Syringe Injection of Water into the Trunk: A Rapid Diagnostic Test for Citrus Blight

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ABSTRACT

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A diagnostic test for citrus blight is described in which a 30-ml plastic syringe was used to inject water under pressure through a hole drilled in the trunk of the tree. About 1 ml/sec could be forced into healthy trees, but little or no water could be injected into blighted trees. In Florida and South Africa, water uptake in blight-affected trees as measured by the syringe test or the standard gravity injection method declined with the negative logarithm of the canopy symptom rating. Zinc content of trunk wood, another indicator of blight, increased as a power function of the canopy symptom rating. Visual diagnosis, the gravity injection test, the syringe test, and diagnosis by zinc content concurred in the majority of the cases, but the gravity injection test appeared to be the least reliable. The syringe injection test is simple, fast, requires less equipment, and is at least as accurate as presently available diagnostic techniques for blight.

Citrus blight is a disease of unknown etiology associated with a dysfunction of the xylem (8). Several diagnostic techniques have been developed to identify blight-affected trees and distinguish them from trees affected by other disorders (1,6). Zinc- and water-soluble phenolics accumulate in the trunk wood (8,10), and zinc accumulates in the phloem of blighted citrus trees (2). Affected trees take up little water into the trunk by gravity injection (4) because xylem vessels of the trunk are plugged (5,14). Gravity injection tests and analyses for zinc have been useful in equating blight, sandhill decline, and young tree decline, which are indistinguishable by all known criteria, and for separating them from declines caused by citrus tristeza virus, psorosis, Phytophthora foot rot, nematodes, and flooding damage (1,4,9,16). These tests have also been helpful in associating tree declines in South America and South Africa with citrus blight in Florida (7,12).

In spite of the general utility of the gravity injection and zinc tests to diagnose blight, interpretation of the results is sometimes uncertain. Zinc levels in trunk wood vary greatly from grove to grove and tree to tree. In Argentina, zinc levels in wood are low and there is considerable overlap between levels in healthy and declining trees (11; L. W. Timmer, *unpublished*). Moreover, analytical equipment for zinc determination is not available in all areas. We found that although blighted trees seldom took up water by the gravity injection method, healthy trees in some groves or at some times also took up little water, making results difficult to interpret. The gravity injection method requires heavy, cumbersome equipment and usually 24 hr is needed for accurate readings.

The syringe injection method for identification of citrus blight described in this report is based on a technique originally described to inject insecticides into trees (3). Diagnostic tests on trees in various stages of decline are evaluated and their relative effectiveness compared.

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MATERIALS AND METHODS

Syringe injection technique. To measure water uptake by this method, a 3-cm-deep hole was drilled in the trunk 25–40 cm above the bud union, using a 3.2-mm-diameter (1/8-in.) bit and a battery-powered drill. The hole was freed of sawdust with the drill bit and the mouth of the hole was widened slightly to accommodate the entire syringe tip. A 30-ml Pharmaseal Stylex disposable syringe with a luer-tip without a needle was filled with 10 ml of water. The plastic tip of the syringe was then fitted snugly into the hole in the trunk. The barrel of the syringe was steadied with one hand while the operator applied the maximum pressure possible to the syringe plunger. The average pressure applied by an adult male operator was measured at 7.75 kg/cm^2. Greater pressure resulted in bending or breaking the syringe plunger. The time required to inject 10 ml of water or the amount injected in 30 sec was recorded and the data were expressed in milliliters per second.

Results obtained by two operators on 10 healthy and 10 blight-affected trees in a Florida citrus grove were compared.

Standard diagnostic techniques. Analysis of zinc content of trunk wood was conducted as described by Wutscher et al (10). Water uptake by gravity injection was determined as described by Cohen (4).

Symptoms and tree selection. Trees for comparing diagnostic tests were selected. Trees with psorosis bark lesions, Phytophthora foot rot lesions, heart rot, or mechanical damage were not used. All groves used in Florida and South Africa were on rootstocks resistant to citrus tristeza virus. Trees were rated visually for blight severity on a scale of 0 = healthy; 1 = mild—small leaves with symptoms of zinc deficiency, short internodes, slight wilt but little or no thinning of the foliage; 2 = moderate—small leaves, often flaccid, with zinc deficiency symptoms, sparse canopy with some twig dieback; and 3 = severe—thin canopy, substantial dieback, trunk sprouts common. Trees intermediate between these categories were rated at 0.5, 1.5, or 2.5 as appropriate.

Comparison of diagnostic techniques. Diagnostic tests were compared in three sweet orange (*Citrus sinensis* (L.) Osb.) groves in Florida, two on rough lemon (*C. jambhiri* Lush.), and one on Carrizo citrange (*C. sinensis* × *Poncirus trifoliata* (L.) Raf.) rootstock. Trees were rated for symptom severity and the zinc and syringe tests were conducted on at least five trees in each symptom category as follows: category 0, 63 trees; 0.5, 15; 1.0, 12; 1.5, 6; 2.0, 6; 2.5, 5; and 3.0, 5. Gravity injection tests were not conducted on all trees, but the following numbers of trees in each symptom category were tested: category 0, 37 trees; 0.5, 6; 1.0, 12; 1.5, 6; 2.0, 6; 2.5, 5; and 3.0, 5.

In South Africa, sweet orange trees on rough lemon rootstock in 16 groves were tested. Syringe and zinc tests were conducted on the trees as follows: category 0, 108 trees; 1.0, 11; 1.5, 19; 2.0, 46; 2.5, 15; and 3.0, 24. Gravity injection tests were conducted on the following number of trees in each category: category 0, 45 trees; 1.0, 6; 1.5, 4; 2.0, 19; 2.5, 7; and 3.0, 4.

Diagnostic tests were compared by plotting zinc level or water uptake against severity of blight symptoms. The curve that best fit the data was found, the theoretical line determined, and correlation coefficients calculated separately for each country. Curves were developed from all data, whereas points are the means for each symptom severity rating. Thus, curves do not appear to fit the points in all cases because most of the trees were in the 0 and 0.5 or 2.0 and 3.0 categories. Because water uptake by gravity injection and the syringe method depend on the same mechanism, data from these two methods were compared directly by linear regression and correlation coefficients were calculated.

Diagnostic techniques were compared by designating each tree as positive or negative for blight by visual symptoms and by each of the diagnostic criteria. Trees were considered to be blight-affected if they had more than 3.0 μg/g of zinc in the trunk wood, took up less than 50 ml/24 hr by gravity injection, or less than 0.3 ml/sec by syringe injection. Threshold levels were established on the basis of previous work for standard methods (4,7,8,10–12,15) and on experience for the syringe injection technique. Because there is no absolute method for identifying blighted trees, the percentage of concurrence among the various diagnostic procedures was calculated to compare the reliability of the tests (Table 1).

Table 1. Percentage of concurrence between symptomatology and diagnostic tests for identifying citrus blight in Florida and South Africa

	Florida		South Africa	
Diagnostic methods	Concurrence (%)	Trees (no.)	Concurrence (%)	Trees (no.)
Symptoms vs. zinc	76	112	93	204
Symptoms vs. syringe	96	112	95	204
Symptoms vs. gravity infusion	80	77	76	84
Zinc vs. syringe	87	112	89	204
Zinc vs. gravity infusion	69	77	74	84
Syringe vs. gravity infusion	79	77	70	84

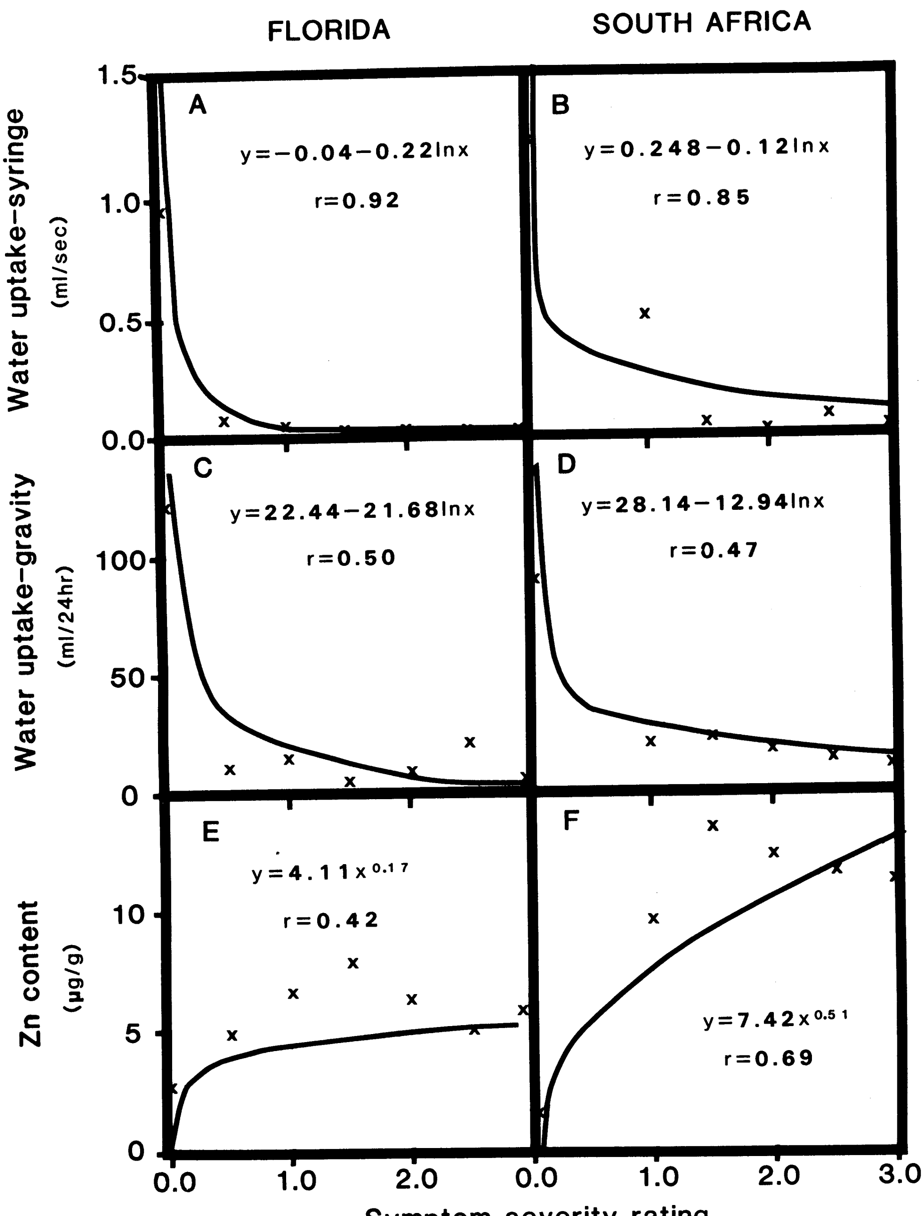

Fig. 1. Relationship of **(A and B)** water uptake by syringe injection, **(C and D)** water uptake by gravity infusion, and **(E and F)** the zinc content of trunk wood with symptom severity of citrus blight (0 = healthy to 3 = severe) in Florida and South Africa. All correlation coefficients are significant at $P = 0.01$.

RESULTS

Performance of syringe test. Diagnoses using the syringe injection method agreed with visual diagnosis by experienced observers. Blighted trees rarely took up water, even with the maximum possible force applied. Healthy-appearing trees took up an average of more than 1 ml/sec in all trees tested in both countries. Nearly all healthy trees took up a minimum of 10 ml in the 30-sec test period. In contrast, moderately or severely blighted trees usually took up no water, or occasionally, 1–2 ml in 30 sec. Most mildly affected trees did not take up water, but a few accepted up to 5 ml in the test period. Repeated tests at various sites on the trunks of mildly affected trees (0.5 severity category) indicated that uptake varied among sites from 0 to more than 1 ml per sec.

The average amount of water injected into healthy trees by two operators varied slightly. Operator 1 injected an average of 1.04 ml/sec, with a standard deviation of 0.58 into 10 healthy trees, and operator 2 injected 1.16 ml/sec, with a standard deviation of 0.45 in the same trees. Neither operator was able to inject water into any of the 10 blighted trees.

Diagnostic tests and symptom severity. Uptake of water by pressure injection with the syringe was highly correlated with the negative logarithm of the canopy severity rating (Fig. 1A,B). Absorption of water by gravity injection followed a similar pattern (Fig. 1C,D) but did not correlate as highly with symptom development. Although the equations differed slightly for water uptake of trees in Florida and South Africa, there was no substantial difference in behavior of blighted trees in the two countries. The relationship between water uptake by gravity injection and syringe injection was linear, with a correlation coefficient of 0.98 ($P < 0.01$) in Florida and 0.94 ($P < 0.01$) in South Africa.

The zinc content of trunk wood increased as a power function of the canopy severity rating (Fig. 1E,F). Zinc levels in the trunk wood of blighted trees were generally lower in Florida than in South Africa. Differences in zinc levels between blighted and healthy trees were greater and zinc levels correlated more highly with symptom severity ratings in South Africa than in Florida.

Water uptake by the syringe method correlated more highly with blight severity ratings than did zinc levels or water uptake by gravity injection (Fig. 1).

Accuracy of diagnostic tests. Because there is no absolute test to determine whether a tree has citrus blight, the agreement between blight identification by symptoms and by the diagnostic techniques was compared by calculating the percent concurrence between tests. Diagnosis by the zinc test agreed with visual diagnosis more frequently in South Africa than in Florida (Table 1). This was probably attributable to the greater differences in zinc content of trunk wood between healthy and blighted trees in South Africa. The syringe test agreed with diagnosis by visual symptoms in 94–96% of the cases in Florida and South Africa but the gravity injection test agreed in only about 76–80% of the cases. The syringe test concurred with the zinc test in 85–90% of the trees tested. The gravity injection test concurred with visual diagnosis and the other tests least frequently. Most of these cases were attributable to apparently healthy trees that failed to take up the required 50 ml of water.

DISCUSSION

The syringe injection technique is a rapid, accurate technique for confirming visual diagnosis of citrus blight. The equipment required is simple, inexpensive, and highly portable. The entire test requires less than 5 min per tree, including preparation time.

The zinc technique is useful in blight diagnoses but has limitations. It is highly effective in areas such as South Africa, where zinc levels in wood are low in healthy trees and high in blighted trees. In some Florida groves and in South American groves, however, zinc levels in wood of individual healthy and blighted trees overlap even though averages for groups of trees differ significantly. Thus, diagnosis of blight in individual trees requires corroboration by other techniques. Because zinc usually does not accumulate in trunk wood of blight-affected seedling trees and accumulation is less pronounced in blight-affected trees on certain rootstocks (2), zinc levels in the phloem as well as the xylem may have to be determined. Zinc tests appear to be specific for blight, but the mechanism of zinc accumulation and its relationship to symptomatology are unknown.

Water uptake tests are more likely to be accurate indicators of blight in that the symptoms are attributable to restricted water transport to the canopy due to plugging in xylem vessels of the trunk (5,13–15). With gravity injection tests, however, healthy trees sometimes take up little water, giving false positive diagnoses. Use of pressure in the syringe test avoids this problem and uptake apparently depends only on the presence of unobstructed vessel elements.

Uptake by gravity injection is not affected by other disorders such as tristeza, Phytophthora foot rot, nematodes, or other problems that are phloem or root disorders (4). Preliminary unpublished results with the syringe test indicated that water uptake of trees affected by citrus greening disease, tristeza, nematodes, or water damage was similar to that of healthy trees. Trees affected by psorosis frequently do not take up water, however, presumably because trunk wood is heavily impregnated with gum. Thus, caution should be used in diagnosing blight by water injection where psorosis is a common problem.

We feel that the syringe injection technique in combination with careful visual examination of trees is highly reliable for diagnosing citrus blight. Where a high degree of certainty is needed, such as in experimental plots, zinc content of trunk wood can be a useful additional criterion.

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