulled and treated areas

The program for control as recommended by the Industry-Wide Spreading Decline Technical Committee requires that a resistant rootstock, either 'Milam' or 'Ridge Pineapple', be planted after pulling an infested grove and fumigating the soil if the grower desires to receive testing services of the program. At present, only 'Milam' is being utilized in this program.

The future in the use of resistant rootstocks

The possibility that 'Milam', because it is a rough lemon type, will prove susceptible to young tree decline; the ever-present specter of nematode biotypes; and the risk of depending on any 2 resistant rootstocks, indicates a need for additional rootstocks. I believe that the most significant progress, in developing new rootstocks, can be made in the area of hybridization by combining material of known resistance with citrus cultivars for which there is some data on rootstock potentialities.