Phytophthora Studies on Citrus Rootstocks

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Introduction

The common and widespread occurrence of phytophthora fungi that are pathogenic to citrus continues to have a great influence on the world rootstock situation. While much can be done to prevent serious infection by adopting safer cultural practices and by using suitable preventative measures, the only certain solution to a severe phytophthora disease problem is to use more resistant rootstocks. Unfortunately, few citrus species or relatives are highly resistant to phytophthora, and much of this material is unsatisfactory from other standpoints. Thus, in practice, many susceptible rootstocks still have to be planted because of their superiority in other more essential respects.

The severity of phytophthora attack varies according to local climatic and edaphic conditions. In citrus producing areas of the world that have well-drained and well-aerated soils, and where rainfall is not too frequent, it may still be reasonably safe to use as rootstocks any but the very highly susceptible varieties. Where conditions are more favorable for disease development, as on soils that remain wet for long periods following rain or irrigation, it may be unwise to use even those rootstocks that are only moderately susceptible to phytophthora. Such considerations affect the latitude that can be permitted in different citrus producing areas with respect to the screening out of phytophthora susceptible material in rootstock improvement programs.

In Florida, serious losses generally occur only where highly susceptible varieties have been used as rootstocks. Having made this statement, I must hasten to add that much of the more serious damage caused by phytophthora in local nurseries and groves involves primarily the scion portion of the trunk and not the rootstock. This is not always fully appreciated and in many cases the rootstock variety is wrongly blamed for the attack. The question as to how much importance should be attached in Florida to the selection of rootstocks that are highly resistant to phytophthora will be considered later in this talk; because it will be possible to examine this subject objectively only after considering in some detail what is known about the host-parasite relationship and the possible effects of phytophthora on tree performance.

Parts of tree affected by phytophthora

Certain parts of citrus trees are more susceptible to phytophthora attack than others, and the names given to the different expressions of the disease syndrome vary accordingly. Particularly prone to attack on such highly susceptible species as sweet orange are the feeder roots and the bark of the crown roots and lower part of the trunk. Older parts of the root system between the fibrous roots and the region where the roots start to form the crown portion of the trunk are affected less frequently. If these older roots do become infected, the lesions tend to be small and self-limiting, giving rise to a symptom that has been described as frog-eye (7). Bark on the lower part of the trunk is potentially more susceptible to infection than bark below ground (9). This is one of the reasons why trees should be planted no deeper than they stood in the nursery, the aim being to reduce as much as possible the amount of highly susceptible stem bark in contact with the soil.

In some very wet areas of the world, infection of bark can occur quite high on the trunk and even on the limbs. Under Florida conditions, however, the bark of even the highly susceptible scion varieties seldom becomes infected, provided that it is separated from the soil by at least 3 inches of resistant rootstock bark.

On the more resistant rootstock varieties, rotting of the bark on the crown roots and the trunk is uncommon. Some infection may, however, still occur on the feeder roots of such varieties, thereby permitting the fungus to build up and threaten the bark of a susceptible scion variety if it is close enough to the ground. Thus, even on so-called resistant rootstocks, it is very important to bud trees as high as practical.

The various names given to the different types of damage caused by phytophthora are as follows: When infection is confined to the feeder roots, the resulting injury is called "feeder or fibrous root rot". If the damage to the root system is more extensive and yet does not extend upwards to the crown roots, it is described as "phytophthora root rot". Damage to the bark of crown roots and/or base of the trunk is called "phytophthora gummosis, collar rot or foot rot" and it is the last mentioned name that is generally used in Florida.

The term "foot rot" originated in Florida in the last century and was intended to describe a bark rotted condition on the trunk base or crown roots. Unfortunately, this term has at times been used interchangeably with root rot. Such broad use of the term foot rot tends to obscure the real nature of the phytophthora problem as it faces us in Florida. Hereafter in this talk, "foot rot" will be used to describe a diseased bark condition on the crown roots or lower part of the trunk, as distinct from feeder root rot. It will also be used to describe the situation where the disease actually started on, or remained confined to, the scion portion of the trunk.

Life cycle of phytophthora and mode of infection

Two species of phytophthora pathogenic to citrus have been recorded in Florida; they are *Phytophthora parasitica* and *P. citrophthora*. The latter is rarely encountered in this State (12), but is a serious problem in certain other citrus producing areas of the world. In Florida, *P. parasitica* has been detected in the soil of almost every grove and nursery tested. It has not, however, been detected in soil samples collected from any sites where citrus has never been planted, suggesting possibly that this fungus can only survive and multiply in the presence of a citrus substrate. Growth in the soil itself is very restricted, but the fungus can survive for limited periods in the soil away from the host substrate in the form of drought-resisting resting spores (11). Very little inoculum arises from the diseased bark and direct spread of infection from the bark of diseased trees to that of healthy trees is probably unlikely.

Invasion of host tissue is by motile, free-swimming spores known as zoospores. These are produced in fruiting bodies called sporangia that grow out in profusion from the surface of infected feeder roots and from germinating resting spores. Water, and not just high humidity, is the key factor in almost all stages of the life cycle. Water is required for sporangial formation, dispersal of zoospores as well as for spore germination.

Zoospores swim through the soil water and, if they get close enough to young roots, they become attracted by a chemotactic response to the region of cell elongation immediately behind the root tip (14). On older roots and stems, penetration apparently occurs only where there is a break in the bark (1, 13). Direct penetration of stems is, however, possible on young shoots before a protective barrier of corky tissue starts to form in the outer layer of the stem cortex (13). A strong attraction of zoospores to freshly made breaks in the bark of older roots (1) and stems has been observed (13).

It has long been recognized that foot rot often commences at locations where the bark has been damaged as, for example, during cultivation or after removing unwanted shoot growth. But such openings in the bark are not the only ones that allow fungal entry. Penetration can also occur through growth cracks (13). Such naturally formed cracks have been detected by using the triphenyl tetrazolium chloride (TTC) technique (13). This colorless chemical turns red when it comes into contact with living cells. Because aqueous solutions are unable to penetrate intact bark, the appearance of a red stain in the underlying living cells after applying TTC to the surface of the trunk provides evidence for the existence of some kind of break in the outer corky layer of the bark.

In inoculation tests with zoospore suspensions carried out at the Lake Alfred Research and Education Center (AREC), relatively few uninjured sweet orange trees developed foot rot, whereas the disease incidence increased to almost 100% on trunks that had been intentionally injured before inoculation (13). These results have served to strengthen the contention that avoidance of mechanical injury can greatly reduce the incidence of foot rot.

The association between foot rot and low budding

The bark of most commercial scion varieties is highly susceptible to Phytophthora infection. Thus, even if trees are growing on resistant rootstocks, there is still a chance that foot rot can occur if the tree has been budded too low and/or planted too deeply. One of the problems we have experienced in Florida in carrying out meaningful surveys to determine the involvement of the rootstock in foot rot outbreaks, is that because of low budding it is frequently impossible to determine whether the infection started above or below the bud union. The true situation can be further obscured by the fact that, by the time the disease becomes evident, the roots may have already died from starvation due to the lack of food translocation from the tree canopy into the roots.

Young trees can succumb to stem girdling in a matter of only a few months, and after the root system has died, it is virtually impossible to be certain of the cause of death. Nevertheless there is a common tendency to blame such "root rot" on phytophthora invasion of the rootstock.

Another factor that may influence the true phytophthora picture in Florida is that some rootstocks, because of certain practical problems in the nursery, tend to be budded lower than others. Generally, rough lemon seedlings are budded lower than sour orange, and this may account, at least partly, for the observation that trees on rough lemon develop foot rot more frequently than trees on sour orange. But other explanations for the reputedly high susceptibility of rough lemon to foot rot are also likely, as will be discussed later.

The relative importance of foot rot and feeder root injury in Florida citriculture

The economic effects of foot rot in citriculture are much more apparent than those due to partial destruction or temporary loss of feeder roots following phytophthora attack. Even after allowing for the fact that a great deal of foot rot could be avoided by using safer cultural practices, we know that it is still unwise to plant trees on highly susceptible stocks. Highly susceptible varieties, therefore, need to be excluded from any rootstock improvement program.

In contrast to foot rot, the economic significance of feeder root infection in Florida is uncertain. Feeder root infection is, of course, important from the standpoint that it builds up inoculum to infect the trunk of the tree. Considerable doubt remains, however, as to whether the temporary loss of feeder roots that can occur during spells of wet weather really has any long-term effect on tree growth. It could be conjectured that a partial loss of root absorbing surface at times when the soil is excessively wet would be much less serious than such a loss (as could be caused by other agents) when soil moisture levels are low. The ultimate effect on tree performance would probably depend on the ability of a rootstock to produce new roots rapidly as the soil dries out and the conditions become less favorable for the phytophthora pathogen.

Reliable and meaningful data on the importance of feeder root rot would be very difficult to obtain. Growth comparisons between potted trees growing in phytophthora-infested and noninfested soil would hardly be translatable to field or even nursery conditions. Nor would the results of soil fumigation tests necessarily provide the required information because such treatments can affect tree performance through their other effects on the soil microbiology.

Foot rot is often observed on trees that had been growing exceptionally vigorously up to the time that extensive trunk girdling caused the trees to go into a decline. Apparently, the destruction of feeder roots that must have been occurring in such groves for some time prior to the foot rot attack had little, if any, effect on tree growth. Perhaps the situation could be different on sites with high water tables, but here the urgency would be for better land drainage rather than to try and solve the problem only by using stocks that are highly resistant to phytophthora. In the long term, feeder root infection in itself may not significantly affect tree performance, except in excessively wet groves and in nurseries that are irrigated too frequently.

Procedures for testing the relative susceptibility of rootstocks to phytophthora

Methods used to determine the relative susceptibility of rootstocks to phytophthora need to provide the following information: 1) the relative rate of feeder root destruction under conditions favorable for phytophthora attack; 2) the rate at which a root system is able to regenerate new roots following a reduction in disease pressure; 3) the susceptibility of the crown roots and trunk to the foot rot phase of the disease syndrome and; 4) the ability of the rootstock to produce callus tissue around the diseased bark, thereby preventing extensive girdling or renewed foot rot activity at a later time.

Programs for testing the relative susceptibility of rootstocks to phytophthora attack have been in operation in several citrus-producing areas of the world for many years (2, 3, 4, 6, 8, 10). Two basic inoculation procedures have been used; one to test the susceptibility of the roots and the other to test the susceptibility of the stem bark to infection.

For testing the relative susceptibility of the roots to phytophthora attack, young seedlings or rooted cuttings are first grown in a sterile medium until a large number of healthy feeder roots have been produced. The plants are lifted carefully to preserve as much of the root system as possible, and the entire root system is then placed in a tank containing aerated water, to which zoospore-producing inoculum is added. After leaving the roots in the inoculation tank for a day or more, the plants are planted in pots in the greenhouse or in nursery beds outdoors. In order to encourage further infection and to provide optimum conditions for disease development, the plants are watered more frequently than actually required for growth. Under such heavy disease pressure, young plants of highly susceptible and even moderately susceptible varieties can be rapidly killed. The results of such feeder root inoculation tests have been measured variously in terms of plant survival, the amount of root rot, or as a reduction in root and shoot growth. A major weakness with this kind of test is that it is difficult, or perhaps even impossible, to determine the root regenerating capacity of the stock. The method is therefore useful for assessing high levels of susceptibility or resistance, but has limited value for determining the likely field behavior of those many rootstocks that fall in between these 2 extremes.

Tests to determine the relative susceptibility of stem bark to phytophthora have been carried out in the past as follows (2, 8, 10): A small disk of bark extending down to the cambium is removed from the trunk and a disk of agar culture of phytophthora is inserted in its place. The area is then bound with waterproof wrapping material. After suitable periods, the wrapping is removed and the distance that the rotted bark extends away from the point of inoculation is measured. Useful though this stem inoculation technique has been, it certainly does not stimulate very closely the natural infection process in which zoospores are the main, and perhaps the only kind of infective propagule.

Methods currently being investigated at LAREC to determine the relative susceptibility of rootstocks to foot rot

Since 1969, attempts have been made at Lake Alfred to devise a more natural procedure for determining the susceptibility of the trunk bark to infection, using zoospore suspensions as inoculum. The first requirement was to develop some system whereby large numbers of zoospores could be brought into contact with stem bark without also having to immerse the root system in the inoculum. This was achieved on young potted plants in the greenhouse by building a watertight collar around the stem with segments of Tygon tubing. The resulting collar, which rested on the soil surface, was sealed at the base by pouring in a melted 1:1 mixture of paraffin wax and vaseline. A measured volume of zoospore suspension of standardized concentration was then poured into each collar. The results of tests using this technique (13) showed that 1) after the periderm had formed, no infection could occur unless a break existed in the outer bark; 2) relatively large numbers of zoospores were required for infection to occur at all; 3) an injury rendered the stem susceptible to infection for up to 2 weeks after it had been made and; 4) the chances of infection were greater when the injury extended into the cambium. It was found that only a minute cut in the bark was required for fungal penetration. The procedure eventually adopted for uniformly wounding the stems in variety susceptibility tests consisted of making 2 or 4 vertical cuts with the point of a scalpel blade. These cuts extended up the stem for a distance of 25 mm above the soil surface. Disease ratings were based on the distance that diseased bark extended laterally from each vertical cut.

For inoculating larger trees outdoors with zoospores, a much simpler technique has been successfully used (13). Four vertical cuts were first made at the base of the trunk with a pointed pocket knife blade. A 6-inch high collar of absorbent cotton was wrapped around the base of the trunk, which was then banked with soil to the top of the cotton collar. After saturating the cotton with lake water or rain water, a freshly prepared zoospore suspension was poured around the bark in such a manner that it would run down the stem over the areas containing the vertical cuts.

Microscopic examinations revealed that only a short period of wetting was required for infection (13). Zoospores settled on the exposed inner bark tissue almost immediately. They germinated in less than 30 min, and the germ tubes mostly penetrated the living tissue adjacent to the cut in less than 60 min after inoculation. Sufficient fungal growth within the bark to cause foot rot only occurred, however, if the bark was kept moist for a further period after fungal penetration. This was achieved by pouring small amounts of water over the cotton 2 or 3 times a day for the next 2 days. Thereafter, the collars were kept moist during dry weather by applying overhead sprinkler irrigation as required. Trees were inoculated mostly in June-July. On susceptible varieties, foot rot developed very rapidly and the trunk of 1-3 year old trees often become completely girdled in less than a month. Lesion development started to slow down by October-November, and callus tissue began to develop at the periphery of the rotted area. The soil banks were then removed and the amount of trunk girdling and callus formation was recorded.

The reaction of some currently used and experimental rootstocks to zoospore inoculation has now been studied both in the greenhouse and in the field. Some varieties showed the same relative response in both environments. For example, 'Pineapple' orange seedlings always showed a highly susceptible reaction both when tested as young seedlings in the greenhouse and as older plants in the field. At the other extreme, trifoliate orange, 'Carrizo' and 'Troyer' citranges consistently showed a highly resistant reaction. With some clones, however, particularly within the rough lemon group, different reactions occurred between the greenhouse and outdoor tests. For example, certain rough lemon types that had appeared moderately susceptible in greenhouse tests showed a highly resistant reaction in the field tests (Whiteside, unpublished data), suggesting that the bark had become more resistant with age. The reverse situation has also occurred. One rough lemon variant that appeared to be only moderately susceptible in the greenhouse tests showed a highly susceptible reaction in field tests. Such discrepancies have cast doubts on the reliability of greenhouse tests on young plants for determining the likely "field" reaction of rootstocks to phytophthora attack.

Because of the current importance of rough lemon in Florida citriculture and the great variability that exists in this species and the contradicting reports that have been made concerning its susceptibility to foot rot, special attention is being given at Lake Alfred to the field testing of different rough lemon cultivars for susceptibility to foot rot.

A major problem in the growing of rough lemon as a tree outdoors in Florida is the very stunted growth that is caused by the almost constant alternaria leaf spot attack. Because this disease is very difficult to control by fungicide spraying, the obvious solution was to bud the greenhouse-grown trees with sweet orange before planting outdoors. This enabled the inherently vigorous rough lemon stock to grow as fast as, and even faster than, trees on other rootstock. It was mainly because of the special problem with rough lemon that the decision was made to bud all rootstock varieties with sweet orange before testing them for foot rot susceptibility. But another advantage in making the tests on budded trees soon became apparent. By making inoculations across the bud union, a further check could be obtained as to whether a rootstock had failed to develop foot rot because of inoculation failure or because it was truly genetically resistant. This inoculation technique ensured that there would be a double chance of foot rot developing on the stock; either by direct penetration of the rootstock at the time of inoculation, or by growth of the fungus across the bud union from the highly susceptible and almost invariably infected sweet orange bark.

To date, perhaps the most surprising result that has been obtained from these field inoculations is the apparently high resistance to foot rot of most (though not all) sources of rough lemon. This rootstock has earned a reputation for being moderately to highly susceptible to foot rot in some citrus growing areas of the world. This view is also widely held in Florida, although the experience in many local groves does not really support this contention. Many cases have been observed locally in which foot rot has remained confined to the scion portion of the trunk on trees growing on rough lemon stock. Perhaps the apparent contradictions in the behavior of rough lemon stock are related to the great genetic variability which occurs within this species. Differences in the susceptibility of feeder roots to infection have previously been reported (5). In field tests at Lake Alfred large differences in the incidence of foot rot have been observed between clones of rough lemon (Whiteside, unpublished data). In fact, some clones appeared to be as susceptible to foot rot as sweet orange. The majority, however, appeared to be moderately resistant to this disease. Therefore, at least some of the locally severe outbreaks of foot rot that have occurred in Florida could, in fact, have been due to the unwitting use of highly susceptible clones of rough lemon. Rootsprout propagations have been made from several groves in which foot rot has occurred on supposedly rough lemon stock. The relative susceptibility of these clones will eventually be determined in controlled field inoculation tests.

The citrus rootstock-phytophthora situation in Florida in perspective

The only long-term practical method for preventing foot rot and phytophthora root rot is to use sufficiently resistant rootstocks and to bud the trees at least 3 inches above ground level. In view of the Florida habit of budding trees very low, it is perhaps surprising that foot rot has not become an even greater problem in our citrus groves. Probably what prevents this happening is the sandy nature of the soil and the fact that the bark on the trunk seldom stays wet for long periods after rain. In fact, infection usually starts on those parts of the crown roots and trunk lying below ground level. If infection does occur above ground, this is usually associated with the piling of plant residues or soil against the trunk.

In spite of the sandy and well-drained properties of the soil, experience has shown that the incidence of foot rot on highly susceptible citrus species and varieties can be very high. Thus, it is important to avoid using such kinds of citrus as rootstocks if at all possible. The question arises, however, as to how much resistance or tolerance to phytophthora is really required under Florida conditions. Any species that is as highly susceptible to foot rot as sweet orange, grapefruit, and smooth lemon certainly needs to be avoided. But it is doubtful whether there is any justification for going to the other extreme and retaining as possible rootstock candidates only those varieties that are highly resistant to phytophthora. Certainly in Florida this does not seem to be necessary. For commercial planting, rootstocks have to meet many other requirements, including resistance to some diseases that are even more devastating than phytophthora. Therefore, high resistance to phytophthora frequently becomes a secondary consideration in the choice of rootstock. However, it is important to know whether a candidate rootstock is highly susceptible to foot rot under grove conditions. It is also important to know what capacity an easily infected variety has for sealing off areas of diseased bark by producing callus tissue. Reliable information on these matters can probably only be obtained by testing the susceptibility of stem bark under field conditions, and after budding the tree with a locally used scion variety. It is to be hoped that the inoculation techniques now being tested at Lake Alfred might prove useful in these respects.

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