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Project number PC 57 a

Project title: Control of *Puccinia horiana* Henn.(white rust) on chrysanthemum with *Verticillium lecanii* (Zimm.) Viégas

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Key words: Chrysanthemum, Integrated Pest Management, pesticides, aphid, thrips, whitefly, pesticide compatibility, glasshouse trials, humidity.

Application of results

Verticillium lecanii is a fungal pathogen which is used to control insect pests, it is also reported to infect rusts on several crops including cereal rust, chrysanthemum white rust and coffee rust. This project has evaluated the potential for using *V. lecanii* against white rust on protected chrysanthemum crops. Results showed that commercially available strains of *V. lecanii* could infect white rust although they were unable to prevent or control the spread of the disease. Pesticides compatible with *V. lecanii* include Afugan, Torque and Dimilin, whereas Rovral was moderately safe, Tilt and Repulse were harmful. Results from previous trials (HDC PC/18d) have shown that *V. lecanii* can control several insect pests providing the humidity is high enough. The current work indicates that if white rust should occur it will increase if elevated humidities are being used and that suitable curative fungicides are not, as yet, compatible.

Scope and objective

Chrysanthemum white rust (*Puccinia horiana*) is a major disease and is particularly active during periods of high relative humidity (Rh). To counter these conditions many growers use a crop blackout system made of woven material designed to reduce Rh. These woven screens, by lowering Rh, effectively prevent the use of the insect pathogen *Verticillium lecanii* for controlling insect pests. Current research, funded by the HDC at Littlehampton, has shown that raising Rh under the blackout screen at night by water-fogging will activate *V. lecanii* allowing biological insect control. However, there is obviously concern that raising Rh will increase outbreaks and spread of white rust. Previous work has indicated that *V. lecanii* can grow parasitically on white rust and provide a measure of control. Thus a combined integrated biological pest and disease control programme is a potential possibility, alternately selective fungicides may be integrated to prevent or control white rust.

Summary

From these trials it was shown that commercial strains of *V. lecanii* (Vertalec, Mycotal and Microgermin) were unable to prevent or control white rust spread and development throughout the trial. However, all strains did infect white rust pustules, with Vertalec killing aphids on the same plants. In fact the control glasshouses frequently became heavily infected with *V. lecanii* and on one occasion almost killed the white rust inoculum. Isolates were taken from infected rust pustules and stored for future characterisation and application in the search for a suitable strain. This, if integrated fully with existing commercial products, could be used to prevent and control white rust and glasshouse chrysanthemum pests simultaneously.

Humidity was found to be the key factor in the development of white rust, with much higher levels of infection occurring in houses with humidity in excess of 95%. Nutrients contained within the commercial products of *V.lecanii* to enhance its growth in the absence of a suitable host did not significantly increase infection of white rust. Similarly water sprayed onto leaves each evening to create a 'wet leaf' during the night did not increase infection risk when compared to dry leaves at the same humidity.

Several pesticides were screened for their effect on the fungal pathogens, and a novel test method was developed for the Mycotal strain. Whitefly scales attached to their host leaf were mounted on agar, pesticides could then be applied at various times before and after treatment with *V. lecanii*. The pesticides found to be safe for integration with *V.lecanii* were Afugan (pyrazophos), Torque (fenbutatin oxide), and Dimilin (diflubenzuron) with Rovral (iprodione) moderately safe and Tilt (propiconazole) and Repulse (chlorathalonil) harmful. Koppert (the suppliers of Vertalec and Mycotal) have also screened several pesticides for integration with *V. lecanii*, their results confirm those in this project.

Figure 1 shows the results of a preventive spray against white rust. In this trial clean chrysanthemum plants were sprayed with each of the commercial strains of *V. lecanii*. Immediately after spraying a white rust infected plant was placed amongst them to determine if the *V. lecanii* could grow on the sprayed leaves and prevent infection spreading. The results indicate that no disease prevention was obtained, in fact the white rust was heaviest on the *V. lecanii* sprayed plants than on the water treated plants. This

could be due to there not being enough time to allow the *V. lecanii* spores to germinate before the white rust began to infect the clean plants. Or that nutrients in the *V. lecanii* formulations were encouraging the white rust to grow leading to a more rapid infection than on the water sprayed plants. Another reason could have been simply due differences in environmental conditions amongst the plants.

Because of this result the trial was repeated but time was allowed for the *V. lecanii* spores to germinate also trials were conducted with the nutrients alone as they would have been in the formulated material. Throughout the trial pustules of white rust infected with *V. lecanii* were frequently found and isolates collected for future investigation.

Table 1 shows how ineffective the commercial strains of *V. lecanii* were in controlling white rust when applied to freshly broken (erupting) spores. The mean number of white rust infected leaves on each plant are presented at various times after treatment with *V. lecanii*. The plants were infected with white rust and allowed to develop until the spores were at the point of erupting to produce the next infection. Sprays of *V. lecanii* were applied in an effort to determine how effective the products may be in preventing the established infection from continuing and spreading to new growth.

Action points

Pest control with *V. lecanii* has been shown to give good and reliable results when elevated humidities are used to provide the fungus with necessary conditions for germination and infection. However, the same conditions will exacerbate any white rust infection present in the crop. The approved fungicide for control of this disease on chrysanthemum is propiconazole (Tilt) but it has been shown to be incompatible with *V. lecanii* for about 2 weeks. Other fungicides, suitable for control and prevention of other diseases are compatible providing that no tank mixes are made and that at least 3 days elapse between sprays of *V. lecanii* and pesticide. In general, most insecticides and acaricides are also compatible but none should be tank mixed with *V. lecanii*, the exception being *Bacillus thuringiensis* which can be mixed to control several species of caterpillars. The above summary of results indicates that strains of *V. lecanii* are able to infect white rust, these are being kept in liquid nitrogen at HRI Littlehampton for future work.

Introduction

Puccinia horiana Henn. was first found in England in 1963, (Baker, 1967) having previously been confined to China and Japan.(Hiratsuka, 1957). This fungus causes white rust on chrysanthemum plants and if left unchecked can destroy crops. Despite quarantine measures and a Ministry of Agriculture, Fisheries and Food (MAFF) statutory eradication campaign (Lelliot, 1984) it has continued to reappear. After a 5 year test period, propiconazole proved to be an effective curative fungicide (Dickens, 1990) and with measures to reduce relative humidity in the crop, disease incidence and severity were reduced.

The fungus *Verticillium lecanii* which can be used to control aphids and thrips on chrysanthemum using a system which involves high r.h regimes (Helyer & Chambers, 1991; Helyer, Gill & Bywater, 1992), also has the ability to parasitise a range of rust fungi including *P.horiana* (Whipps, 1992). Both fungi require high humidity for germination to take place. This project examines effectiveness of *Verticillium* to control *P.horiana* using the commercial strains of Vertalec, Mycotal and Microgermin, including integration with some chemicals.

Glasshouse trials conducted during project

1 - Preventive - To determine whether *Verticillium lecanii* can prevent the growth and spread of *Puccinia horiana* on chrysanthemum.

2 - Preventive - To determine whether pre-germinated *V.lecanii* can prevent the growth and spread of *P.horiana* on chrysanthemum.

3 - Control - To determine whether *Verticillium* can control developing *P.horiana* on chrysanthemum.

4 - To determine whether nutrients present in commercial products of *Verticillium* affect the growth and spread of *P.horiana.*

5 - To determine what effect *Verticillium* with nutrients, and nutrients only have on the growth of *P.horiana* with both treatments kept at the same humidity.

6 - To determine whether *P.horiana* growth and spread is increased by water sprayed onto leaves.

Pesticide screening in Laboratory/Glasshouse trials

7 - To determine the effect of the chemicals Afugan, Rovral and Tilt on *V.lecanii.*

8 - To determine the effect of Afugan, Rovral, Tilt, Torque, Dimilin and Repulse have on *V.lecanii* infection of *Trialeurodes vaporiorum* (whitefly) scales.

9 - The effect of Afugan, Rovral, Tilt, Torque, Dimilin and Repulse on the growth of *T.vaporiorum* scales.

10 - To determine the effect of Repulse and Tilt on *V.lecanii* infection of aphids, in the glasshouse.

Materials and Methods

Trial 1

Cuttings of chrysanthemum variety Mundial were planted in pots 11cm square by 12cm deep in compost containing 50:50 peat:grit. Two groups of eight uninfected 40cm tall plants and one group of eight uninfected 25cm tall plants were set out on benches covered with capillary matting. Plots were formed as a 3 x 3 open square in each of eight isolated glasshouses. Water was applied twice daily and plant feed was applied once a week by irrigation, consisting of Nitrogen 200ppm, Potassium 150ppm, Phosphorus 15ppm, Magnesium 20ppm and Iron 1ppm. At the beginning of the trial the tall plants were 11 weeks old, and the small plants 7 weeks old (no pest or disease preventative sprays were applied before the trials began). These plants were then sprayed with either Microgermin at 1.5g per litre, Mycotal at 1g per litre, Vertalec at 2g per litre or water. There were 2 replicates, each in a separate house. A heavily infected white rust plant was then immediately placed in the centre of each group. Humidity was raised each night for 15 hours to greater than 95% Rh by enclosing all the plants in a polyethylene tent. All leaves on the plants were observed and scored for presence of white rust infection regularly.

Trial 2

One glasshouse was used to spray 64 clean plants with either Microgermin, Mycotal, Vertalec or water, all at the same rates as trial 1. All 3 products are formulated with a source of organic nutrients (skimmed milk and barley flour) to aid the growth of *V.lecanii*. Variety, pot sizes, compost and irrigation were all as trial 1. Plants were 9 weeks old and between 30-35 cm high. After spraying, upper and underside of leaves, humidity in the house was raised each night for 5 days, 12 hours at a time, to greater than 95% Rh by a time controlled water spray fogging machine, allowing *V.lecanii* to pre-germinate and grow on the leaves.

After 5 days the plants were transferred to 8 separate glasshouses, 8 plants into each house. Each treatment was replicated in a separate house. A heavily infected white rust plant was immediately placed in the middle of each group. Humidity was raised for 15 hours each night and all leaves on the plants were observed and scored for white rust infection regularly.

Trial 3

Two groups of four white rust infected plants were set out on benches as a 2 x 2 open square in each of six isolated glasshouses. One group in each house was sprayed with either Microgermin, Mycotal or Vertalec. The other group in each house was sprayed with water as a control. There were two replicates of each treatment with one water control in each house.

Once the white rust infected plants had been sprayed with either V.lecanii treatments or the water controls, 4 uninfected plants, were placed amongst them to determine the spread of the disease making it a 3 x 3 open square, bringing the
total number of plants in each group to eight. White rust infected plants were 9 weeks old and 30 cm high when *Verticillium* treatments were applied. The uninfected plants were 7 weeks old and 25 cm high. Humidity was raised each night for 15 hours and all leaves on the plants were observed and scored for white rust.

Trial 4

One group of eight uninfected tall (40 cm) plants and one group of eight uninfected small (25 cm) plants were set out on benches covered with capillary matting. Plots were formed as 3 x 3 open squares in each of eight isolated glasshouses. At the beginning of the trial the tall plants were 11 weeks old and the small plants 7 weeks old. Plants were sprayed with nutrients only and carriers (i.e no live *V.lecanii* spores) from either Mycotal or Vertalec products, skimmed milk or water. Skimmed milk was at 1.5 g per litre, the other treatments were at the same rate as trial 1. There were 2 replicates of each treatment. An infected white rust plant was then placed in the centre of each group. Humidity was raised each night for 15 hours and leaves were scored for white rust infection.

Trial 5

Three groups of eight healthy plants were set out on benches as a 3 X 3 open square in each of six isolated glasshouses. Plants were 40 cm tall and 11 weeks old. One group of plants in each house was sprayed with either Vertalec or Mycotal, one with nutrients only from Vertalec or Mycotal and one with water as a control. There were three replicates of each treatment with one water control in each house. A heavily infected white rust plant was placed in the centre of each group. Humidity was raised each night for 15 hours for four days and then raised 24 hours a day for the other three. This was done to speed up any white rust spread that was occurring. Leaves were scored for white rust infection.

Trial 6

Two groups of 13 healthy, uninfected (with white rust) 50 cm tall plants were set out on benches with capillary matting in each of 6 isolated glasshouses. Plots were formed as a 5 x 3 rectangle, each with two open spaces into which infected white rust plants were placed, making a total of 15 plants in each group. Plants were 40 cm tall and 13 weeks old when used.

One group of plants in each house were sprayed every evening with water on the upper and underside of leaves with the other group left dry. Humidity was then raised for 15 hours throughout the night every night. Leaves were scored on sample dates for white rust infection and any differences between the wet and dry leaves monitored.

Trial 7

Petri dishes containing Potato Dextrose Agar, 39 g per litre) and Aureomycin (19.19 mg per litre) were sprayed through a Potter tower with 1.5 ml of either Vertalec (2 g per litre/100%strength), Mycotal (0.01 g per litre/0.01% strength) or water. One day later 1.5 ml of the fungicides Afugan (0.5 ml per litre),Rovral (1 g per litre) and Tilt (0.4 ml per litre + 0.1 ml per litre of Pbi spreader) plus a water control were each sprayed separately through a Potter tower onto the *V.lecanii* and water treated Petri dishes. Three replicates were set up for each treatment. Chemicals were also sprayed, onto other *V.lecanii* and water control plates after 3 and 5 days. This was repeated but in reverse with Vertalec, Mycotal and water sprayed onto dishes, 1, 3 and 5 days after being sprayed with chemicals. Petri dishes were then kept in an incubator at 22°C and observations and a score made every0 day for presence of *V.lecanii* colonies. At first, presence or absence of colonies was noted and then levels of growth from 3 for the highest amount of growth down to 0 for no growth. Levels were estimated by area of plate covered by *V.lecanii* and measured by comparing plates with each other (see Table 2). A separate number of plates were also sprayed with water or chemicals only as a guide to the background population of

microorganisms present in the atmosphere at the time that could inhibit *V.lecanii* growth.

Trial 8

Sections of tobacco leaves with a known number of *Trialeurodes vaporariorum* (whitefly) scales were placed on agar (agar technical 15 g per litre) just at the point of setting in Petri dishes. 1.5 ml of Mycotal (1 g per litre/100% strength) was then sprayed through a Potter tower onto all plates. Three days later 1.5 ml of the chemicals Afugan, Rovral and Tilt at the same rates as trial 7 and Torque (0.5 g per litre), Dimilin (0.3 g/l), Repulse (2.2 ml/l) and 2 water controls were sprayed separately through the Potter tower onto the same plates, with 3 replicates of each. There were 2 tests (at different times) with 3 chemicals tested and 1 water control in each. This was also repeated in reverse (as in trial 7) with chemicals being sprayed 3 days before Mycotal. Plates were kept at 23°C with 16 hours daylight. Regular observations were made and scales infected with Mycotal were counted and means calculated from the 3 replicates.

Trial 9

Sections of tobacco leaves with a known number of whitefly scales were cut out and placed on molten agar in Petri dishes, as in trial 8. Thirty scales were chosen at random from each section on each plate and measured. Mycotal was then applied (as trial 8) to 3 different plates. Afugan, Rovral and Tilt were then also applied (as trial 8) in the same way, with 3 replicates of each. Plates were kept under the same conditions as trial 8 and measurements made 8-9 days later. After a further 7 days emergence of adult flies from scales was counted. There were 2 tests (at different times) with 3 chemicals tested plus 1 water control in each. Results were calculated from the means of the 3 replicates.

Trial 10

Two groups of 13 uninfected (with white rust) 50 cm tall plants were set out on benches with capillary matting in each of 6 isolated glasshouses. All plants were heavily infested with healthy aphids (*Aphis gossypii*). Plots were formed as a 5 x 3 rectangle, each with two open spaces, into which infected white rust plants were placed, making a total of 15 plants in each group.

Repulse (2.2 ml per litre), Tilt (as trial 7) and a water control were applied to 2 houses each. After 3 days Vertalec (as trial 7) was then applied to 3 of the houses, 1 to each treatment, and then after 6 days to the remaining 3. This method was repeated in a separate experiment with Vertalec being applied 11 and 14 days after the treatments. Sampling involved collecting 10 leaves from each group of plants and counting aphids alive and aphids infected with *V.lecanii.*

Results and Discussion

Trial 1 (Fig 1)

Pre-treatment of healthy chrysanthemum plants with commercial formulations of *V.lecanii* failed to prevent subsequent infection by *P.horiana* and white rust development. A faster rate of infection occurred on the *V.lecanii* sprayed plants than the water control. Although *V.lecanii* was found to have infected white rust on all plants in all of the houses, including the controls, it did not eradicate it.

On day 6 (13-7, Fig 1), white rust levels were lower in the *V.lecanii* treated houses than the control houses. Infection then spread rapidly in the treated houses with the spread slower in the control. Fourteen days after the treatments were applied in the *V.lecanii* sprayed houses, all plants were heavily infected with white rust. It took an extra 20 days for infection to reach the same level in the control houses, at which time the trial was terminated. Small plants were more susceptible to infection than large plants. All leaves were infected at the same rate on small plants but on the large plants, new young leaves quickly became infected, whereas old leaves were more resistant. In all cases white rust infection was higher in the middle to the top half of the plants, with the lower leaves more resistant. Aphids on plants in all the houses eventually became infected with *V.lecanii*, an indicator of its presence and also a possible reason for its spread into the control houses later the white rust stock house. It was thought that a variety of reasons could have accounted for the heavy infection of white rust on all *V.lecanii* sprayed plants with a less and a later appearance on the water sprayed controls -

a) The *V. lecanii* spores did not have enough time to germinate on the leaves before white rust spores began to infect the plants.

b) The nutrients in the formulated products encouraged the rust spores to germinate faster than the water controls leading to more rapid disease development in the presence of the *V.lecanii* preparations.

c) Different temperature and humidity conditions occurred in the separate houses leading to different patterns of white rust infection.

Trial 2 (Fig 2)
In trial 1 white rust infected plants were introduced to *V. lecanii* sprayed plants at the same time. No control of white rust occurred and the disease spread to all plants. This trial aimed to determine whether *V. lecanii* which had been allowed to pre-germinate on the leaves prior to being exposed to white rust, could prevent its growth and spread. No prevention or control of white rust was achieved by the pre-germinated *V. lecanii*. As in trial 1 a faster rate of infection occurred on the *V. lecanii* sprayed plants than the water control, but not at such a high level. Infection took 2 days longer than trial 1 to reach an equivalent level. Twenty-three days after plants were exposed to white rust and 28 days after the spray treatments, the trial was stopped. *V. lecanii* was growing on the white rust but not eradicating it, and the sites of infection on the plants were the same as in trial 1.

Trial 3 (Table 1)
In trials 1 and 2 *V. lecanii* was sprayed onto healthy plants in an attempt to prevent subsequent white rust infection, growth and spread. No disease control was achieved but *V. lecanii* was found to be growing on white rust pustules. This trial aimed to determine whether *V. lecanii* sprayed directly onto freshly broken white rust pustules could control disease.

No control was achieved and white rust infection continued to increase on all white rust infected plants and spread to adjacent uninfected plants. By the end of trial 3, *V. lecanii* was eventually found to have infected the white rust in all houses, including the "clean" white rust stock house. Isolates were collected, taken mainly from the stock and control

houses, with a few from Mycotal and Vertalec houses, (Microgermin strains were largely inactive) and were sub-cultured and stored for future characterisation testing and application.

V. lecanii spread rapidly through the rust colonies and no uninfected white rust could be found. Nevertheless, white rust colonies continued to develop and erupt, despite the presence of *V. lecanii* on the oldest pustules. All the houses were cleared and fumigated with formaldehyde, before re-starting with a fresh supply of white rust.

Trial 4 (Fig 3a,3b)

In trials 1 and 2, plants that had been sprayed with *V.lecanii* showed a higher level of white rust growth and spread than the water control sprayed plants. This trial aimed to determine whether the nutrients contained within *V.lecanii* products had an effect on white rust growth.

No definite differences were found between the development of white rust in the *V.lecanii* nutrient sprayed houses and the water control sprayed houses. However, control replicate 2 showed a higher level of infection than control replicate 1 and Mycotal nutrients replicate 2 showed a higher level of infection than Mycotal replicate 1. Also, infection in Mycotal replicate 2 was higher than in any other house (these differences could be due to differing humidity conditions).*V.lecanii* was not observed in any of the nutrient sprayed houses. As in previous trials small plants were more susceptible to white rust infection than large plants. Large leaves on the large plants were mainly resistant to white rust infection (apart from Mycotal.replicate 2), whereas all young leaves on the large plants became infected. Sites of infection on plants were the same as in trial 1.

Isolates of *V.lecanii*, *Acremonium*, *Cladosporium* and*Botrytis* growing on the white rust were taken from two separate white rust stock houses. These were isolated onto P.D.A / Aureomycin plates and then sub-cultured onto slopes for identification and possible future application.

Trial 5 (Fig 4a,4b)

In previous trials higher levels of white rust growth have been noticed in houses with higher levels of humidity. To determine the effect *V. lecanii* and the nutrients contained within *V. lecanii* have on the growth of white rust this trial aimed to eliminate the different humidity conditions plants would be under in separate houses by applying three different treatments to three groups of plants within the same house. No differences in white rust infection were observed between the separate treatments throughout the trial with all plants becoming infected. However, there was a higher rate of white rust infection in the Mycotal houses than the Vertalec. In each house the *V. lecanii* and nutrient sprayed plants showed a slightly higher rate of infection than the nutrients only and water control sprayed plants, with the nutrients only showing a slightly higher level of infection than the water. No control of white rust was noticed in any of the houses.

Trial 6 (Fig 5)

There was a constant, slightly higher rate of white rust infection noticed on the plants sprayed with water. However, overall, no definite differences in the levels of white rust infection were noticed between the wet and dry plants.

In both Vertalec sprayed houses and the Mycotal 1 sprayed house there was a slightly higher rate of white rust infection on the plants sprayed with water than on the

plants left dry. In the Mycotal 2 and control house 1, white rust infection was slightly higher on the dry plants, apart from the last sample in the control house when the wet leaves became more infected. In the other control house infection was slightly higher on the wet leaves, apart from the last sample when infection became slightly higher on the dry.

Trial 7 (Table 2)
Rovral and Tilt did not affect growth of *V. lecanii* 5 days after Vertalec had been applied and 3 - 5 days after Mycotal. When Rovral and Tilt were applied before any *V. lecanii* it was found to be more harmful and at no time were they safe to use with growth of *V. lecanii* suppressed for up to 5 days. Afugan had a slight effect when applied before *V. lecanii* but this could have been due to yeasts and fungi inhibiting its growth. Apart from this Afugan was safe to use at any stage and had no effect on the growth of *V. lecanii*.

The Table 2 shows mean levels of growth 7 and 14 days after application of *V. lecanii*. Humidity could not be measured in the Petri dishes but condensation was always present, indicating high humidity all the time and perfect conditions for *V. lecanii* growth. Water or chemical only sprayed plates had different yeasts and fungi growing on them, except on the Tilt sprayed plates and to a lesser degree the Rovral plates. This could have inhibited the growth of *V. lecanii*.

Trial 8 (Table 3)
Torque and Dimilin had no effect on Mycotal infection of whitefly scales. Afugan and Rovral were found to be moderately safe but in an inconclusive test due to low levels of infection in the controls. Tilt and Repulse were found to be harmful.

In the first test when Mycotal was sprayed before the chemicals, Afugan and Rovral did have an effect, while Tilt prevented Mycotal infection completely. This contradicted slightly with trial 7 where Afugan did not affect growth of *V. lecanii*. When the chemicals were sprayed before Mycotal, Afugan and Rovral did not affect infection of whitefly scales but Tilt was found to prevent *V. lecanii* infection. The second test showed Torque and Dimilin not to affect infection of whitefly scales in either, with slightly less levels of infection though, when Dimilin was sprayed before Mycotal. Repulse was found to decrease infection of whitefly scales in both cases, especially when applied before Mycotal.

Trial 9 (Table 4)
From this trial Afugan and Rovral did not affect development of whitefly scales. Torque had some effect and Tilt, Dimilin and Repulse were harmful.

Trial 10 (Fig 6a,6b)
Lower numbers of aphids infected with *V.lecanii* were found at all times on plants that had been sprayed with Repulse and Tilt, previous to *V.lecanii*, with less after 3 or 6 days. Infection of aphids by *V. lecanii* was higher when sprayed 11 or 14 days after the treatments, but was still less than the controls where there was 100% infection. *V. lecanii* was found to be growing on white rust pustules, but growth and spread occurred in all cases except on plants sprayed with Tilt, where it was almost completely suppressed.

Acknowledgements
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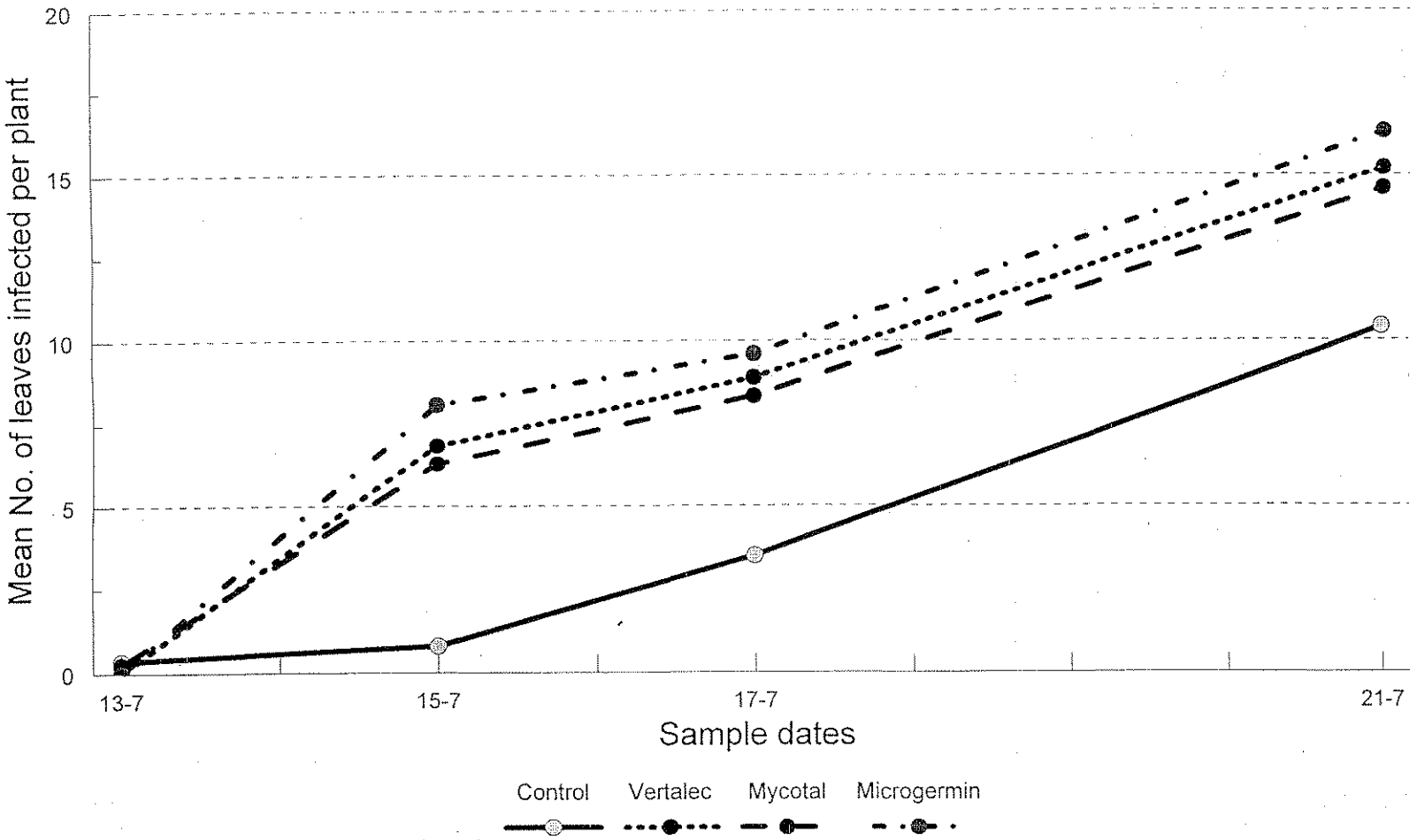
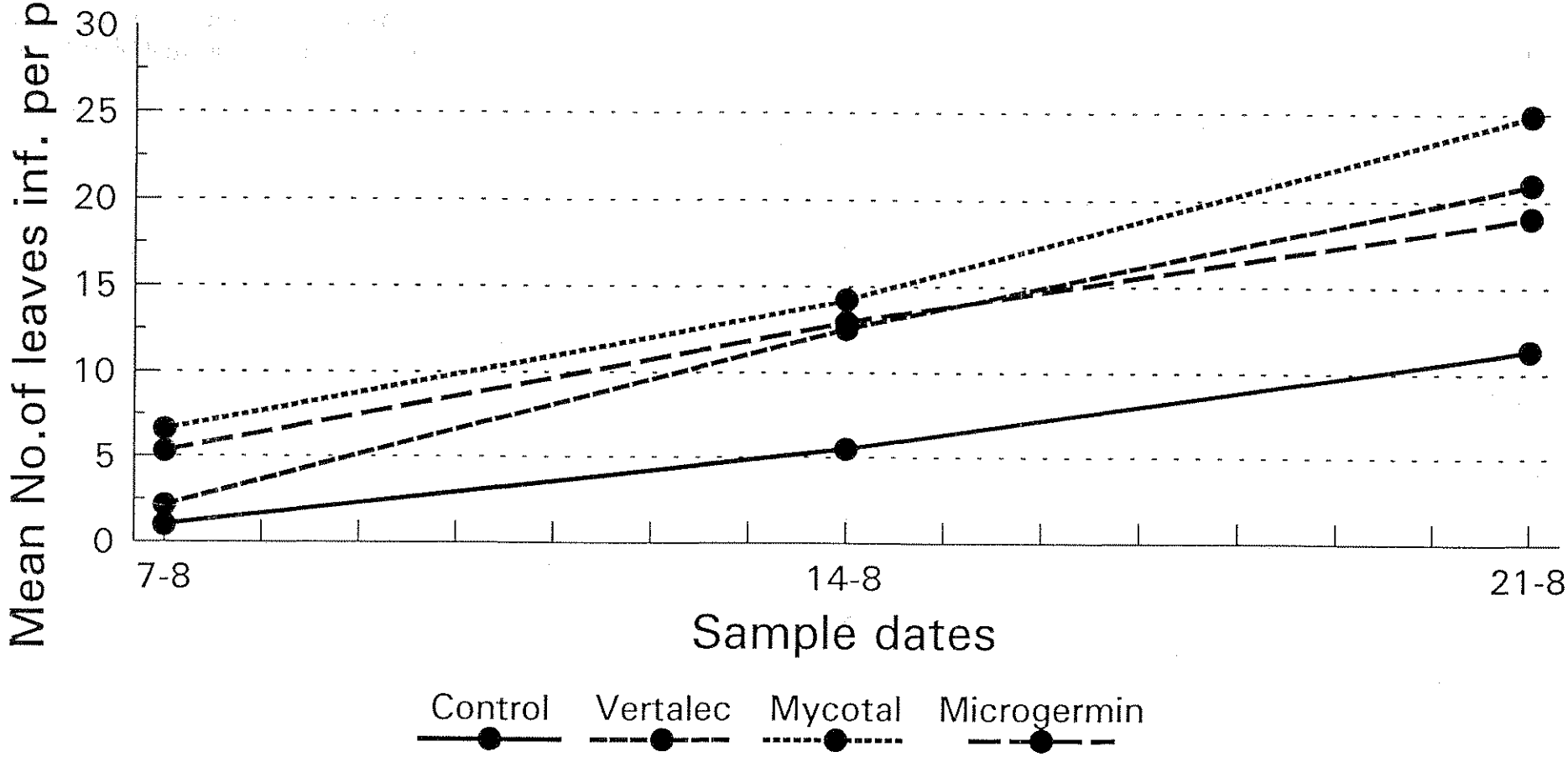
Fig 1 - Trial 1 - Preventative treatment of *V. lecanii* against white rust. Mean numbers of leaves infected with white rust per plant, after application of treatments.

Note that infection is greater in all the *V.lecanii* treated houses than the control



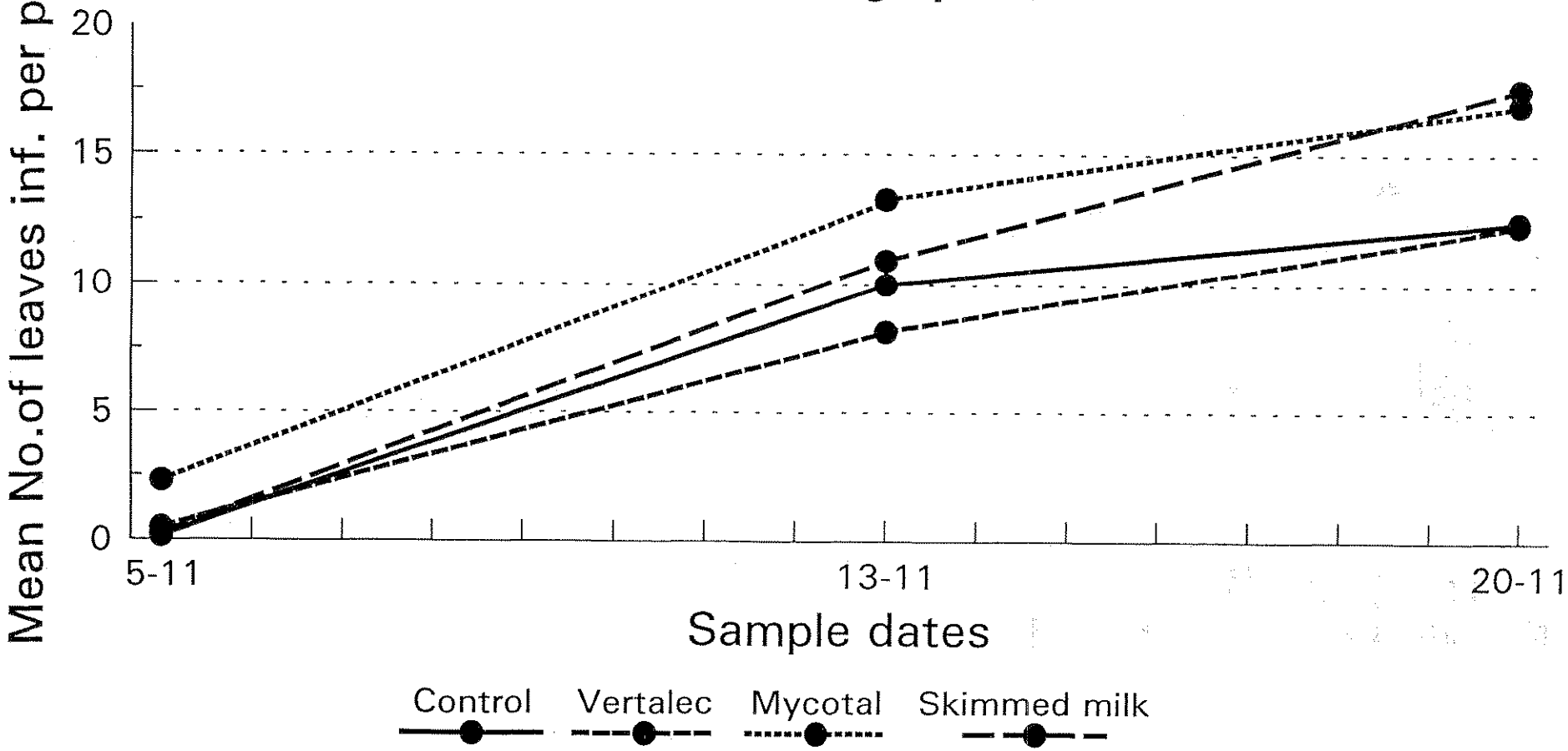
Date of V.lecanii treatment - 7/7/92. Number of leaves at final sample - Control 30. Vertalec 25. Mycotal 25. Microgermin 25.

Fig 2 - Mean numbers of leaves infected with white rust per plant, after application of treatments.



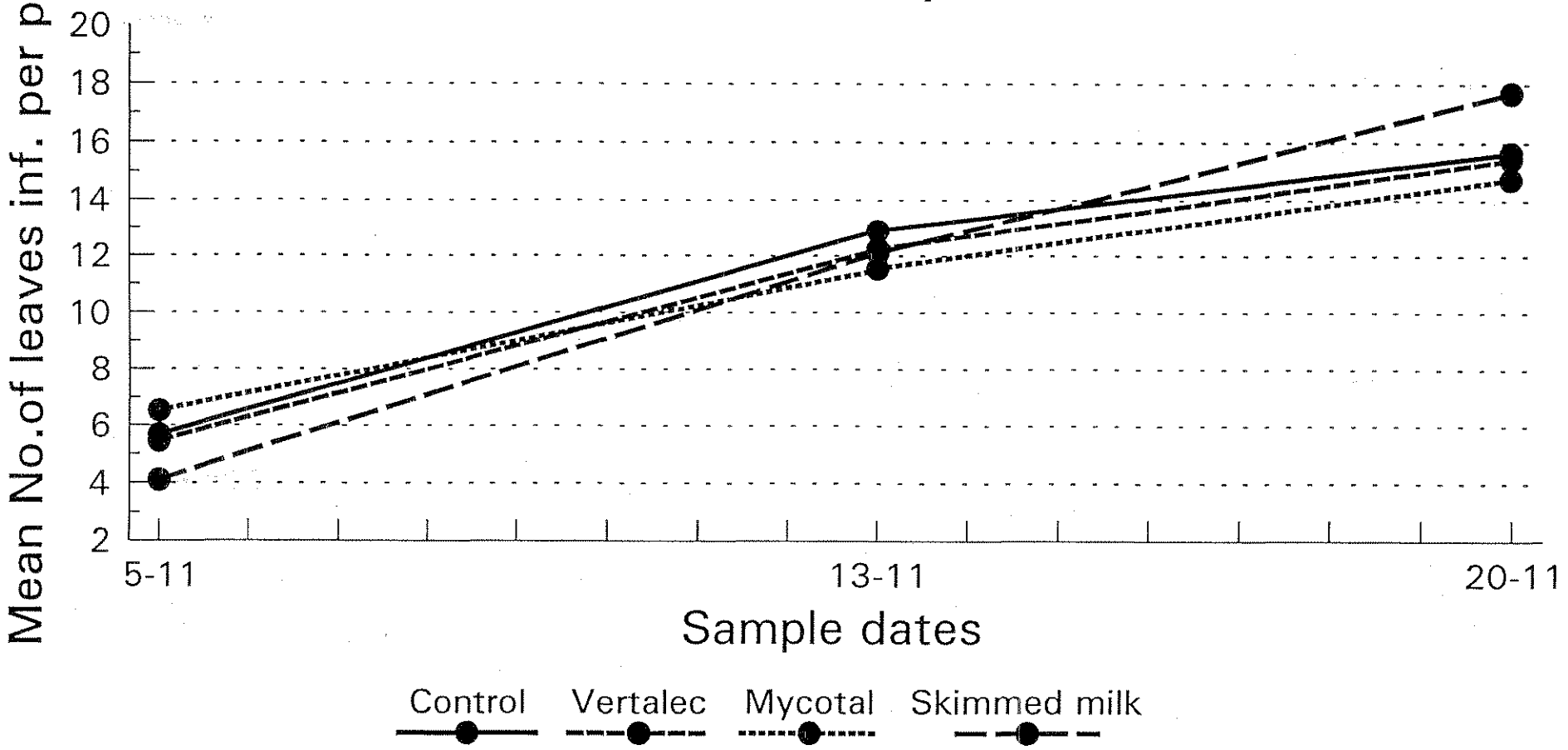
Date of initial V.lecanii treatments - 24/7/92
Date sprayed plants introduced - 29/7/92
Number of leaves per plant at final sample - 55

Fig 3a - Mean numbers of leaves infected with white rust per plant, after application of treatments. - Large plants.



Date of V.lecanii treatments -19/10/92 - 9/11/92
Number of leaves per plant at samples -19,28,31

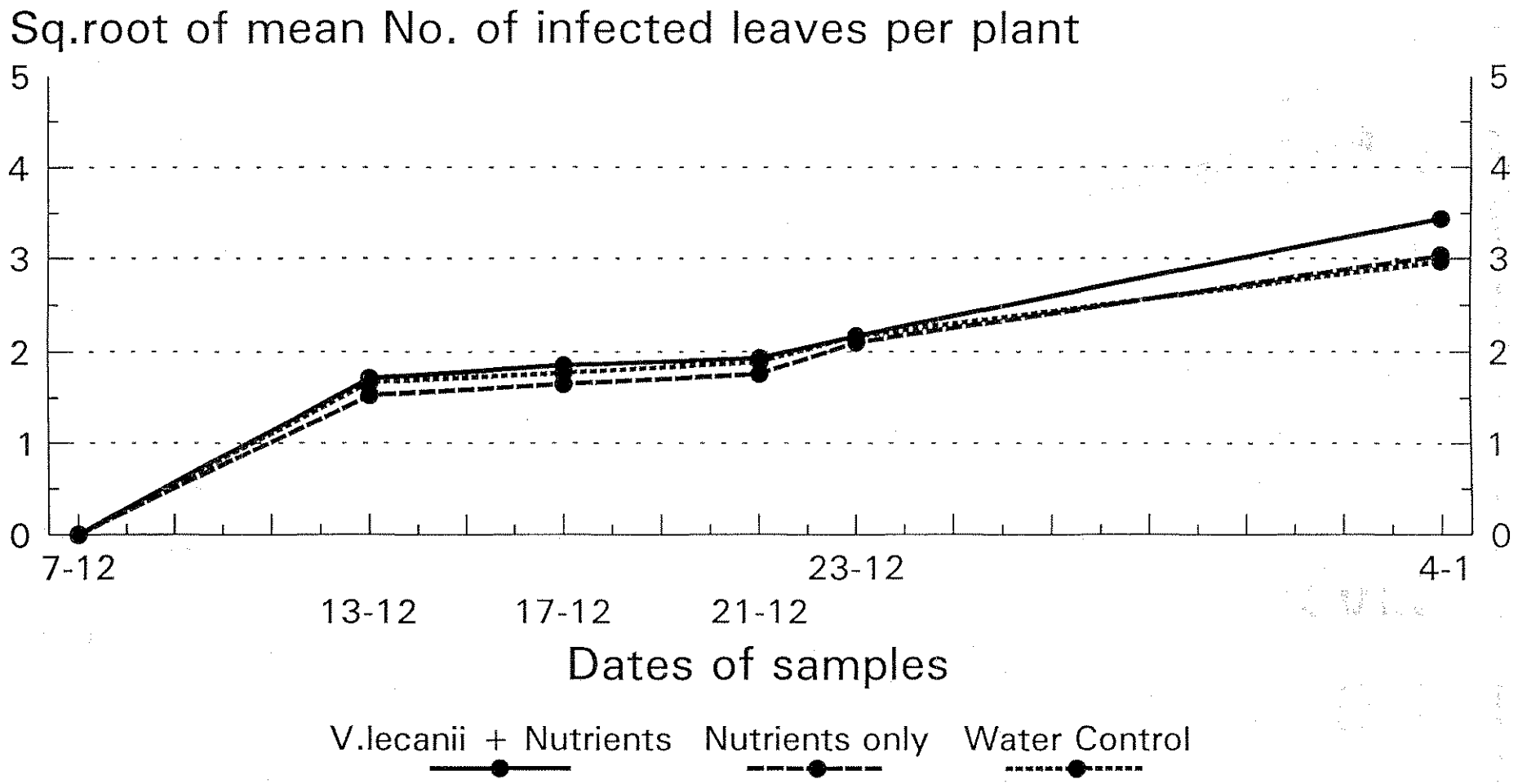
Fig 3b - Mean numbers of leaves infected with white rust per plant, after application of treatments. - Small plants.



Date of V.lecanii treatments -19/10/92 - 9/11/92
Number of leaves per plant at samples -19,23,26

Fig 4a - Vertalec - Mean numbers of leaves

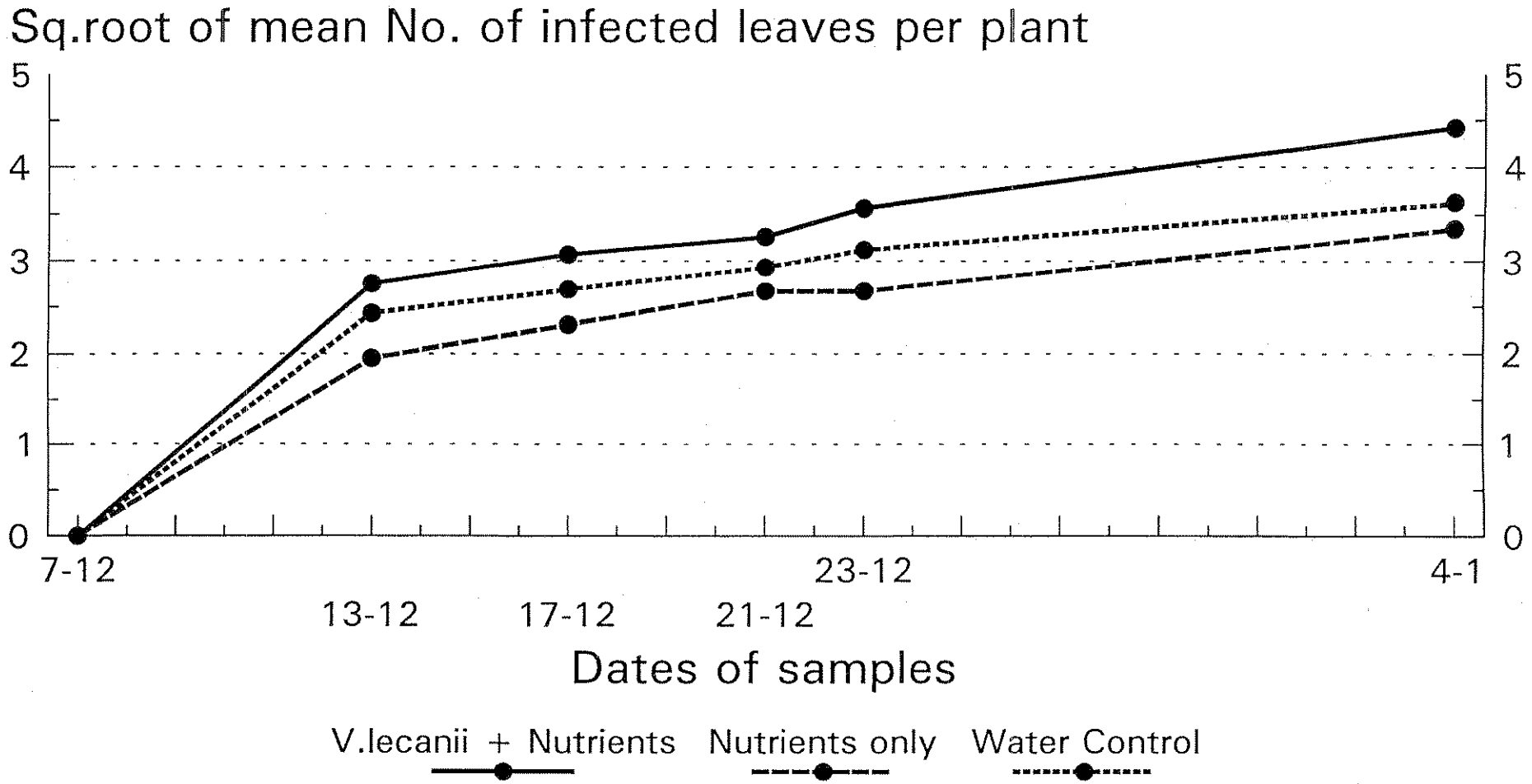
infected with white rust per plant, after application of treatments.



Dates of treatments - 27/11 - 18/12

Fig 4b - Mycotal - Mean numbers of leaves

infected with white rust per plant, after application of treatments.



Dates of treatments - 27/11 - 18/12

Fig 5 - Mean numbers of leaves infected with white rust per plant.

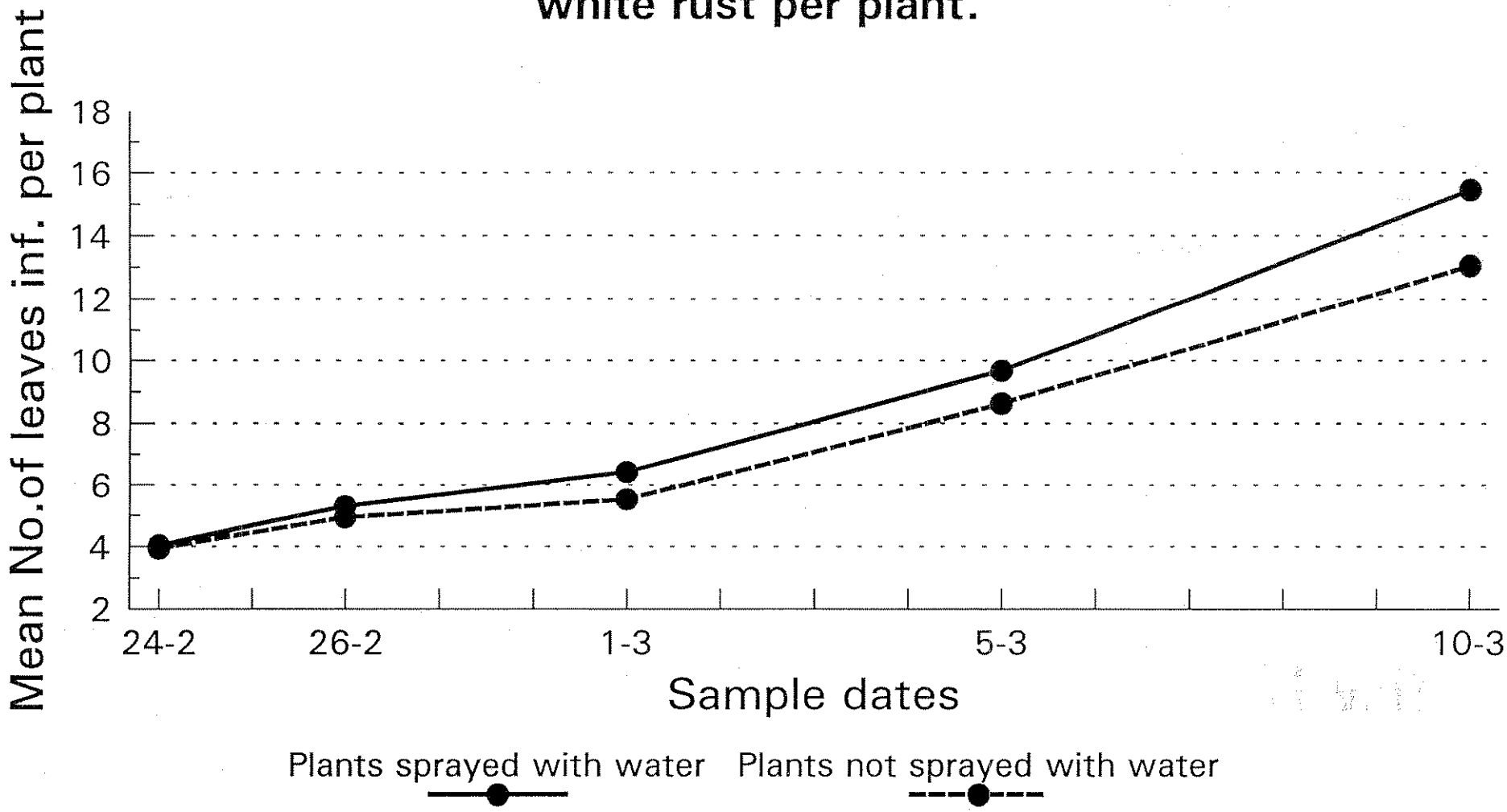
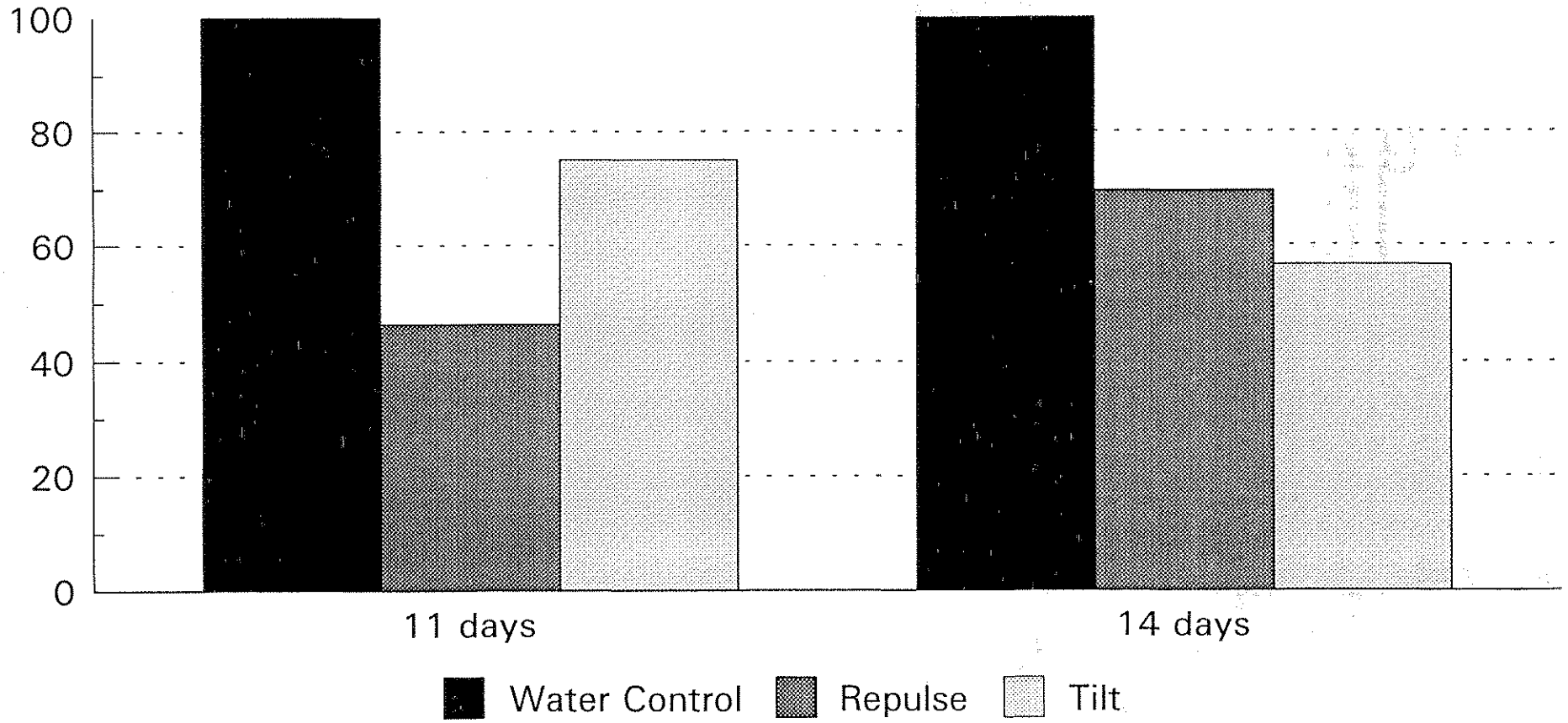


Fig 6b - Mean percentages of aphids infected

with Vertalec, after Vertalec was sprayed onto plants 11 and 14 days after treatments.



Final sample - 19 and 16 days after Vertalec applications

Table 1. **Results of Trial 3 where *V. lecanii* was applied to freshly broken white rust pustules: Mean numbers of leaves infected with white rust per plant, comparing treatments with control**

Sample Dates	*V. lecanii* Formulation		Control
29-7	Vertalec	2.3	4.0
	Mycotal	3.3	5.8
	Microgermin	6.0	5.6
3-8	Vertalec	17.6	15.4
	Mycotal	12.2	12.5
	Microgermin	14.4	14.5
7-8	Vertalec	24.4	22.4
	Mycotal	19.7	19.9
	Microgermin	21.9	21.9

Date of *V. lecanii* treatments - 22/7/92
Number of leaves on plants at final sample - 35

Table 3

Percentage levels of whitefly scales infected with *V.lecanii* after Mycotal had been applied 3 days before and after applications of Afugan, Rovral, Tilt and a water control.

Percentage of whitefly scales infected with Mycotal - 4/3/93

Mycotal sprayed	- 22/2/93	**Chemical sprayed**	- 22/2/93
Chemical sprayed	- 25/2/93	**Mycotal sprayed**	- 25/2/93
Control	- 23.8 %		30.8 %
Afugan	- 6.05 %		21.4 %
Rovral	- 5.99 %		31.4 %
Tilt	- 0 %		4.6 %

Percentage levels of whitefly scales infected with *V.lecanii* after Mycotal had been applied 3 days before and after applications of Torque, Dimilin, Repulse and a water control.

Percentage of whitefly scales infected with Mycotal - 12/3/93

Mycotal sprayed	- 2/3/93	**Chemical sprayed**	- 2/3/93
Chemical sprayed	- 5/3/93	**Mycotal sprayed**	- 5/3/93
Control	- 66.9 %		50.4 %
Torque	- 57.7 %		54.2 %
Dimilin	- 72.8 %		33.7 %
Repulse	- 11.8 %		0 %

Table 4. The effect of Afugan, Rovral, Tilt, Torque, Dimilin, Repulse and water controls on the growth of whitefly scales.

	Mean scale size	Mean scale size	% Emergence of whitefly
	22/2/93	3/3/93	11/3/93
Control	0.36	0.57	39.5 %
Afugan	0.37	0.51	32.5 %
Rovral	0.39	0.51	39.5 %
Tilt	0.39	0.42	7.5 %
	2/3/93	10/3/93	17/3/93
Control	0.4	0.57	76.7 %
Torque	0.4	0.56	35.3 %
Dimilin	0.42	0.53	9.9 %
Repulse	0.425	0.49	6.9 %