South African Avocado Growers' Association Yearbook 1992. 15:18-23

Effect of different dosages of methyl bromide on nursery pathogens

L C McLean and J M Kotzé
Department of Microbiology and Plant Pathology, University of Pretoria 0002

ABSTRACT

The effect of different concentrations of methyl bromide fumigations on the survival of Phytophthora cinnamomi *and* Fusarium solani *was evaluated in two types of nursery medium. Samples of fungus inoculum which were placed in shade cloth bags were "planted" at certain predetermined positions in the. medium. The methyl bromide fumigations were done in wooden bins with a volume of 1m^3 at six different concentrations. The inocula were removed after aeration and tested for viability. Bait techniques for detection of* Phytophthora cinnamomi *were evaluated*

INTRODUCTION

Root rot of avocados (*Persea Americana* Mill) caused by *Phytophthora cinnamomi* Rands can be easily distributed through infected nurseries (Kotzé *et al,* 1987). SAAGA introduced the Avocado Plant Improvement scheme in 1984 (Partridge, 1984) and one of its important aims is to establish disease free nurseries.

Many rivers are heavily infested with *Phytophthora cinnamomi* and *Pythium spp* (Kotzé, 1985). Riversand which make out an important part of nursery medium is therefore a primary source of inoculum when introduced into the nursery and has to be sterilised prior to use. River water should also be avoided for irrigation in the nursery unless it is filtered or chlorinated before use (Kotzé *et al,* 1987; Maas & Kotzé, 1990).

Methyl bromide (MBr) is currently being used as a soil sterilant in nurseries (Kotzé *et al,* 1987; Munnecke & Ferguson, 1953; Timmer & Leyden, 1978). The most general application is the cold gas method where MBr in cans (681 g), under natural pressure, is used (Technical brochure, Landkem; nursery survey conducted).

MATERIALS AND METHODS

Two experiments were conducted using two different types of nursery medium. The nursery mixture used in the first experiment consisted of composted sugar cane fibre (known as "mila") and riversand. The riversand was obtained from the Ramadiepa River on Westfalia Estate, Duiwelskloof. The mila as well as the riversand was sifted with a 15 mm x 15 mm sieve prior to mixing. The mixing ratio was 1:1.

The nursery medium used in the second experiment consisted of composted, milled pine bark and riversand. These components were also sifted with a 15 mm x 15 mm sieve and mixed at a ratio of 1:1. The soil moisture content should be in the order of

about ⅔ field capacity (Technical brochure, Landkem). Field capacities for both nursery mediums were determined by drenching the mixtures with water, in small buckets with drainage, and left for three days until all excessive water was drained off.

The mixture's moisture content was then determined by using a "Ultra X infra-red moisture tester". A sample of 10 g was weighed and then dried in the tester for 10 min at 110°C. This was done five times for each batch and the average value then determined. This percentage value was taken as 100% field capacity and a value was calculated accordingly for 66% field capacity.

The mixture was tested prior to fumigation and the moisture content determined. Water was then added to the mixture if necessary. The mixture was usually drier than $^{2}/3$ field capacity.

The bins used for the fumigation was built from wooden boards (thickness = 25 mm) with the following inner dimensions: 4170 mm x 600 mm (width) x 450 mm (depth). When filled up to 400 mm it had a volume of 1 m^3. All the bins (six of them) were a standard size and placed on a level concrete slab (Figure 2). Only enough working space was left between the bins.

Two different fungi were used in these fumigations to determine viability. The first, *Phytophthora cinnamomi* Rands, was used for its consistent occurrence in avocado nurseries and it causing root rot of avocado (*Persea americana* Mill). The second fungus, *Fusarium solani,* a pathogen of citrus was used because it readily forms of chlamydospores. Cultures were maintained on potato dextrose agar (PDA).

Inoculum was prepared by growing the pathogens on sterilised millet seed. A 500 mℓ erhlenmeyer flask was used and filled with 180 g millet seed; 90 mℓ water was added and a cotton plug put in the flask and then covered with aluminium foil. These flasks were then sterilised at 121 °C for two hours. After three days the millet seed in the flasks were inoculated with 7-day-old cultures growing on PDA by cutting it in small pieces and mixing it into the millet seed. This was done in a laminar flow cabinet under sterile conditions.

Chlamydospores of both *P cinnamomi* and *F solani* were produced on medium of low nutritional status and incorporated in the inoculum mixture before fumigation. *P cinnamomi* was grown on cornmeal agar for 15-30 days and then covered in the petri dishes with VB juice broth (Mircetich *et al,* 1968). VB juice was not available and a vegetable extract used as babyfeed (Vita juice) was used instead.

The broth was prepared as follows: The juice was centrifuged for 10 min at 1 720 g and the supernatant filtered through Whatman no 42 paper five times. Two grammes $CaCO_3$ was suspended in 900 mℓ demineralised water for 30 min and then filtered through Whatman no 42 filter paper. Vita juice (100 mℓ) was then added to the filtered $CaCO_3$ suspension to make up 1ℓ. The solution was then autoclaved for 15 min at 121 °C. The medium pH was 4,5-4,7 (Mircetich *et al,* 1968). When added to the cultures on cornmeal agar, chlamydospore formation took place from 3-5 days, with a high yield reached after 10 days.

F solani was also grown on cornmeal agar and about 2 to 3-week-old cultures were used in the inoculum to ensure excessive chlamydospores. Four-week-old fungus

mycelium growth on millet seed was used and 500 mℓ millet seed was mixed with 4,5 ℓ unsterilized nursery medium to make out the inoculum (10%). Ten cornmeal plates were cut into small blocks and added per 5 ℓ inoculum mixture. Samples of about 300 mℓ were made up by putting the inoculum mixture in shade cloth bags (100 mm x 150 mm) (60% shade netting was used). Coloured plastic tags were used to distinguish between the different fungi.

These samples were placed at two different depths and five different locations with these locations being the same for all the bins. The top layer was placed 10 cm from the surface and the bottom layer 10 cm from the bottom. It was not placed on the bottom, for methyl bromide will accumulate due to gravitation. The first and fifth samples in the bin were placed 20 cm from each side, one sample was placed in the centre and the other two (nr's 2 & 4) halfway between the centre one and the one on the outside. These five samples were in the centre of the width of the bin.

Evaporation pans to be used in the fumigation process were made by cutting 5 ℓ plastic buckets in half and inserting a 25 cm long 12 mm black poly-ethylene tubing by making a small hole in the top on one side of the bucket. This piece of tubing serves as a guide for the methyl bromide applicators' pipe. The evaporation pans were placed in the centre of the bins directly above the centre sample. Methyl bromide cans (Landkem) with a nett weight of 681 g was used.

Methyl bromide in cans is under natural pressure and in the liquid form. A special applicator (Figure 1) was used, which punctures the can and releases the liquid methyl bromide through a nylon tube (Figure 1) with a inner diameter of 8 mm (Technical brochure, Landkem). This applicator contains the nursery medium and is operated from outside the bin.

Transparent low density poly-ethylene (LDPE) plastic sheeting was used to cover the fumigation bins. The thickness of this sheeting was 180 μm (Figure 2). The plastic sheets were inspected before each fumigation and any small holes which occurred due to handling were taped with plastic tape. Sun rays are let through the transparent plastic, resulting in a greenhouse effect (Mahrer, 1979) and the subsequent temperature increase in nursery medium. The nursery medium's temperature was determined each time before fumigation and was never less than 20°C (Table 1).

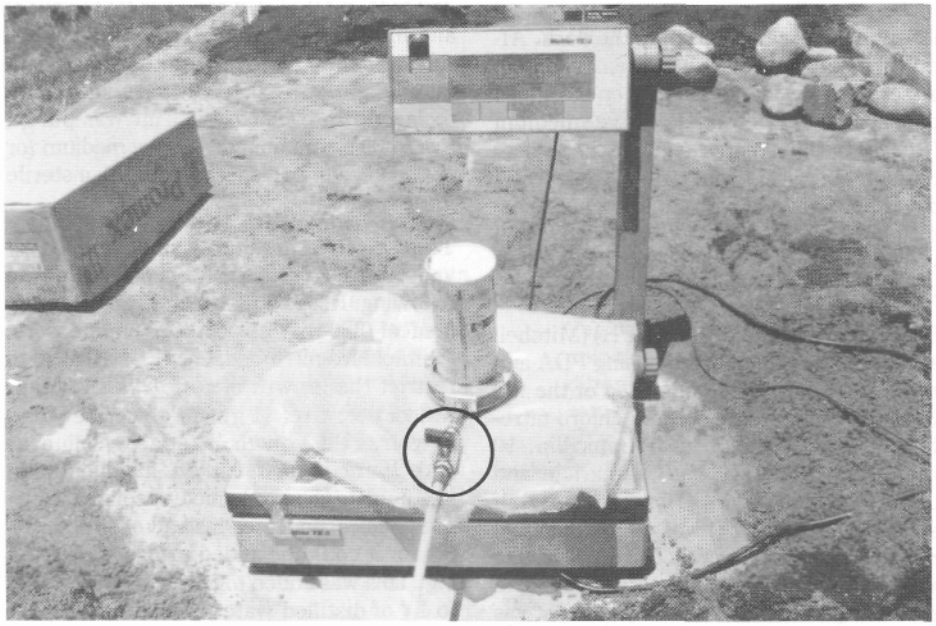

Fig 1 A special applicator with a valve (circled) is used to release measured amounts of methyl bromide through the nylon tube. A 6 kg Mettler scale was used. The plastic underneath the MBr can is to protect the scale should any MBr leak out.

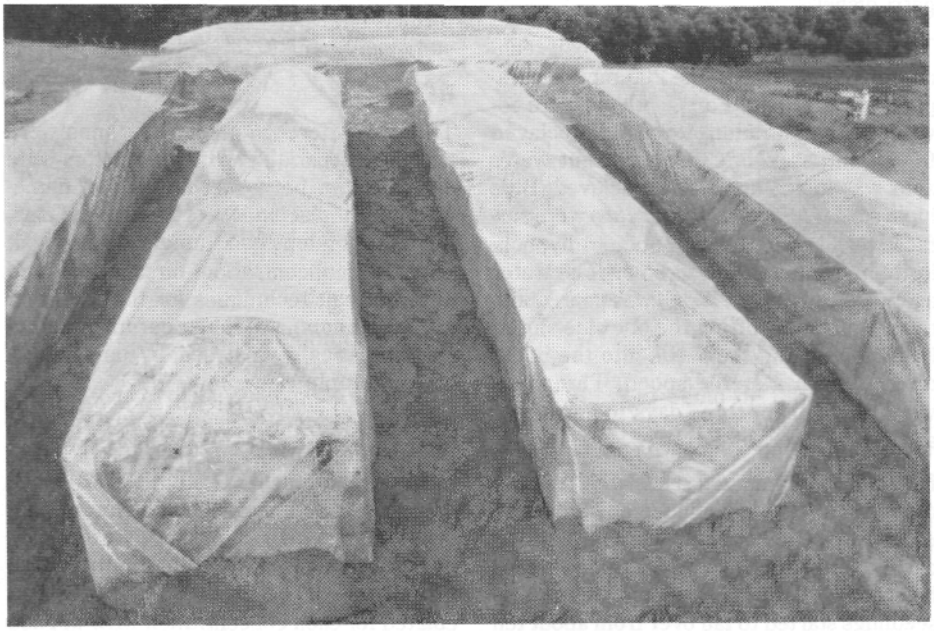

Fig 2 Bins covered and sealed off are left for 48 hours after fumigation.

Wire loops made from a 2,5 mm galvanized wire were put on the sides of the bins at four locations to form a canopy to lift the plastic sheeting about 200 mm from the nursery medium (Figure 2). This airspace was created to ensure effective diffusion of methyl bromide gas on top of the medium. The end of the applicator's nylon tubing was

now inserted in the evaporation pan via the black guiding pipe and the other end brought downwards next to the bin and led away. The edge of the plastic sheeting was sealed off with wet sand on the concrete floor with only the nylon tubing coming out (Figure 2).

TABLE 1 Difference in temperature before and after fumigation at different depths

Different fumigations	Degrees Celsius at *10 cm depths*	
	10 cm – before	10 cm – after 2 days
Pine bark 1	26,5	38,1
Pine bark 2	31,3	40,2
Pine bark 3	29	41,7
Pine bark 4	31,5	36
Mila 1	32	43,2
Mila 2	20,5	29,3
Mila 3	30,8	44,2
Mila 4	29,7	38,7

The applicator was fitted with an in-line valve to shut off the flow of MBr and an easy coupling as used by the compressed air industry (Figure 1). A male coupling was fitted onto the nylon tube that was inserted in the bins. The MBr cans was packed on ice to cool them off and therefore reduce the pressure in the cans. A 6 kg Mettler scale with l g divisions was used to measure the amount of MBr applied (Figure 1).

Once the can was pierced with the applicator, it was put on the scale and by opening the valve slightly, the amount of MBr released was registered by the scale. When the correct amount of MBr was released, the valve was closed again and after all the MBr in the pipe was evaporated, the applicator was uncoupled from the tube and the tube removed from the bin without disturbing the edge sealed with the sand. Now it was on to the next bin and the same procedure repeated.

Six different concentrations were used with the first, 0 g/m^3, being the control. The other five concentrations were; 150, 300, 450, 600 and 750 g/m^3. Four replicates were done for each fumigation. This was used according to a nursery survey conducted in early 1991 where the most nurseries use a concentration of 400 800 g/m^3. The legal registered dosage for *P cinnamomi* in avocado nursery soil is 150 g/m^3 (Vermeulen *et al,* 1990).

The MBr is then released into an evaporation pan where it evaporates from the liquid form into a gas and diffuses evenly through the air space and the nursery medium. A halide detector lamp was used to determine leaks in the applicator system (Munro, 1964). A change in the flame colour could give an idea about the amount of MBr present in the air. The rubber seal was replaced after every second fumigation as a safety factor for one is not advised to restrict the flow of methyl bromide from a can

when using this special applicator. That is also why the cans are cooled on ice before being used.

The fumigations usually took place between 10 am and 2 pm. Plastic covering was left on the bins for 48 hours and removed in the later afternoon when the area was evacuated to aerate the nursery medium for 24 hours before the samples were removed. Temperatures were measured before fumigating and then after the covers were lifted, monitored in the control bins.

Retrieving of samples were done 24 hours after the covers were lifted. Samples were combined in the following way: The outer two samples (each the same distance away from the point of application) were combined. The two samples halfway between the point of application and the outer samples were combined and the middle sample under the point of application was retrieved on its own. This was done separately for the two layers in each bin. Each combined sample was put in a marked plastic bag and immediately put in the shade from where it was taken to the laboratory.

The sequence wherein samples were retrieved was as follows. First the sample underneath the evaporation pan (sample no 3), then the two samples halfway between the point of application and the end of the bin (they were combined to form sample no 2) and lastly the two samples at the ends of the bin (also combined to form sample no 1). The bin with the highest concentration of MBr applied was started with and the control bins was done last as to avoid contamination of samples. Hands were sanitised with a chlorine mixture (5 g HTH commercial chlorine/ℓ water). Sanitation was done between the different layers and the different bins. The two different layers of fungus inoculum (top and bottom) were evaluated seperately for every bin.

Two tests were done on the *P cinnamomi* samples both using bait techniques. Polystyrene cups (250 mℓ) were used for both. 100 mℓ of a sample was put in one cup and 200 mℓ of the same sample put in another cup, both were marked. The samples were then left in the cups for ten days to aerate and kept moist with sterile water.

The first test (avocado leaf disc bait technique) was based on the one used for citrus (Grimm & Alexander, 1973). This was done by covering the 100 mℓ soil sample with sterile distilled water and stirring it well with a sterile glass rod. Avocado leaf discs were then punched out with a sterile cork borer. The leaves were obtained from a Fuerte tree that was not sprayed with chemicals and picked from chest height and higher. Ten discs were put on the water and left floating for three days in the dark. After three days the discs were removed with a sterile pincette, clotted dry on sterilized paper and put on a selective medium (PARPH). The plates were incubated at room temperature and observed for 2-7 days and examined with a microscope to determine any *P cinnamomi (Pc)* mycelial growth. Every disc that showed *Pc* growth counted for ten percent on every petri dish.

This selective medium (PARPH) (Mitchell *et al,* 1986) was modified by using PDA and not Cornmeal agar. It consisted of the following: 100 mg/ℓ Penta-chloro-nitrobenzene (PCNB), 250 m/ℓ Ampicillin, 10 mg/ℓ Rifampicin, 10 mg/ℓ Pimaricin and 0,15 mℓ/ℓ (54 ppm) Hymexazol. Half strength PDA (20 g/ℓ) was made up in 800 mℓ distilled water and sterilized. Distilled water (200 mℓ) was also sterilized in a separate flask. When the 200 mℓ water was cooled to about room temperature, the above ingredients were added.

This mixture was then added to the PDA as soon as it was cooled to about 50 °C and poured into petri dishes.

The second test (Lupin seedling bait technique) was done with the 200 mℓ soil sample (Darvas, 1979). Foil pie plates were covered with aluminium foil and autoclaved for 15 min at 121 °C. One percent water agar was mixed and also autoclaved. Blue lupin seeds *(Lupinus angustifolius)* were surface sterilised with 2,5% Sodium hypochlorite in a laminar flow cabinet. The water agar (cooled to about 50°C) was poured into the pie plates by lifting the one side slightly and spreading the seeds into the liquid medium with a sterile spoon. The plates were then left for three days to germinate at room temperature.

Five sterile germinated seeds with the radicle between 10 and 20 mm long were planted in every cup by inserting only the radicle in the soil. The cups were then watered but not flooded with sterilized water. The seedlings started to fall over from about ten days up to 23 days. When a seedling fell over, the lesion at the soil surface, as well as the adjacent healthy looking part of the stem, was removed and the surface sterilized in 2,5% sodium hypochlorite for two minutes. It was then washed in sterile water for one minute and cut into five pieces. These pieces were plated out on PDA or PARPH. The plates were incubated at room temperature and observed from 2-7 days and microscopically examined for any *Pc* growth. Every lupin seedling in a cup counted for 20% infection and if one out of five pieces plated out on a petri dish showed *Pc* growth it was taken as 20%.

The soil samples containing *F solani* were also aerated for ten days prior to testing for viability. Two soil dilutions were done for each sample and 10 g of a sample was added to 90 mℓ 0,15% wateragar (autoclaved) and mixed thoroughly. 10 mℓ of this mixture was then added to another flask containing 90 mℓ 0,15% wateragar (autoclaved). In each case 0,1 mℓ was put on a petri dish containing selective medium for *Fusarium* spp and spread out with a sterile glass hockey stick.

This selective medium was developed by van Wyk *et al* (1986) and consisted of the following: Rose bengal (0,5 g) and Chlorampenicol (250 mg) as bacterial suppressors; Pentachloro-nitro-benzene (PCNB) (1 g) to restrict the growth of *Rhizopus* and other Mucorales; Urea (1 g) and glycerine (10 g) to restrict the growth of aerial mycelium as with *Verticillium dahliae;* L-alanine (0,5 g) to stimulate the production of conidia. Chloramphenicol was used instead of streptomycin for it could be added prior to autoclaving. This was added together with 15 g agar to 1ℓ of distilled water and autoclaved for 20 min at 121°C.

The petri dishes were observed from 2-10 days and microscopically examined to identify *Fusarium solani.* All the colonies that formed on a petri dish were counted and statistically analyzed.

RESULTS

The two different nursery mediums were evaluated separately. Different factors were evaluated for both the fungi tested namely: different placements of fungi in the bins, the effect of depth on fumigation survival, effect of concentration on survival and different

bait techniques for the detection of *P cinnamomi.* All the experimental data was statistically analysed by using Duncan's multiple range test.

F solani was only evaluated for the control and the first fumigation (150 g/m^3). All of other fumigations resulted in 100% control of the fungus. The effect of different concentrations of MBr on the survival of *F solani* in pine bark is depicted in Figure 3. The mean value of the four replicates was calculated for each concentration MBr applied and plotted on a graph. There is a significant difference in the amount of colonies surviving the fumigation between the control and the 150 g/m^3 application. There is also a statistical difference between the named two applications and the rest of the applications (4), where no survival *of F solani* was found.

Figure 4 shows the effect of MBr concentrations on survival *of F solani* in mila nursery medium. In this case the control was not statistically different from the 150 g/m^3 application but these two were statistically different from the rest of the fumigations where *F solani* did not survive.

Figure 5 shows the amount of surviving colonies of *F solani* for the 150 g/m^3 treatment on the three positions in pine bark. Statistically, there is no difference between the survival of the fungus samples in the three positions. Figure 6 depicts the same information for the 150 g/m^3 treatment in mila nursery medium. Here is also no statistical difference between the survival of *F solani* at the three different positions in the fumigation bin.

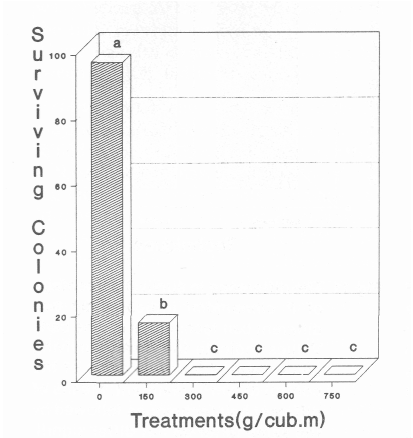

Fig 3 Effect of methyl bromide treatments on the survival of *F solani* in pine bark. Values indicate the mean surviving colonies of four replicates. Means of different treatments followed by the same letter do not differ significantly (P = 0,0001) according to Duncan's multiple range test.

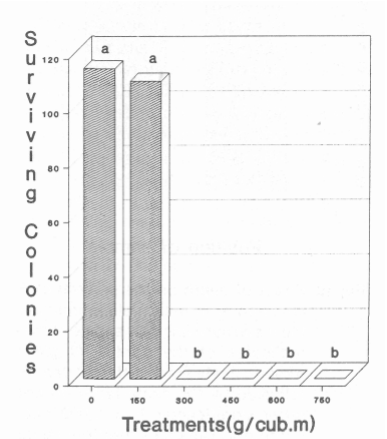

Fig 4 Effect of methyl bromide treatments on the survival of *F solani* in mila. Values indicate the mean surviving colonies of four replicates. Means of different treatments followed by the same letter do not differ significantly (P = 0,0001) according to Duncan's multiple range test.

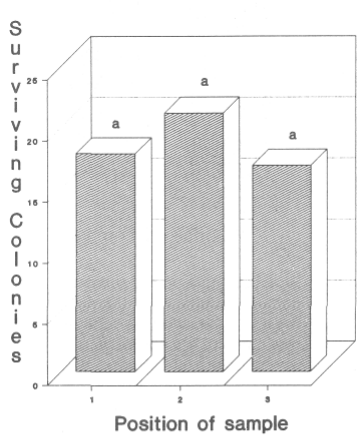

Fig 5 Effect of distance from the point of application of methyl bromide on the survival of *F solani* in pine bark. Values indicate the mean surviving colonies of four replicates of the 150 g/m^3 treatment. Means of different distances followed by the same letter do not differ significantly (P = 0,8537) according to Duncan's multiple range test.

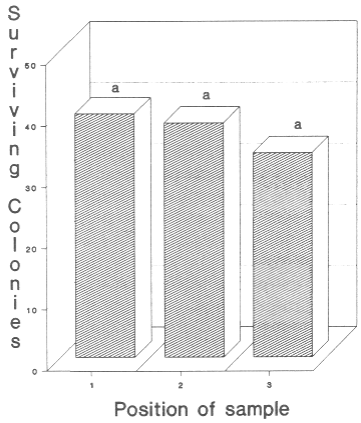

Fig 6 Effect of distance from the point of application of methyl bromide on the survival of *F solani* in mila. Values indicate the mean surviving colonies of four replicates of the 150 g/m^3 treatment. Means of different distances followed by the same letter do not differ significantly (P = 0,8592) according to Duncan's multiple range test.

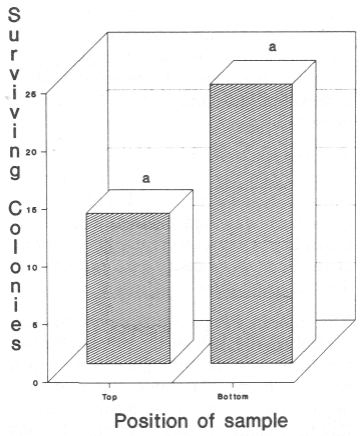

Fig 7 Effect of depth on the survival of *F solani* in pine bark. Values indicate the mean surviving colonies of four replicates of the 150 g/m^3 treatment. Means of different depths followed by the same letter do not differ significantly (P = 0,0873) according to Duncan's multiple range test.

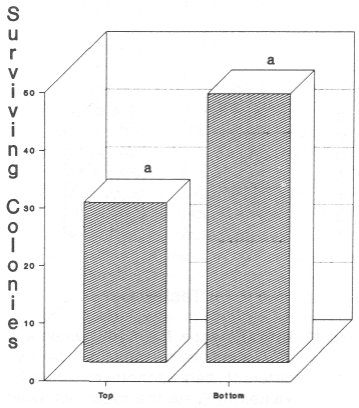

Fig 8 Effect of depth on the survival of *F solani* in mila. Values indicate the mean surviving colonies of four replicates of the 150 g/m^3 treatment. Means of different depths followed by the same letter do not differ significantly (P = 0,0553) according to Duncan's multiple range test.

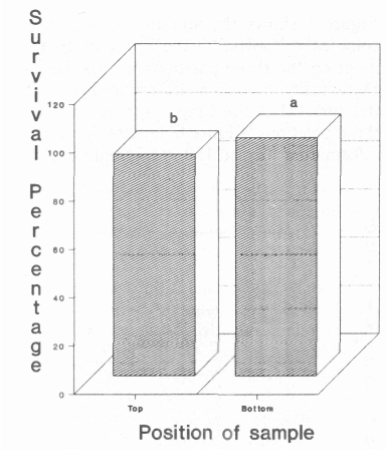

Fig 9 Effect of depth on the survival of *P cinnamomi* in pine bark. Values indicate the mean surviving percentage of four replicates of the control (0 g/m^3) treatment. Means of different depths followed by the same letter do not differ significantly (P = 0,0282) according to Duncan's multiple range test.

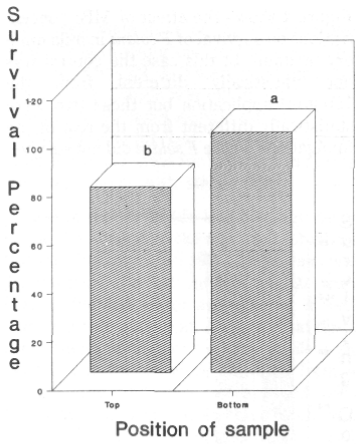

Fig 10 Effect of depth on the survival of *P cinnamomi* in mila. Values indicate the mean surviving percentage of four replicates of the control (0 g/m^3) treatment. Means of different depths followed by the same letter do not differ significantly (P = 0,0001) according to Duncan's multiple range test.

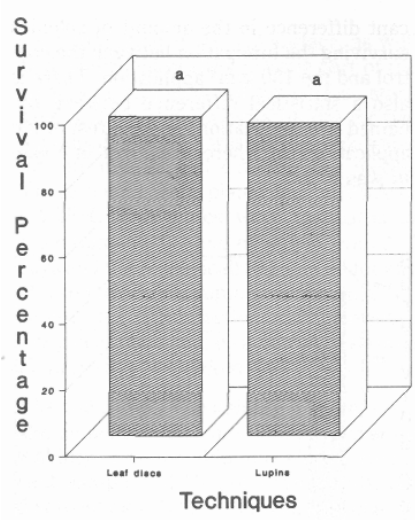

Fig 11 The difference in survival percentage of *P cinnamomi* indicated by two different bait techniques in pine bark. Values indicate the mean surviving percentage of four replicates of the control (0 g/m^3) treatment. Means of different bait techniques followed by the same letter do not differ significantly (P = 0,5287) according to Duncan's multiple range test.

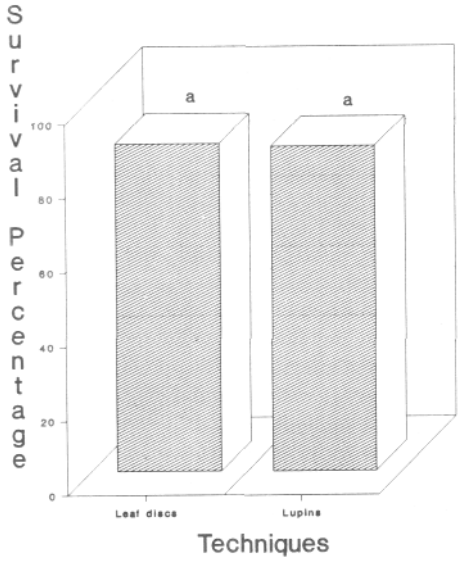

Fig 12 The difference in survival percentage of *P cinnamomi* indicated by two different bait techniques in mila. Values indicate the mean surviving percentage of four replicates of the control (0 g/m^3) treatment. Means of different bait techniques followed by the same letter do not differ significantly (P = 0,8960) according to Duncan's multiple range test.

Figure 7 shows the difference in the mean values of surviving colonies of *F solani* in pine bark at the two different depths for the 150 g/m^3 treatment. The same information is depicted in Figure 8, with mila used as nursery medium.

The survival of *P cinnamomi* was evaluated as described earlier and plotted as a percentage. No survival was observed for any of the bins fumigated and therefore only the control bin's values were used in the evaluation. This will be discussed thoroughly under the discussion.

The difference in percentage survival in the control bin (0 g/m^3) for the top and the bottom layers is shown in Figure 9 for the pine bark medium. The same information is depicted in Figure 10 with the difference that the fungus samples were placed in mila medium.

Figure 11 shows the two bait techniques used for the pine bark medium. Statistically, there is no difference in the survival percentage indicated by the two different techniques. Figure 12 depicts the bait techniques as used for the mila medium. There is no statistical difference in the survival percentage indication.

DISCUSSION

No survival of *F solani* was found in any fumigation higher than 150 g/m^3. This indicates effective fumigation and the sensitivity of *F solani* at concentrations higher than 150 g/m^3 applied to pine bark nursery medium (Figure 3).

The mila medium's result as depicted in Figure 4 shows no statistical difference between the control and the 150 g/m^3 fumigation for survival of *F solani.* There is a difference between the named two and the rest of the treatments. This indicates that in the mila medium more colony forming units (cfu) of *F solani* survived the 150 g/m^3 treatment. This could be the result of lower temperatures obtained in the medium during fumigation.

The lateral movement of methyl bromide for the 150 g/m^3 treatment of *F solani* is evaluated in Figure 5 for the pine bark medium. There was no statistical difference between the three different locations of fungus samples. This indicates an effective lateral diffusion of methyl bromide and can be the result of the raised plastic cover. The same result was obtained for the mila medium according to Figure 6. No statistical differences between the three locations also indicate an effective lateral movement of methyl bromide.

Downward movement of methyl bromide was evaluated for the 150 g/m^3 treatment *of F solani* in pine bark and shown in Figure 7. There was no statistical difference in the amount of colonies surviving the fumigation in the top and the bottom layers. This clearly indicates even distribution of methyl bromide due to gravitation and mass flow (Brown & Rolston, 1980). The same result was obtained for the 150 g/m^3 treatment in the mila medium according to Figure 8. There was also no statistical difference between the top and bottom layers.

P cinnamomi did not survive any of the fumigations with MBr and only the results obtained from the control treatment was used in the statistical analysis. It was found

during laboratory experiments conducted by Munnecke *et al* (1978) that *Pc* chlamydospores were less resistant to MBr than mycelium and infected roots. This was done using a flow chamber and the fungus was exposed to gaseous MBr in compressed air.

LD90 values were determined when exposed for 12 hours to a certain concentration of methyl bromide (Munnecke *et al,* 1978). The LD90 value for chlamydospores was obtained at about 8 000 µℓ MBr ℓ air. The LD90 values for infected roots and mycelium were 11 500-11 900 µℓ MBr /ℓ air.

There was a difference in the percentage of *Pc* surviving in the top and the bottom layers of the control bin where no MBr was applied. Figure 9 clearly shows a statistical difference between the top and the bottom layer for the pine bark medium. The top layer had a lower percentage of *Pc* surviving than the bottom layer.

This could be the result of high temperatures in the medium under the transparent plastic (Table 1). The same result is depicted in Figure 10 for the mila medium, with a definite statistical difference between the top and bottom layers. Fräser fir seedlings were used in an experiment to evaluate hot water treatments for eradicating *P cinnamomi* from the roots (Benson, 1978). It was found that 100% inactivation of *Pc* was obtained at 39°C with a hot water dip treatment for 90 min. The time was decreased to 4,5 min for treatment at 44°C. This is an indication of thermal inactivation of *Pc* by soil solarization obtained under the transparent plastic. It was also found by Pinkas *et al* (1974) that *Pc* was inactivated with solarization and that only 10% of the pathogen propagules in naturally infested soil survived heating for four hours at 36°C.

The two different bait techniques used to detect any *Pc* survival were statistically analysed and shown for the pine bark medium in Figure 11 and the mila medium in Figure 12. There was no statistical difference in the survival percentage detected by the two different techniques.

It is important to keep a certain amount of MBr under the plastic cover for a certain period of time. This is the whole basis of fumigation with methyl bromide (Quayle & Knight, 1921). The CT value which is the result of concentration x time indicates that the higher the concentration, the shorter the period of exposure to MBr needed for the same result. The LD90 value for *Pc* infected roots for a concentration of 5 900 ppm was obtained after 27 hours (Munnecke *et al,* 1974) while the LD90 value at a concentration of 11 500 ppm was obtained after 12 hours (Munnecke *et al,* 1978).

The use of transparent plastic is important not only because of its lesser permeability to MBr than coloured plastics (Code of practice for the application of fumigants, SABS), but also for the greenhouse effect that results (Mahrer, 1979).

Apart from the ability of high soil temperatures to kill fungal pathogens, it also benefits the distribution of MBr in the nursery soil. This was clearly illustrated when *Pythium ultimum* growing on agar discs was exposed to a MBr concentration of 25 000 $\mu\ell$ MBr/ℓ air. The LD90 value was obtained after nine hours at 5°C and after about 2,5 hours at 30°C (Munnecke & Bricker, 1978).

REFERENCES

BENSON, D M 1978. Thermal inactivation of *Phytophthora cinnamomi* for control of Fräser *Fir root rot. Phytopathology.* 68,1 373 - 1 376.

BROWN, R D & ROLSTON, D E, 1980. Transport and transformation of methyl bromide in soils. *Soil science.* 130(2), 68 - 75.

DARVAS, J M, 1979. Lupine bait technique for the semi-quantitative analysis of *Phytophthora cinnamomi* and other root pathogens in avocado soils. S *A Avocado Growers' Assoc Yrb* 3, 29 - 30.

GRIMM, G R & ALEXANDER, A F, 1973. Citrus leaf pieces as traps for *Phytophthora parasítica* from soil slurries. *Phytopathology* 63, 540 - 541.

KOTZE, J M 1985. Strategy for combating avocado root rot. S *A Avocado Growers' Assoc Yrb* 8, 13 - 14.

KOTZÉ, J M, MOLL, J N & DARVAS, J M 1987. Root rot control in South Africa: Past, present and future. *S A Avocado Growers' Assoc Yrb* 10, 89 - 91.

MAAS, E M C & KOTZÉ, J M, 1990. Recommendations for the control of root rot. S *A Avocado Growers' Assoc Yrb* 13, 6 - 7.

MAHRER, Y 1979. Prediction of soil temperatures of a soil mulched with transparent polyethylene. *Journal of applied meteorology* 18, 1263 - 1267.

MIRCETICH, S M, ZENTMYER, G A & KENDRICK, J B Jr 1968. Physiology of germination of chlamydospores of *Phytophthora cinnamomi. Phytopathology* 58, 666 - 671.

MITCHELL, D J, KANNWISCHER-MITCHELL, M E & ZENTMYER, G A 1986. Isolating, identifying and producing inoculum *oí Phytophthora* spp. Pages 63-66 in: Methods for Evaluating Pesticides for Control of Plant Pathogens. K D. Dickey, ed. APS Press, St. Paul, MN. 312pp.

MUNRO, HAU 1964. Manual of fumigation for insect control, pp 294 in FAD Studies no. 56.

MUNNECKE, D E & FERGUSON, J 1953. Methyl bromide for nursery soil fumigation. *Phytopathology* 43, 375 - 377.

MUNNECKE, D E & BRICKER, J L 1978. Effect of temperature on response of *Pythium ultimum* to methyl bromide. *Plant disease reporter* 62(7), 628 - 629.

MUNNECKE, D E, BRICKER, J L & KOLBEZEN, M J 1974. Dosage response of *Phytophthora cinnamomi* to methyl bromide. *Phytopathology* 64, 1 007 - 1 009.

MUNNECKE, D E, BRICKER, J L & KOLBEZEN, M J 1978. Comparative toxicity of gaseous methyl bromide to ten soilborne phytopathogenic fungi. *Phytopathology* 68, 1210 - 1216.

PARTRIDGE, C J 1984. Plant improvement scheme-long term planning. S *A Avocado Growers' Assoc Yrb 7,* 23.

PINKAS, Y, KARIV, A & KATAN, J, 1984. Soil solarization for the control of *Phytophthora cinnamomi:* thermal and biological effects. *Phytopathology* 74, 796 (abstr).

QUAYLE, H J & KNIGHT, H 1921. The use of gas-tight fumigation covers. *The California citrograph* (April) 196 - 197.

TIMMER, L W & LEYDEN, R F 1978. Relationship of seedbed fertilization and fumigation to infection of sour orange seedlings by *Mycorrhizal* fungi and *Phytophthora parasítica. J Amer Soc Hort Sei* 103(4), 537 - 541.

VAN WYK, P S, SCHOLTZ, D J & LOS, 0 1986. A selective medium for the isolation of *Fusarium* spp. from soil debris. *Phytophylactica* 18, 67 - 69.

VERMEUELEN, J B, SWEET, S, KRAUSE, M, HOLLINGS, N & NEL, A 1990. *A guide to use of pesticides and fungicides in the Republic of South Africa.* Department of Agricultural development, p32.