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LUPINE BAIT TECHNIQUE FOR THE SEMIQUANTITATIVE ANALYSIS OF *PHYTOPHTHORA CINNAMOMI* AND OTHER ROOT PATHOGENS IN AVOCADO SOILS

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OPSOMMING

'n Aangepaste lupien-lokaastegniek was op Westfalia Landgoed op groot skaal gebruik vir die semi-kwantitatiewe ontleding van P. cinnamomi en ander grondpatogene in avokado gronde. Die tegniek is waardevol vir die diagnose van die siekte, sells voor die verskyning van bogrondse simptome en is relatief goedkoop. Toekomstige chemiese beheermaatreels kan gebaseer word op opnames wat op hierdie manier gemaak word.

SUMMARY

A modified lupine bait technique was employed at Westfalia Estate on a large scale for the semi-quantitative analysis of ***Phytophthora cinnamomi*** *and other soil pathogens in avocado soils. It has proved to be valuable for the diagnosis of the disease even before the appearance of symptoms on above ground parts of the tree. This pathological soil analysis is relatively inexpensive. Future chemical control measures will be based on findings of this type of survey.*

INTRODUCTION

One of the most frequently encountered difficulties in experiments with soil pathogens is monitoring their populations in the soil. Recovery is mainly limited to qualitative methods. In most scientific papers, *Phytophthora cinnamomi* is simply referred to as being present or absent.

Isolation techniques vary from direct isolation from infected root pieces (Crandall, 1948; Zentmyer, Gilpatrick & Thorn, 1960) to bait techniques with pineapple (Anderson, 1951), apple (Campbell, 1949; Zentmyer *et al.,* 1960), avocado fruit (Zentmyer *et al.,* 1960; Zentmyer, 1973; Brodrick, Zentmyer & Wood, 1976), the susceptible avocado relative, *Persea indica* (Zentmyer *et al.,* 1960), Jacaranda seedlings (Brodrick *et al.,* 1976) and lupine seedlings (Chee & Newhook, 1965; Rodger, 1972; Pratt & Heather, 1972; Shepherd & Pratt, 1974; Walker Kirby & Grand, 1975; Brodrick *et al.,* 1976; Kliejunas & Ko, 1976; Donald & von Broembsen, 1977; Hoitink, van Doren & Schmitthenner, 1977). These techniques have been worked out for qualitative analysis, some of them may however be used for semi-quantitative surveying when there are considerable differences in population and the sample size is large. The soil dilution plate technique employed by Hendrix & Kuhlman (1965) together with the soil sieving method of

McCain, Holtlzman & Trujillo (1967) using selective media are more suitable for quantitative analysis, but are restricted in large scale practical applications, due to cost and time.

In these investigations we applied a method for the early detection of root rot and a semi-quantitative technique for the monitoring of *Phytophthora cinnamomi* in the soil which is inexpensive and can be used on a commercial scale.

Zentmyer & Mircetich (1966) found that *Phytophthora cinnamomi had* a limited mycelial growth through non-sterile soil and that the inoculum source should be within 3 cm of the site of the material to be invaded. In other words if a bait plant, like lupine is planted in infected soil, it will be vulnerable to attack by the fungus from a tube of soil with a 3 cm radius around the roots. The aim is not to establish the exact amount of propagules in the soil (the number of simultaneous attacks on one root cannot be detected) but rather to obtain the percentage of the lupine seedlings killed by the fungus or the percentage infected trees in an orchard and to use these data for comparisons. Similar attempts were made by Hoitink, van Doren & Schmitthenner (1977) who recovered the pathogen from soil artificially infected with various levels of inoculum and found a correlation between inoculum level and number of killed lupine seedlings.

Procedure

Soil samples were collected from the area between the tree trunk and the drip line. Soil was taken from the root zone, but large pieces were excluded. Samples were placed in 8 cm plastic cups with a volume of 300 ml. The soil was moistened and planted with 5 pre-germinated blue lupine *(Lupinus angustifoliusi* seedlings, 1 in the centre and 4 on the perimeter. Lupine seedlings were sterilized by 0,1% Hg CI_2 solution for 1 minute, rinsed with sterile water and germinated on an agar medium in Petri dishes (Vaartaja & Cram, 1956). Normal emerging seedlings with approximately 2 cm radicals were planted in the soil sample. Seedlings were watered regularly but not flooded to reduce the possibility of zoosporos moving to plants further away from the inoculum source. Diseased lupine plants were removed from the cups, surface sterilized with 0,1% Hg CI_2 for 10 seconds and plated on Potato Dextrose Agar. A few days later the isolated pathogens were identified. In this survey soil samples were taken from the drip area of 5 trees per ha. but not less than 20 samples from a block.

INTERPRETATION OF RESULTS

Phytophthora cinnamomi caused the earliest damping-off of lupines, followed by *Pythium* spp. *Rhizoctonia, Fusarium, Cylindrocladium, Macorphomina, Cylindrocarpon* killed the seedlings later. The percentage seedlings destroyed by *Phytophthora cinnamomi ana* the other root pathogens was determined. If *Phytophthora* killed lupine seedlings, or the number of infected trees exceeded a certain percentage, Ridomil treatment was recommended for the whole orchard, if below that, only the apparently sick trees were treated. None of the other pathogens were assayed at this stage. It is anticipated that this technique of disease detection, combined with an effective chemical treatment can check the disease at an early stage and prevent yield losses which might

otherwise occur if trees are only treated after appearance of root rot symptoms. The method is also suitable for evaluating the efficacy of fungicidal soil treatments (Darvas, 1978).

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