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FAILURE TO CONTROL *PHYTOPHTHORA CINNAMOMI* AND *PYTHIUM SPLENDENS* WITH METALAXYL AFTER ITS PROLONGED USE

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OPSOMMING

Bestandheid van Phytophthora cinnamomi *teen metalaxyl is bewys in avokado gronde wat met die middel vir vyf jaar behandel was. Indikasies is gevind dat* Pythium splendens *weerstand opbou in dieselfde* grand, *maar bestandheid is nie eksperimenteel bewys nie.*

SUMMARY

Resistance to metalaxyl by Phytophthora cinnamomi was *demonstrated in avocado soils treated with the chemical for five consecutive years. Indications for resistance build-up were observed with* Pythium splendens *in the same soils, but this resistance was not experimentally confirmed.*

INTRODUCTION

A decrease in the efficacy of metalaxyl against Phytophthora root rot of avocados was observed after the second year of its continued use at Westfalia Estate. This was associated with a progressively shortened post-treatment inhibition of the fungus by the chemical in the soil (Darvas, 1982).

This is a report on further investigations to evaluate the degree of resistance build-up in *Phytophthora cinnamomi* and *Pythium splendens* to metalaxyl in avocado soil treated experimentally for five years with the chemical and also to determine the loss of efficacy in commercial orchards which had received metalaxyl for various periods of time.

MATERIALS AND METHODS

In the laboratory tests which were carried out to determine the degree of reduced efficacy of metalaxyl, soil samples were used from block 4A of Evenrond Section of Westfalia Estate, Northern Transvaal. Samples were taken in August 1982 from under untreated trees and from trees treated with metalaxyl at 2,5 g ai per m^2 for five years from September 1977 until January 1982 (Darvas, 1983).

Two soil samples of about five kg each were drawn from the root zone under the canopy area of four trees in each treatment. Soil samples were thoroughly mixed within treatments giving a compound sample for the control and another for metalaxyl treated trees. All visible root pieces were removed and the samples were then placed in 300 ml plastic cups, using 100 cups filled with test soil for each treatment. The 100 cups were divided into groups of 20 cups each and each group received a different concentration of metalaxyl drench. The treatments were as follows: drenching of the test soil in the cups with 25 ml aqueous solution of 0, 0.2, 2, 20 and 200 ppm ai metalaxyl. Solutions were made-up by using the Ridomil 25 WP formulation. Five pre-germinated lupine *(Lupinus angustifoliusi* L) seedlings were planted in the test soil of each cup according to the technique of Darvas (1979), wetted with tap water and kept at ambient temperature with diurnal fluorescent lighting. Isolations were made from damped-off seedlings on Potato Dextrose Agar (PDA).

A number of *P cinnamomi* isolates used in studies for the determination of resistance were transferred onto PDA containing 25 ppm Streptomycin and 25 ppm Ampicillin to prevent any bacterial contamination of the cultures. A total of 10 *P cinnamomi* isolates selected over the range of 0 to 200 ppm ai metalaxyl from each treatment were then inoculated into 500 ml Erlenmeyer flasks. Each flask contained 200 ml of autoclaved barley and crushed maize mixture (1:1 ratio) wetted with 200 ml 4 percent V-8 vegetable juice autoclaved water suspension. The medium was completely overgrown by white mycelium of the fungus after three weeks incubation period at room temperature (appr 25 °C). The 10 flask cultures of each treatment were then homogenized in a blender and mixed thoroughly with 50 kg heat sterilized soil collected from a *P cinnamomi free* area with no previous metalaxyl exposure. The soil was placed in 300 ml plastic cups, using 100 cups filled with the inoculated soil for each treatment. The 100 cups were divided Into 20 cup groups each of which received a different concentration of metalaxyl drench. The following treatments were used: 0, 0.2, 2, 20 and 200 ppm ai metalaxyl solution, giving 25 ml for each cup. The test soils were then planted with five pre-germinated lupine seedlings, wetted with tap water and kept at ambient temperature. Isolations from damped-off seedlings were made on PDA and the incidence of *P cinnamomi* was recorded for each treatment.

The extent of Metalaxyl's ineffectiveness under field conditions was investigated by testing soils collected from orchards that had received commercial metalaxyl treatments for various periods of time (0-5 years). Again four trees were sampled in each locality and samples were compounded and 120 plastic cups were filled with soil in each treatment. They were divided into 20 cup groups and each group was drenched with 25 ml of one of the following metalaxyl solutions: 0, 0.5, 5, 50, 250 and 500 ppm ai. The test soils were then planted with five pre-germinated lupine seedlings per cup. Isolations from the damped-off seedlings were made on PDA and the incidence of *P cinnamomi* was recorded for each treatment.

RESULTS

As illustrated in Fig 1, the drenching of the control soil with the 0.2 ppm ai metalaxyl caused only a slight drop in the incidence of *P* cinnamomi, while the 2 ppm dose rate considerably reduced it. However, the latter treatment had no significant influence on the pathogen in the metalaxyl treated soil. The 20 ppm concentration resulted in a total inhibition of the fungus in the control soil, but left the population of the pathogen nearly unchanged in the treated soil in which even after the 200 ppm ai metalaxyl drench the fungus remained fairly active. These findings indicate that there is at least a 100 fold decrease in Metalaxyl's inhibitive effect against *P* cinnamomi in the five year treated soil.

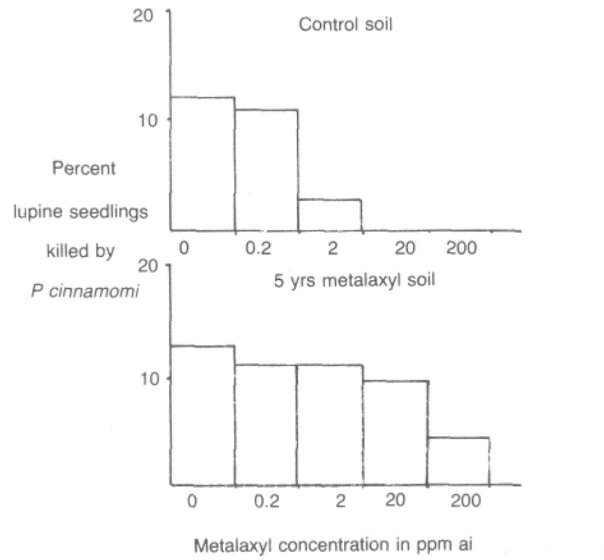

Fig 1: The recovery of *P cinnamomi* from untreated soil and avocado orchard soil treated for 5 years with metalaxyl.

A significantly reduced effect of Metalaxyl against Pythium spenders was also evident in the soil treated with the chemical for five years. (Table 2).

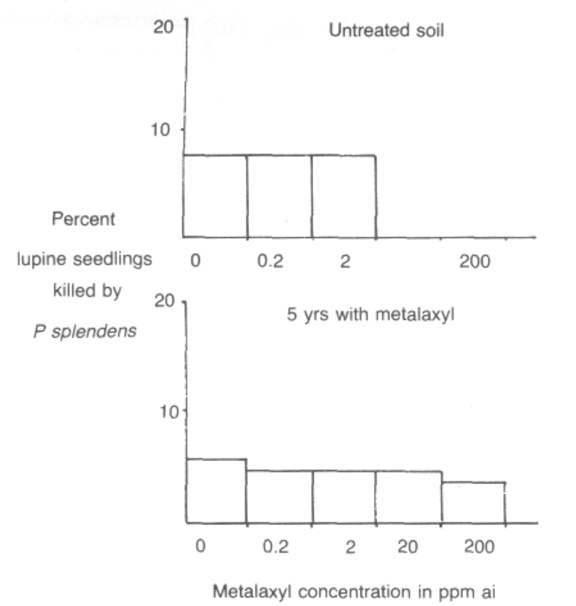

Fig 2. The recovery of *P splendens* from avocado orchard soil which had been treated for 5 years with metalaxyl and untreated soil.

High percentages of the seedlings were killed by *P* cinnamomi when heat sterilized non-avocado soil without previous metalaxyl histories were inoculated with isolates from control and metalaxyl treated trees. Isolates from the control trees were completely inhibited by the 20 ppm dose rate of metalaxyl, while infections occurred even at the highest concentration of 200 ppm drenched soil inoculated with isolates of the fungus from metalaxyl treated soil (Fig 3).

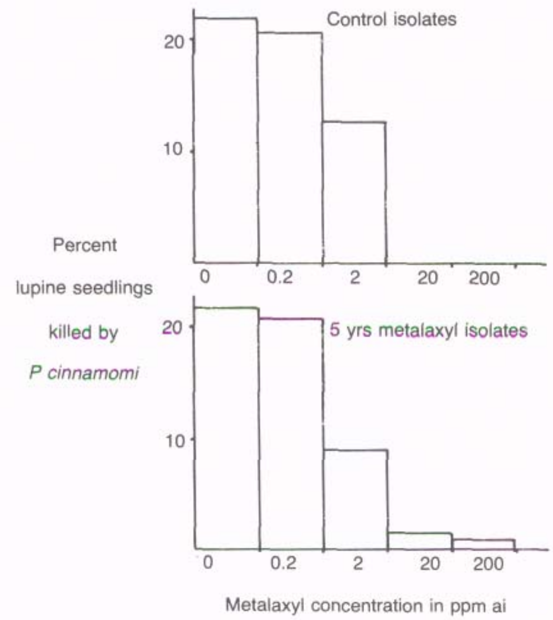

Fig 3: The recovery of *P cinnamomi* from soils inoculated with isolates from control and five years metalaxyl treated soils.

A progressive deterioration in the efficacy of metalaxyl was proved in the soils of commercial avocado orchards with prolonged treatments. There were isolates in some of the treatments (4 years) which remained active at 10000 times of the concentration that inhibited *P* cinnamomi in the control soil (Table 1).

Table 1: The recovery of *P cinnamomi* from avocado orchards treated commercially with metalaxyl for various time periods.

Metalaxyl concentration in ppm ai	Lupine seedlings killed by *P cinnamomi*					
	0 yr	1 yr	2 yrs	3 yrs	4 yrs	5 yrs
0	+	+	+	+	+	+
0.5	+	+	+	+	+	+
5	—	+	+	+	+	+
50	—	—	—	—	t	t
250	—	—	—	—	—	+
500	—	—	—	—	+	—

DISCUSSION

A progressive loss in Metalaxyl's efficacy against *P cinnamomi* due to prolonged usage was first reported by Darvas (1982) and McKenzie and Margot (1982). This has been clearly demonstrated again, using soils taken from under control trees and trees treated with the chemical for five years (Fig 1).

The two major reasons that may account for such loss of efficacy are the buildup of micro-organisms in the soil which utilize metalaxyl as a feed source and the development of resistance in the pathogen against metalaxyl.

McKenzie and Margot (1982) found a shortened half-life of the chemical in soils treated for five years with the product. They suggested that bio-degradation of the chemical could be the cause for Metalaxyl's failure after continued field application and that no resistance could be detected.

While the present study did not include any tests that were aimed at measuring the role of the breakdown of metalaxyl in the soil by micro-organisms, it has conclusively showed the presence of strains or isolates of *P cinnamomi* in the five years treated soils that are highly resistant to metalaxyl. The resistance in some isolates reached at least a 100 fold increase in metalaxyl tolerance (Fig 3).

Snyman and Kotzé (1983) experimented with isolates obtained from the same five year old metalaxyl treated soils and found that metalaxyl failed to control root rot of young avocado seedlings in soils inoculated with *P cinnamomi* isolates from the five year metalaxyl treated soil, whereas fosetyl-Al was effective against the disease.

A progressive deterioration in Metalaxyl's effect under field conditions points again to the necessity to terminate the use of metalaxyl after two or three years' consecutive application of the chemical (Table 1). Studies are continuing on sites used in these experiments to establish whether *P cinnamomi* populations believed to be resistant will return to a susceptible, wild type population after a certain rest period with no metalaxyl applications.

In the root zone of the five year metalaxyl treated trees at the beginning of the experiment, *P splendens* was found to be the most common species, followed by *P spinosum* and *debaryanum.* Initially, all *Pythium* spp were inhibited by metalaxyl, but one species reappeared again in the fourth and fifth year of the treatment and this was *P splendens. P spinosum* and *P debaryanum* are absent in the five year metalaxyl soil indicating either a complete disappearance of these fungi from the soil or a persistant Inhibition by the chemical. *P splendens* showed a trend for tolerance build-up similar to *P cinnamomi* (Fig 2), but since laboratory experiments to inoculate and recover the organism from sterilized soils with the various isolates of the fungus have all failed, the resistance of *P splendens to* metalaxyl remains an assumption.

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