CONTROL OF CHRYSANTHEMUM PESTS WITH *VERTICILLIUM LECANII*

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ABSTRACT

Integrated pest control on chrysanthemum is once again a possibility. *Verticillium lecanii* is one of the key components as, under the right conditions, it will provide good control of pests such as aphids, thrips and whitefly. High humidity for a number of nights per week is critical for reliable pest control with this fungal pathogen. These conditions can be easily and safely obtained by fogging water over the crop at night. Four consecutive nights of high humidity per week or a cycling 2 nights of high humidity and 2 nights ambient have given excellent pest control with no adverse effect to the crop.

INTRODUCTION

The insect pathogenic fungus *Verticillium lecanii* can control several aphid species (Hall and Burges, 1979), western flower thrips (Helyer and Brobyn, 1988) and whitefly (Hall, 1982) on a range of crops. However, the pathogen has strict environmental requirements: temperature 15-25°C and relative humidity of 95% or higher. The humidity levels can easily be achieved when chrysanthemums are grown under polyethylene blackouts but are rarely met in greenhouses using modern growing techniques. Woven thermal screens and polyethylene covered floors (on crops such as cucumber and tomato, not chrysanthemum) were introduced to save energy and reduce crop humidity. We have evaluated methods of raising humidity to the required level for pest control on bed grown chrysanthemums whilst monitoring the crops for increased pest and disease occurrence. *V. lecanii* also has the ability to parasitise a range of plant pathogenic fungi, including *Puccinia horiana* (chrysanthemum white rust) which has the potential to become a major disease at elevated humidities. The possibility therefore exists for a combined pest and disease control programme using *V.lecanii* and raised humidities (Whipps, 1992).

METHODS

Glasshouse experiments

The trial was conducted over almost 2 years and the programme modified as various developments occurred, different humidity regimes and methods of raising humidity were evaluated. The latter stages of the work screened several cultivars of chrysanthemums for any physiological disorders attributable to elevated humidity levels.

Five separate glasshouse chambers were each planted with one 1 x 7.5 m bed of chrysanthemum variety Dark Pink Gin during the first week of each month. Each chamber had 3 beds to allow a continuous planting and cutting of chrysanthemums, thus at least 2 beds were always growing at any given time. The aphids *Aphis gossypii* and *Macrosiphoniella sanborni* were evenly distributed to each bed at fortnightly intervals (100 aphids per bed). Adult thrips (*Frankliniella occidentalis*) were also introduced fortnightly at 200 per bed.

Sampling of pest populations was done weekly by taking 30 leaves at random from each bed. These were inspected under a binocular microscope for number of aphids (2 species), thrips, leafminer, spider mites and also any parasitised or *V. lecanii* infected insects. Ten growing points, buds or flowers were also taken and placed in a turpentine extraction apparatus (Helyer and Brobyn in press) which repelled any live insects away from the plant material. These were counted on wet filter paper under a binocular microscope a couple of days later.

The commercial product Microgermin® (Chr. Hansen Bio Systems) which contains both the whitefly/thrips and aphid strains of *V. lecanii* was applied as a high volume spray (2,500 - 3000 l/ha) fortnightly to the plants (alternate to pest introductions). The product is formulated with a carbohydrate carrier to act as a source of nutrient for the *V. lecanii* on the leaf surface. Sprays were prepared as a concentrate with 1 litre of water 3 - 4 h before application to enable the dried product to absorb water and thus improve spore germination and subsequent infection.

Chamber 1 was a standard check on the older method of chrysanthemum blacking out for short days with black polyethylene and remained as such throughout the trials. Chambers 2-5 were fitted with woven blackout screens (Ludvig Svensson Environmental Screens) LS 11 + 1. These are designed to save energy and reduce crop humidity. LS 11 is aluminium foil strips woven together and LS 1 is woven black polyester strips. The LS 11 is placed above the LS 1 to reflect heat and light, both screens are pulled across the crop simultaneously.

1. Comparison of natural humidity with raised humidity regimes using low pressure misting and overhead sprinkle lines.

Trial 1.

Chamber 2 was to check the effectiveness of *V. lecanii* control under conditions of ambient humidity. Overhead sprinkle lines were installed in chamber 3, these were manually operated just before blackouts went over the crop and again after removal in the morning. In chambers 4 and 5 a low pressure water fog system was installed (Macpenny), this enabled the humidity to be set beforehand and maintained for a set period of time. The Macpenny fog system uses mains pressure water and compressed air to produce a fine fog which is mainly used in plant propagation. Humidity was raised in chambers 3, 4 and 5 for six consecutive nights after application of spores followed by 8 nights of ambient humidity (day humidity remained ambient in all chambers throughout the trial).

The treatments were as follows, each in a separate chamber:

Ch. 1 Polyethylene blackout on hoops, natural humidity with *V. lecanii*
Ch. 2 Woven blackout screens, natural humidity with *V. lecanii*
Ch. 3 Woven blackout screens, raised humidity (sprinkle lines) with *V. lecanii*
Ch. 4 Woven blackout screens, raised humidity (low pressure fog) with *V. lecanii*
Ch. 5 Woven blackout screens, raised humidity (low pressure fog) **no** *V. lecanii*. Duration 4 months.

Trial 2.

The humidity regime was changed from 6 consecutive nights per fortnight to 4 consecutive nights per week (2 X 4 nights per fortnight).
Chambers 1 and 2 as above.
Ch. 3 was changed to a replicate of chamber 2 (*V. lecanii* with natural humidity **no** sprinkle lines).
Ch. 4 and 5 both changed to *V. lecanii* plus raised humidity by low pressure fog. Duration 4 months.

Trial 3.

Following the almost immediate success of the 4 consecutive nights per week programme, it was decided to investigate different humidity regimes, the days and nights off remaining ambient. Commercial interest in methods of raising humidity was being shown by some companies who volunteered to supply equipment for evaluation in later trials.

Chambers 1, 2 and 3 as in trial 2.

Ch. 4 Woven blackout screens, raised humidity (low pressure fog for 3 consecutive nights per week) with *V. lecanii.*
Ch. 5 Woven blackout screens, raised humidity (low pressure fog alternating 2 nights on and 2 nights off) with *V. lecanii.* Duration 3 months.

2 Comparison of humidifying systems.

Trial 4.

The low pressure water misting system and overhead water sprinkle was compared with a high pressure fan assisted system and two spinning disc machines. Tebarint UK provided the high pressure fog system which takes mains water, passes it through 2 ultra fine particulate filters and pressurises to 84-105 Kg/cm^2 (1,200-1,500 p.s.i.). High pressure water is then atomized through nozzles in front of a powerful fan (effective throw 40-45 m). The whole system was computer controlled which made it easier to run on an alternating 2 nights on and 2 nights off programme than the simpler 7 day time clock programme switched to 3 or 4 nights on per week. The other machines use mains pressure water and a centrifugal spinning disc to atomize the water. Mellor and Bromley's machine has a fan behind the spinning disc which distributes the fog throughout the greenhouse, (effective throw 22 m). A ceiling mounted machine was provided by A and A engineering and produces a fine fog which spreads throughout the chamber, effective throw - unknown. Both spinning disc machines were run for 4 consecutive nights on per week.

Ch. 1 and 2 as above.
Ch. 3 Woven blackout screens, raised humidity (Tebarint high pressure system) with *V. lecanii*
Ch. 4 Woven blackout screens, raised humidity (Mellor and Bromley's spinning disc machine) with *V. lecanii*
Ch. 5 Woven blackout screens, raised humidity (A and A engineering Satair system) with *V. lecanii.* Duration 9 months.

3 Comparison of *V. lecanii* strains and effect of raised humidity on chrysanthemum cultivars.

Trial 5

During December 1991 the commercial products Mycotal® and Vertalec® (thrips / whitefly strain 1-72 and aphid strain 19-79 respectively) were re-marketed in the UK. It was therefore decided to use these two products in place of Microgermin® which is not registered for use in the UK. The level of pest introductions were also decreased to 20 adult thrips and 10 aphids of both species per bed each fortnight. This was to determine the efficacy of prophylactic sprays compared with curative control sprays against a large pest population. During the trial eighteen different cultivars of chrysanthemum were screened for their susceptibility to the high humidity required by *V. lecanii* (Table 1). The humidity treatments remained as above throughout trial 5. Duration 5 months.

Table 1. Cultivars tested at high humidity.

Belair	Pale Salmon Snapper
Bright Yellow Delta	Rhino
Bronze Reagan	Roswan
Cream Delta	Snowdon
Dark Pink Gin	Solarama
Daymark	Super White
Gelb Schneestern	Teide
Honey Sheena	White Fresco
Hurricane	Win

Laboratory experiments

The growth of *V. lecanii* mycelium is dependant on high humidity, the precise humidity can be accurately measured by laboratory bio-assay. By adjusting the amount of glycerol in an agar based medium the water activity potential can be calculated (Dallyn & Fox 1980). Plugs are then taken from a colony of *V. lecanii* and placed in the centre of Petri dishes at different water activities. Replicate plates were then sealed in bags and incubated at 23 °C. The diameters of the growing colonies were then measured every 3-4 days, with measurements taken at right angles to each other. The experiment was repeated for both 1-72 (Mycotal) and 19-79 (Vertalec) strains of *V. lecanii* at water activities equivalent to 92 % Rh to 100 % Rh.

RESULTS AND DISCUSSION

Although thrips were introduced to each chamber every fortnight, very low numbers of live or dead thrips were found. This is due to the feeding mechanism of thrips which rasp the leaf cells rather than inserting their mouthparts thus becoming 'fixed' like aphids. Therefore, unlike aphids, most dead thrips are easily dislodged from the leaf. However, in chamber 2 (ambient humidity) live thrips were continually present, thus indicating control in the other chambers. Aphids infected and subsequently killed by *V. lecanii* were distinctive as white fluffy bodies due to the sporulation of the fungi and were thus easily counted as they remained attached to the leaf.

Trial 1

The overhead sprinkle lines in chamber 3 over-watered the crop and caused damage to open flowers, they also failed to provide adequate pest control (both aphids and thrips). High humidity alone (chamber 5) had no effect on the pest populations which rapidly reached excessive numbers. The initial regime of 6 nights high humidity per fortnight failed to give adequate pest control.

Trial 2

After 4 months the raised humidity without *V. lecanii* (chamber 5) became a replicate of chamber 4. This was because pest levels increased rapidly indicating that high humidity alone had little pest controlling activity.

The humidity regime in chambers 4 and 5 was changed in early October to 4 consecutive nights of high humidity per week which rapidly improved control of aphids and thrips (Figs 1 and 2).

Trial 3

When the humidity regime was changed to just 3 consecutive nights per week in chamber 5, aphid and thrip numbers increased to unacceptable numbers. The alternating 2 nights raised humidity followed by 2 days and nights of ambient humidity proved to be as successful for pest control as the 4 consecutive nights of high humidity per week.

Trial 4

The humidity of 95 % or higher has successfully been achieved by producing a fine fog of water under the blackout screen (Fig 3). All methods of raising humidity proved satisfactory and have worked with no mechanical breakdowns. The computer controlled high pressure system is capable of treating over 1 Ha with several fan units to distribute the fog. By running the system for 2 alternating nights on and 2 off the machine is therefore capable of treating double the area (over 2 Ha). Several individual spinning disc machines would be needed to treat a similar area which may prove more costly. However, the hard water of southern England caused large deposits of calcium to form on parts of the equipment. On fan assisted machines, particles of calcium have been blown on to the upper leaves of plants leaving a visible white mark. Polyethylene blackouts as used in chamber 1 achieved high humidity (+ 98%) on most nights, particularly during the summer months (Fig 4) and were used even when the flowers were in full bloom, with no adverse effects. However, during the winter months less water was applied to the plants and more heating occurred leading to condensation on the glass, a lower ambient humidity (Fig 5) and thus a failure in pest control by *V. lecanii*.

Trial 5

The level of pest control achieved with a mixture of Mycotal® and Vertalec® was comparable to that given by the Microgermin® formulation tested earlier. When prophylactic sprays of *V. lecanii* were tested against a low level pest population adequate control was achieved. This indicates that *V. lecanii* can rapidly infect and kill insect pests before they multiply, thus leaving a plant virtually clean of dead bodies. This possibly occurs because *V. lecanii* is formulated with a carbohydrate nutrient which allows the fungus to grow undetectably over the leaf surface. Therefore under conditions of raised humidity the insect is quickly infected and killed.

None of the cultivars tested in table 1 showed any ill effect of being grown at raised humidities when compared to control plants. However, the pest populations were consistently higher on the low humidity (ambient condition) plants due to failure of *V. lecanii*.

Laboratory experiments

The differing amounts of available water in the agar media correspond to the % Rh at 23 ^{o}C required for the spores to germinate. Although this experiment has shown that there is some activity at 92 % Rh (Fig 6), humidities of at least 96 % for the 19-79 strain and 98 % for the 1-72 strain are needed for there to be any substantial growth. This indicates that if infected aphids are found on plants then infected thrips should also be present although they may not be found for reasons explained earlier.

CONCLUSIONS

Control of several insect pests can be easily and safely achieved with fortnightly sprays of *V. lecanii* as high volume applications (2,000 - 3,000 litres water per ha) beginning the week of planting and continuing until harvesting. It should also be possible to make one treatment to the young cuttings during rooting while they are covered with white or clear polyethylene. The entire pest control programme relies on achieving a high relative humidity (Rh) for certain nights of each week. Four consecutive nights of high Rh per week or an alternating 2 nights on and 2 nights off have provided the humidity requirement for continued control of aphids, thrips and whitefly. Other pest organisms such as leafminer and spider mite can be controlled with parasites and predators respectively.

V. lecanii can be used as a curative agent, giving excellent control of high pest numbers by creating an epizootic infection that will proliferate whilst humidity conditions remain high enough. An epizootic is created by the increase in spore numbers from infected insects which rapidly spread through the pest population (Fig 2). The fungus can also be successfully used as a preventive treatment by repeated applications, the presence of a nutrient source allowing fungal growth in the absence of an insect host. Thus when a susceptible organism makes contact with *V. lecanii* spores an infection will occur when the humidity reaches 96 % or higher. Frequent periods of high humidity will therefore increase the probability of rapid pest control before much reproduction occurs.

Raising humidity at night is reasonably practical on most modern commercial nurseries because thermal blackout screens are pulled over the entire crop. This reduces the volume of air requiring humidification, also during the night temperatures are more stable allowing an easy conversion from % humidity to vapour pressure deficit (96 % humidity is equivalent to 0.09 kPa). Other advantages of a computer controlled system may include temperature control by evaporative cooling and the reduction of thermal shock when screens are removed in the mornings.

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Figure legends

Figure 1. (Chamber 3) The upper solid line represents total aphids (healthy and infected dead bodies) the lower broken line is infected aphids. The closer the two lines become indicates better control of aphids. The humidity regime changed on October 2 from 6 consecutive nights per fortnight to 4 nights per week dramatically improving aphid control.

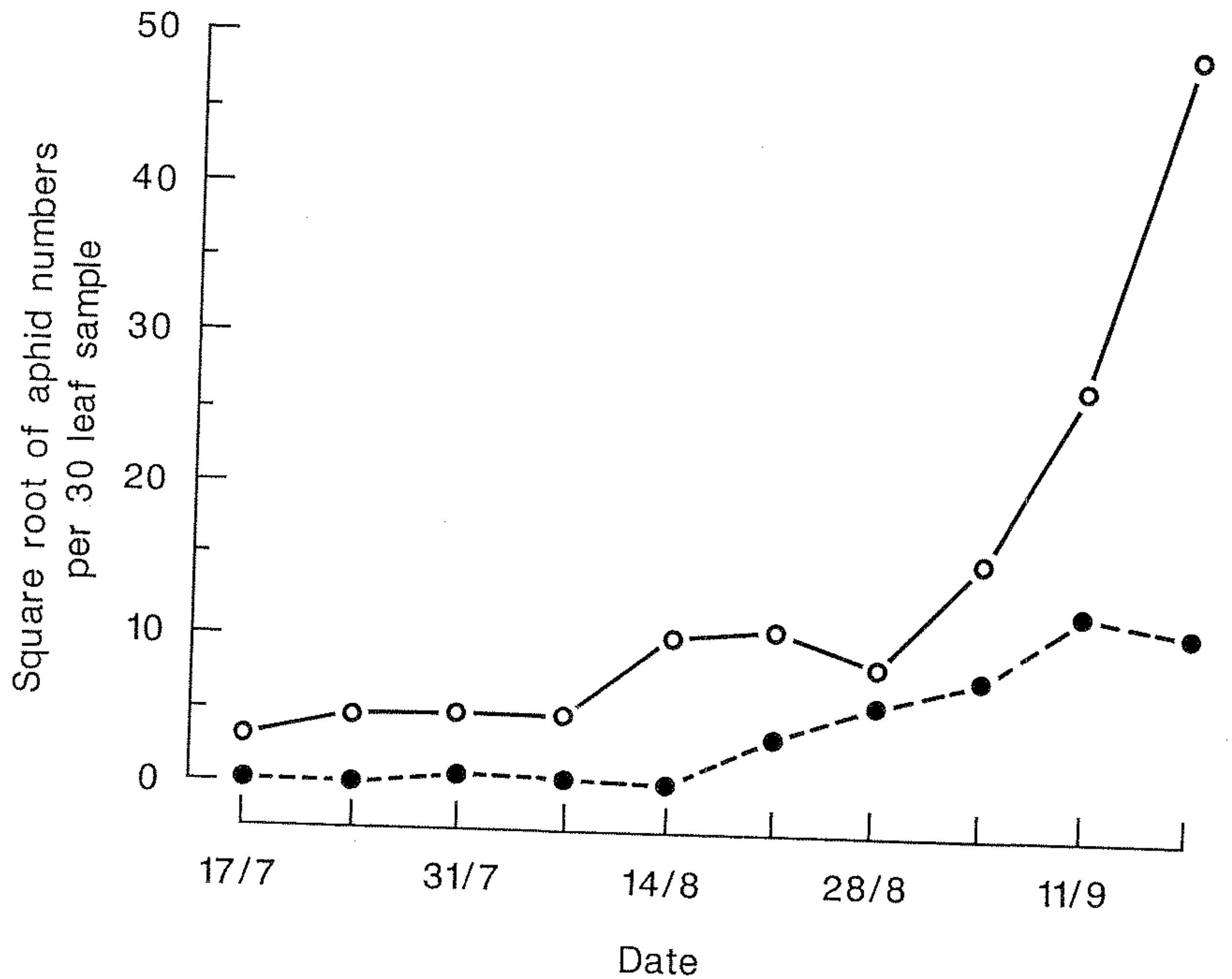
Figure 2. (Chamber 3 - next bed) Aphid control with *Verticillium lecanii* under a regime of 4 consecutive nights of high humidity per week starting on 2 October. The two lines become close during October indicating good pest control, peaks in the graph indicate when extra aphids were introduced to the crop.

Figure 3. Humidity achieved during the winter months using misting equipment for 4 consecutive nights per week. Note ambient humidity at either end of the week and the rapid rise from ambient to 95 % when the machine is activated.

Figure 4. Polyethylene blackouts as used in chamber 1 achieved high humidity (+ 98%) on most nights, particularly during the summer months.

Figure 5. Humidity achieved using polythene blackouts during the winter months when more heating and less water is applied to the plants leading to condensation on the glass and a lower ambient humidity.

Figure 6. Mycelial growth of *Verticillium lecanii* at different water activities (% humidity) and 23 °C. Strain 19-79 grows more rapidly at lower humidity than the aphid strain 1-72 indicating that if infected aphids are found then infection of thrips should also occur.



50
40
30
20
10
0
Square root of aphid numbers per 30 leaf sample
17/7
31/7
14/8
28/8
11/9
Date

Fig 1

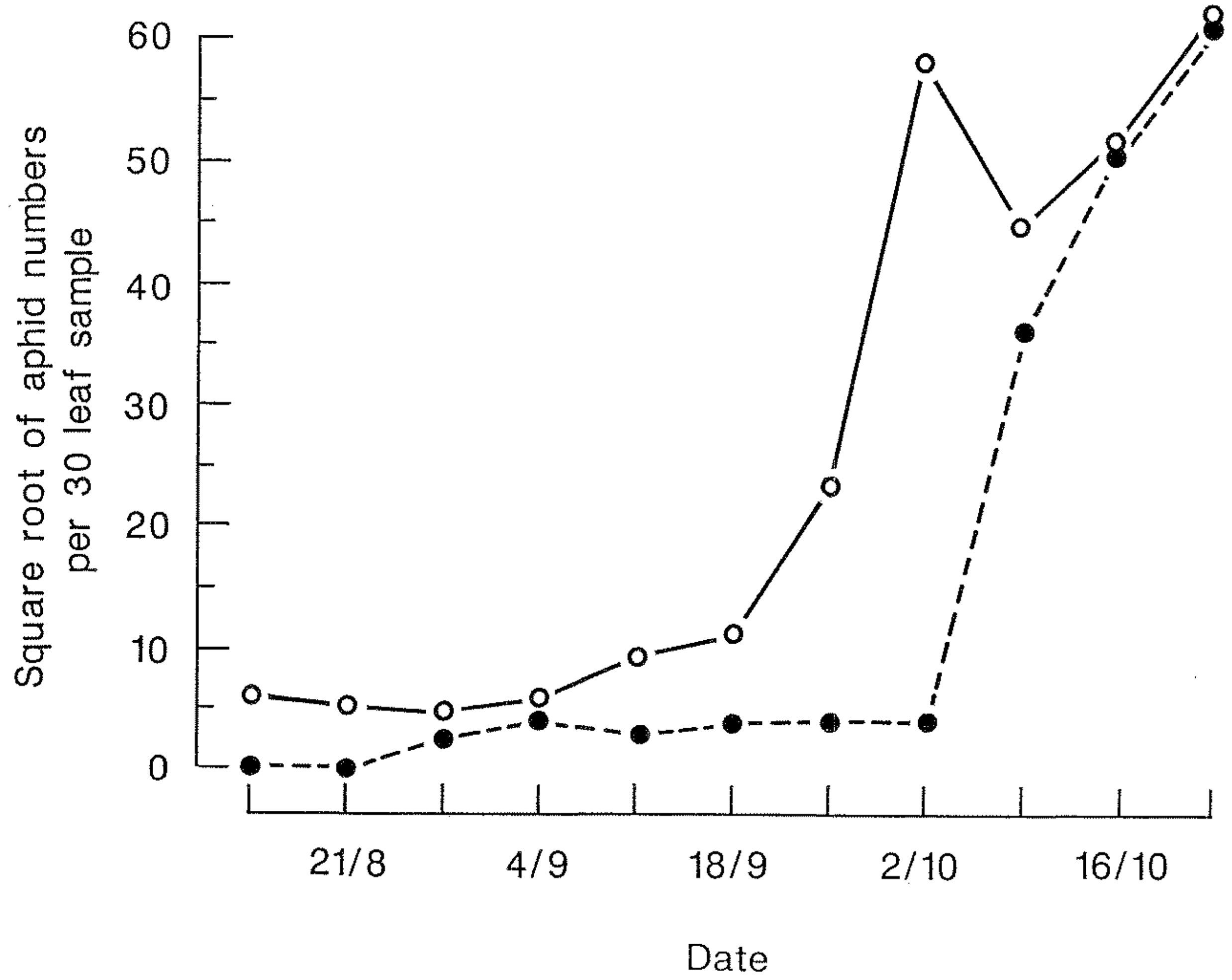
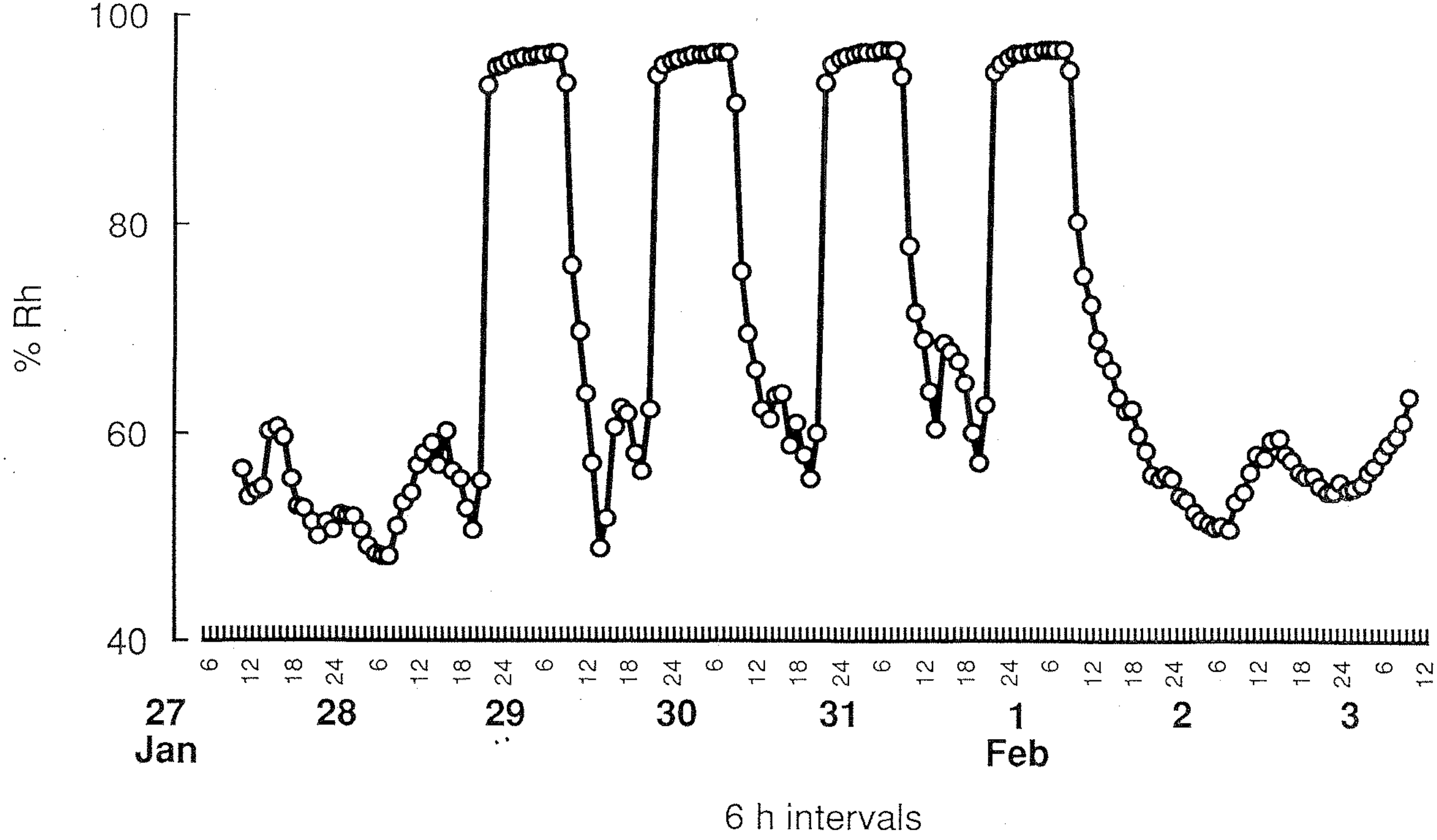
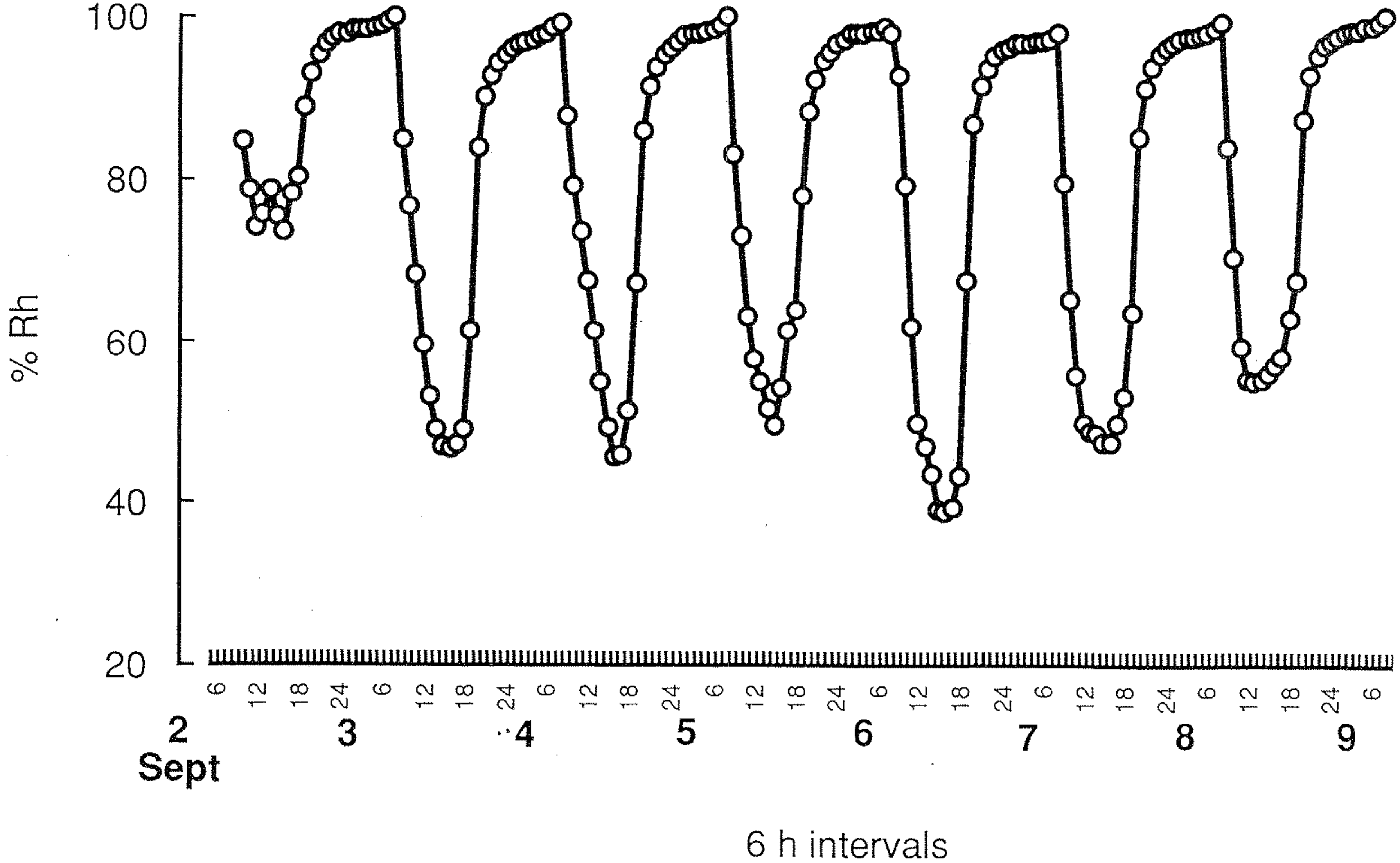

Square root of aphid numbers per 30 leaf sample
60
50
40
30
20
10
0
21/8
4/9
18/9
2/10
16/10
Date

Fig 2

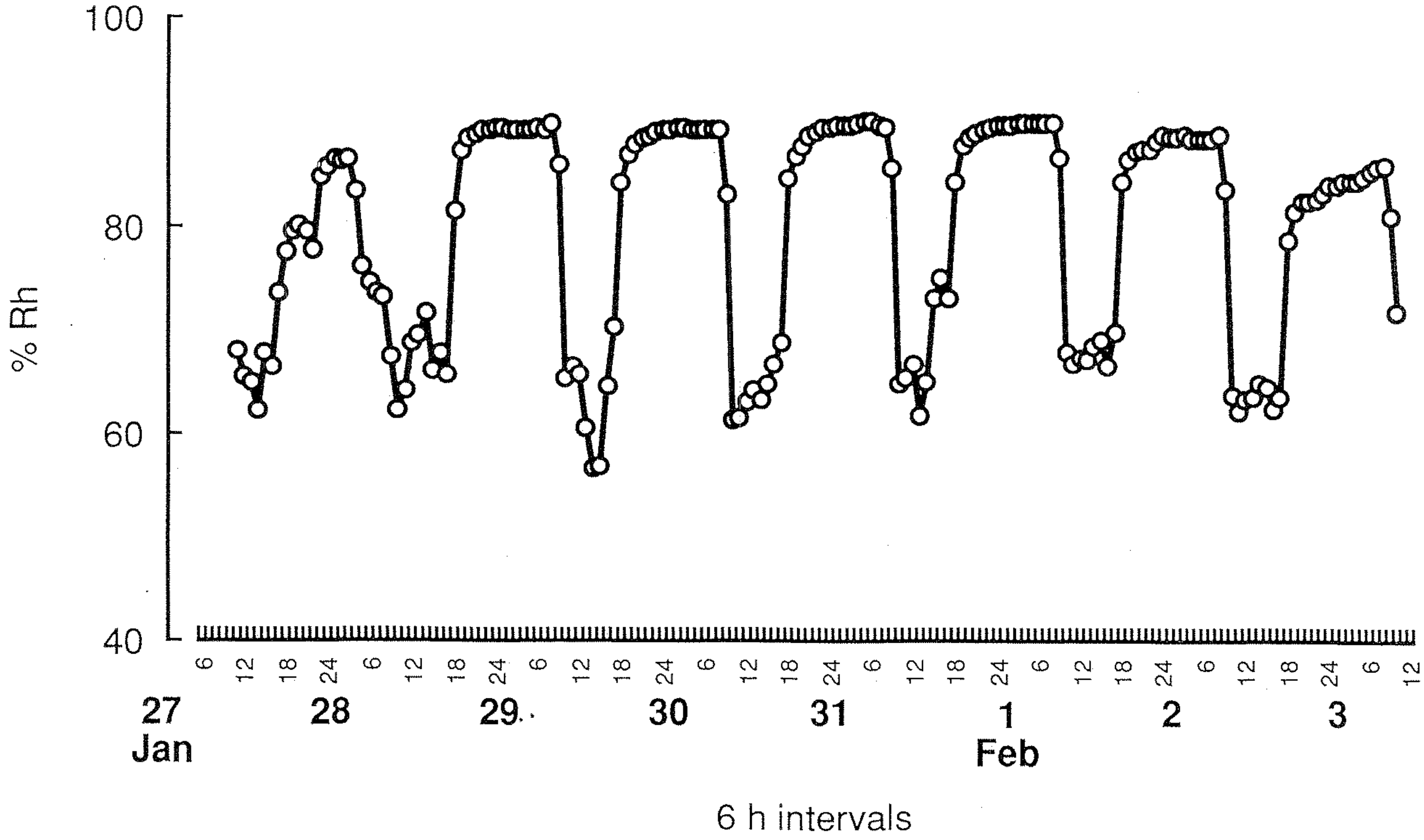
% Rh by fog from 27 Jan - 3 Feb 1992

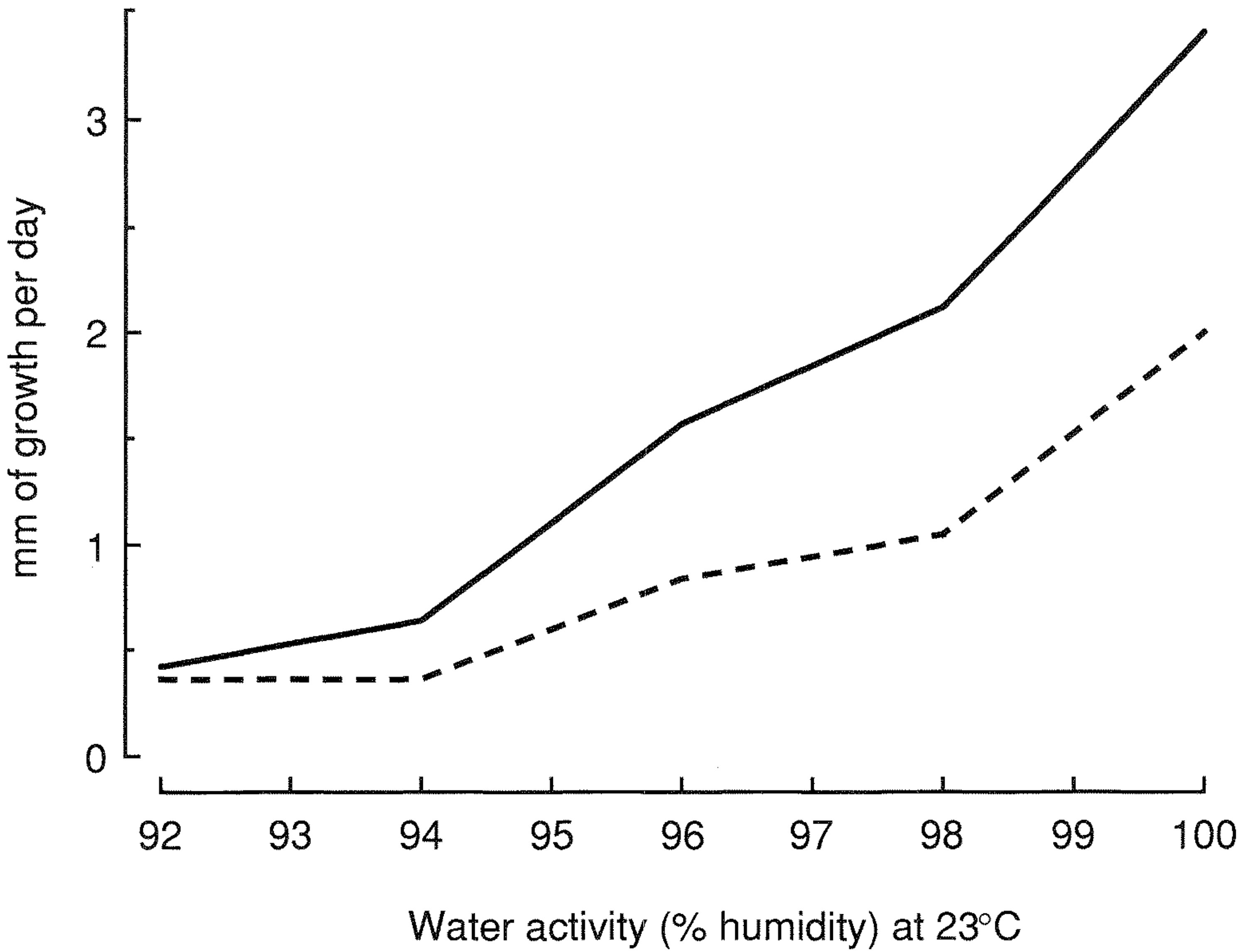


% Rh by polyethylene from 02 - 09 Sept 1991



% Rh by polyethylene from 27 Jan - 3 Feb 1992




mm of growth per day
0
1
2
3
92
93
94
95
96
97
98
99
100
Water activity (% humidity) at 23°C