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EFFECT OF ETRIDIAZOLE AND PYTHIUM ON TOMATOES GROWN IN NUTRIENT FILM

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Summary Etridiazole and an unidentified Pythium sp. pathogenic to tomato were added to nutrient film systems over a period of six months. Although there were some signs of attack by the pathogen yields of tomatoes were unaffected.

INTRODUCTION

Much has been written about the risk of disease in nutrient film systems (Evans, 1977; Staunton & Carmican, 1978; Winsor, Hurd and Price, 1979) yet there have been few catastrophic outbreaks. In this country there have been several incidents involving species of Phytophthora and Pythium which have been contolled with etridiazole. Other incidents with Colletotrichum and Verticillium have failed to develop serious proportions. In New Zealand, however, bacterial wilt led to the death of 20 000 plants in a few weeks (Wordsworth, private comm.). Fortunately the causal organism, Pseudomonas solanacearum, does not occur in this country. With this exception we can ask why, after nearly a decade of experience, has there been so little disease?

In the experiment reported here a tomato pathogen and a fungicide were added to NFT gullies at regular, frequent intervals to observe what effects these would have on the plant growth.

METHODS AND MATERIALS

The tomato plants (cv. Sonatine) used in this study were grown in eighteen nutrient film culture units (10 plants/gully). Each unit was a glass fibre tank containing 200 l nutrient solution (Cooper & Charlesworth, 1977) each with a submersible pump, to force the solution along an ABS plastic pipe to the top of a slope into a 15 cm wide, 6 m long plastic gully. This gully, down which the solution flowed, lay flat upon the floor of the glasshouse which was sloped 1:100. The flow rates were 2 l/min.

Water and salts (as Solufeed F, calcium nitrate and nitric acid) were replaced regularly in calculated amounts based upon daily conductivity and pH readings, which in turn were related to uptake by the plants for transpiration and growth. The conductivity was maintained at 2500 microsiemens and the pH at 5.5-6.0.

Non-nutrient ions such as chlorides and sulphates in the water supply and some added nutrients gradually accumulated and caused higher conductivities than those attributable to nutrient ions only. To counter this the entire solution of each tank was pumped out and replaced after 4 months.

The plants were grown according to the usual commercial practice for moderately early tomato production. Tomato seeds, were sown in mid-December and planted at the

two leaf stage in a peat:sand compost in a whalehide pot (a tarred paper material). During the propagation period carbon dioxide enrichment was used. This stopped when the plants were set out in gullies in January, by which time the first truss was in flower.

Tomato fruit was picked twice a week for ten weeks and the fruit from each gully weighed and graded separately.

Etridiazole, as Aaterra w.p., was added as a creamed suspension in a small volume of hot water (cooled before use) every three weeks to the nutrient reservoir to give a concentration of 30 µg/ml.

Sterilised hemp seeds were scattered over Petri dish cultures of an unidentified Pythium species which had been isolated previously from an NFT system. This fungus severely rotted roots of seedling tomato plants growing in a peat:sand compost. When the hemp seed had become colonised by the fungus the entire content of one dish was laid on the top of the root mat at one of two points in the gullies at the same time that etridiazole was added.

RESULTS

Visual observations.

The plants grew well and, despite the poor light levels in the spring, fruit was satisfactory. The mid-December sowing was four weeks later than is usual for early tomato production so flowering and fruit set was also delayed by four weeks. It is possible that the main period of stress, when an opportunist pathogen can attack, coincides with the maximum fruit load on the plant whilst the quality of daylight is still poor, i.e., March-April. These experimental plants reached their maximum fruit load a month later by which time the quality of daylight was much improved. At this time, however, many of those plants exposed to Pythium inoculations developed magnesium and iron deficiencies with the former being the more severe. To make certain that these elements were available all reservoirs were emptied and refilled with fresh nutrients at the end of May. There was no immediate response to the treatment, suggesting that there had been a substantial loss of root hairs which is typical of Pythium attack. The fungus was readily isolated from these gullies using VP medium (Tsao & Ocana, 1969).

Yields.

The fruit was picked and graded into four classes (3 saleable and waste). No appreciable differences were found either in total yield or waste, either of which might have indicated pathogenic effects (Table 1 and 2). There was a hint of earlier yield and larger numbers of fruits where etridiazole was used alone.

Table 1

Numbers of fruit per tomato plant grown in NFT to which etridiazole and/or Pythium were added

	April	May	June	Total
etridiazole	13.8	37.5	45.9	97.2
etridiazole + Pythium	12.9	39.3	48.6	100.8
Pythium	11.8	38.9	43.2	93.9

Table 2

Mean weight per fruit (g) of tomatoes grown in NFT to which etridiazole and/or Pythium were added

	April	May	June	Whole period
etridiazole	52.3	54.0	58.0	54.1
etridiazole + Pythium	54.0	54.0	57.0	54.0
Pythium	56.7	54.1	62.6	56.7

DISCUSSION

When a root pathogen is active for a long enough period it is usually lethal to its host. If it is active in a sub lethal manner, either because of intense competition from other microorganisms or because of a rapid renewal of roots, host symptoms may be difficult to discern. Wilting is an extreme symptom but less severe effects from the introduced Pythium might have been fewer fruits because of poor flower set or smaller ones because of less assimilate reaching the fruits. Neither of these effects were detectable (Table 1 and 2).

Although some pathogens have been isolated from plants in NFT systems most seem likely to have been introduced in the propagating medium. Growers usually add etridiazole as a routine measure to lessen the risk of damage by Pythium or Phytophthora (Price, 1978). This precaution means that the cause of any plants wilting is probably not fungal and a more likely cause is to be found in the growing conditions or within the plant itself.

It is interesting that the collaborative work of physiologists and pathologists has shown that plants trigger off a self induced wilt (Hurd; Price, 1977) and therefore suffer water stress even though their environment at that moment is generally favourable. In the early days before physiological root death in NFT was understood one of the first clues was the presence of a few turgid plants among thousands of wilting ones; these latter were invariably sterile plants carrying no fruit. Wilting plants recovered when fruits were removed sooner than those with fruits left on. More recently "root death" has been seen on plants late in the season. Among these have been turgid plants which because of chimaera were bearing little fruit. Can it be that root systems of plants differ in susceptibility to disease according to the amounts of assimilate reaching them from the shoot?

In the experiment described here no obvious signs of pathogenic attack were detected. Yet in an earlier experiment tomatoes grown in a solid substrate were severely damaged by the Pythium even though they were young and vigorous.

These results are similar to those of Staunton and Carmican (1978) who used a range of pathogens and can be compared with the pattern of attack by Fusarium on bananas in the wet tropics. When the soil of the banana plantation is saturated in the rains disease is not often seen. As the dry season progresses, i.e. water stress increases, more and more disease occurs. This is only one of the many examples of plant diseases occurring in soils with rapidly fluctuating water contents. Until now the main advantages of NF culture have been the long cropping season, rapid turn round of crop, and low labour requirements. Perhaps a lower disease risk is another advantage.

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CONTROL OF BOTRYTIS CINEREA IN GLASSHOUSE CROPS WITH IPRODIONE APPLIED THROUGH THERMAL FOGGING EQUIPMENT

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Summary Iprodione in a fog formulation (Rovral Fog)(R) has given excellent control of Botrytis cinerea which causes leaf and stem lesions as well as fruit "ghost spotting" of tomato crops. Lesions on layered stems late in the growing season may not be so well controlled by fogging as by HV iprodione sprays, and in this case stems may need separate HV treatment. Compared with HV spraying, use of iprodione fogs can lead to large savings of time, labour and water when treating large glasshouses. Iprodione fog has also been used successfully on ornamental crops.

Resumé L'iprodione utilisé en formulation fumigène (Rovral fog) a donné d'excellents resultats pour le controle de Botrytis cinerea responsable des lèsions de la feuille et de la tige ainsi que des taches fantomes des fruit "ghost spotting" des tomates. Les lèsions de la tige marcottée qui se produisent tard en saison sont moins bien controlées par la formulation fumigène que par pulverisation à haut volume d'iprodione. Dans ce cas, il est donc necessaire d'effectuer un traitement haut volume supplémentaire. L'emploi d'iprodione en formulation fumigène est avantageux par rapport au traitement par pulvérisation haut volume du fait d'un important gain de temps de travail, et de l'eau dans le cas d'applications effectuées dans de grandes serres. La formulation fumigène d'iprodione a également utilisée avec succès sur cultures ornementales.

INTRODUCTION

Iprodione, as a high volume application of the w.p. formulation, Rovral(R) has a widely established use against fungal diseases on a range of outdoor and protected crops, Burgaud et al (1975)

High volume application of iprodione by hand for example to an acre of mature tomato plants under glass can take several hours. However, applying the same material as a fog can take about 20 minutes: There are thus considerable savings to be made of time, of labour and of water by integrating fogging techniques into routine glasshouse spraying.

This paper describes the development of iprodione as a ready-to-use fogging formulation for control of Botrytis cinerea on glasshouse crops to complement the use of the existing w.p. formulation.

(R) Registered Trade Mark

DEVELOPMENT OF IPRODIONE FOGGING FORMULATION

A formulation for fogging was prepared to meet the following requirements: i) An effective Botrytis treatment at reasonable cost; ii) safety to the crop; iii) safety to the operator; iv) a stable ready-to-use solution; v) a dense fog cloud visible to the operator; vi) compatibility with most popular fogging machines.

Items i and ii are discussed under Biological Testing: Items iv, v and vi under Fog Application: Item iii under Toxicological Studies.

TOXICOLOGICAL STUDIES

Acute inhalation toxicity studies on rats produced no macroscopic post-mortem findings 3 weeks after a 4 hour continuous exposure to a fog, maintained by repeated applications of the formulation, totalling $2\frac{1}{2}$ gallons/acre (gpa).

The MAC value for the solvent in use (Maximum Allowable Concentration = the greatest amount to which persons may be exposed for 8 hours a day for a period of months or years without danger to health) is twice the level of formulation applied, for example, to small poly tunnels at 1 gpa, and is nearly 6 times the level that will be applied to single span 1 acre houses (N.B. dose is presently recommended in terms of house area; not house volume).

FOG APPLICATION

Iprodione has a very low solubility in the oils traditionally used to produce dense fogs (e.g. kerosene or paraffin) and it was therefore necessary to utilise a non-toxic solvent for the iprodione with a specific oil to improve fog density. The resultant formulation contained 10% w/v iprodione designed to be applied at a rate of 1 gallon/acre (11.2 litres/ha) giving 1lb a.i./acre (1.12 kg a.i./ha iprodione).

The iprodione fogging solution is suitable for use through thermal "pulse-jet" fogging machines, but is not recommended for use through "exhaust" thermal foggers due to gassing of the solvent, possible risk of chemical decomposition (Burges and Jarrett; private communication) and recorded poor disease control (MacIntyre, 1976). Organic degradation products have not been detected in fogs after passing iprodione through "pulse-jet" foggers.

The density and persistence of a fog tends to be variable but will depend on the droplet size spectrum which in turn is dependent on the type of fogging machine and its setting. Overfeeding a fogging machine will produce very large droplets ("spitting") which can cause severe phytotoxic damage close to the fogger exhaust.

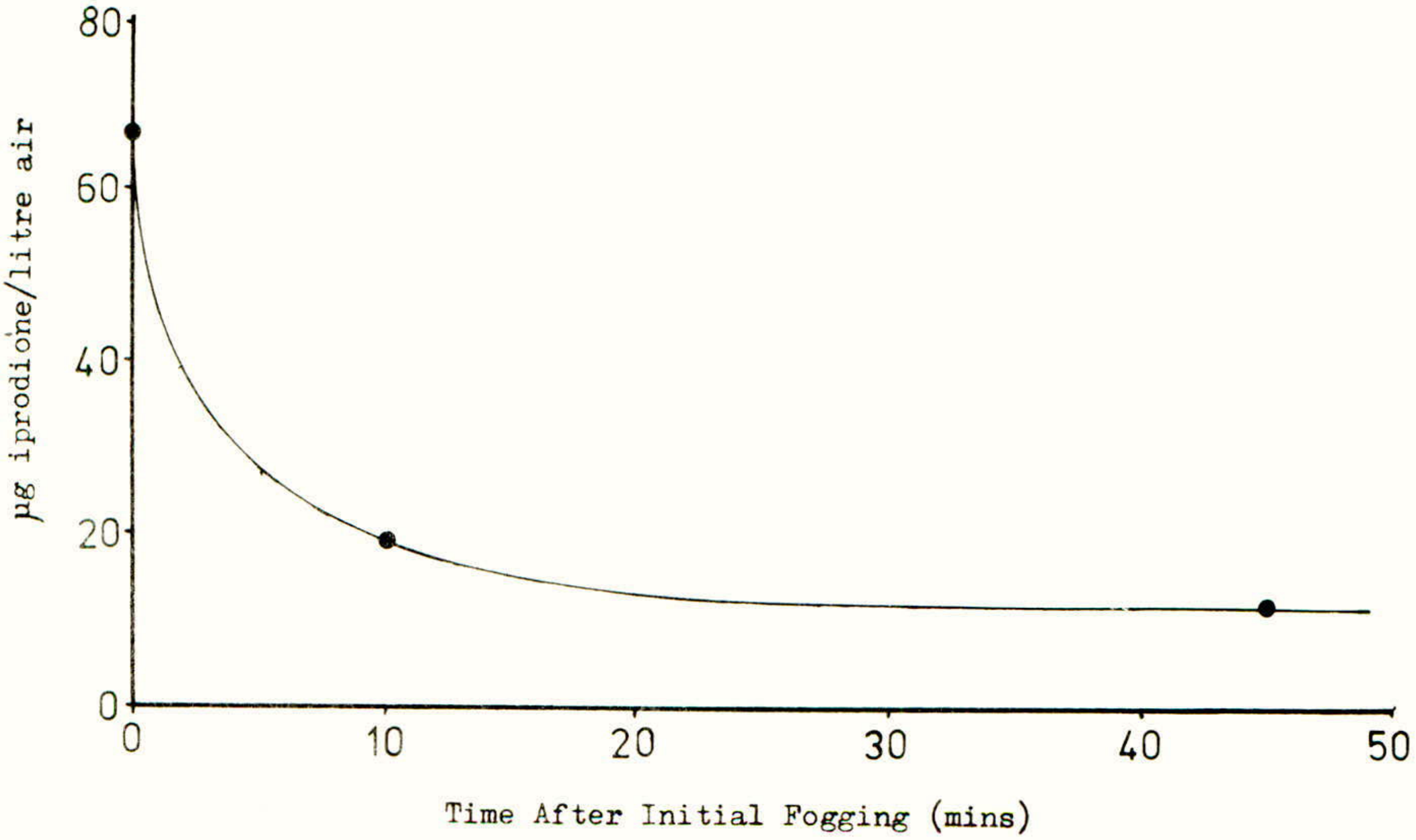
A wide range of droplets is produced in a fog and a typical example of fog fallout, as measured by recovery of airborne iprodione, is shown in Figure 1 where 70% of the iprodione is deposited in the first 10 minutes. Fifteen minutes after fogging, 97% of the airborne droplets were between 2.5 - 7.0 μm diameter. Such a fog is usually visible for at least 2 hours after application.

Studies using a fogging machine at a fixed point (Griffin; private communication) showed that droplets up to 120 μm were deposited at a distance 10 m from the fogger, but up to only 45 μm at a distance of 30 m. Larger fog particles fall out more quickly and therefore applications from a single point can lead to a very uneven distribution of fungicide. The fogging machine must therefore be progressively carried to all parts of the greenhouse under treatment to ensure accurate and even application, (Morgan & Spencer 1978).

Figure 1

RECOVERY OF AIRBORNE IPRODIONE AFTER FOGGING

(Polytunnel treated at a theoretical 66 µg iprodione/litre of air using a Dynafog DH90)



Fogs should be applied in calm conditions and to greenhouses that can be properly sealed against wind and leakages. A high wind can suck a complete application of fog from a poorly sealed house in as little as 10 minutes. Wind can also cause the wholesale displacement of fog within a house so that the upwind half remains untreated.

It is usually preferable to fog during the evening when wind is often slack, when the treated house can be left undisturbed overnight and when the sun will not cause an excessive build-up of heat in the house kept closed for several hours.

BIOLOGICAL TESTING

Varieties of tomato that have been safely treated with iprodione fogging solution are listed in Table 1, Part A.

Table 1

Tomato varieties and ornamental species safely treated with iprodione fogging formulation

Part A) Tomato varieties

Amberley Cross	Alto	Sonatine
Curabel	Nemato	Sonato
Curesto	Odine	Virosa
Eurocross	Sano	96A
Kirdfordcross	Sarina	WW 193

Part B) Ornamental species

Adiantum	Codiaeum variegatum	Monstera deliciosa
Aphelandra squamosa	Cyclamen	Peperomia caperata
Aralia sieboldii	Dieffenbachia	Peperomia hederaefolia
Begonia hybrids	Exacum affine	Peperomia U.S.A.
Begonia rex	Ficus benjamina	Pilea cadierei nana
Boston fern	Ficus robusta	Plumosis
Campanula isophylla	Fuchsia hybrids	Poinsettia
Chamaedora elegans	Hedera	Saintpaulia
Chlorophytum comosum	Hibiscus rosa-sinensis	Sinningia speciosa
Chrysanthemums	Kalanchoe	Solanum capsicastrum

Morgan (1979) has shown that yield and grading of tomato fruit are similar for both fogging and HV iprodione treatments. (Table 2).

Table 2

Yield from Tomato plants (Amberley Cross) treated with iprodione:

Trial at Glasshouse Crops Research Institute UK

Method of application	iprodione kg a.i./ha	Interval between applications (weeks)	Yield	
			weight (kg)*	% of Class I fruit
HV	1.12	2	1058	48.8
Fog	1.12	2	950	50.5
Fog	1.12	1	1033	46.2

* Data for 132 plants

The activity of a programme of iprodione fog, compared with HV sprays containing iprodione as the formulated w.p., against Botrytis leaf lesions, stem lesions and fruit "ghost spotting" on tomatoes is shown in Tables 3 and 4.

Table 3

Control of Botrytis in Tomatoes

Trial at Lea Valley Experimental Horticulture Station UK 1977

Chemical	Application	iprodione kg a.i./ha	No. of Botrytis stem and leaf lesions per 20 plants	No. "ghost spots" per 20 plants
iprodione	HV	1.12	17	24
iprodione	fog	0.96	10	25
dichlofluanid	HV	2.24	28	4
dichlofluanid	fog	1.15	26	4
Untreated	-	-	31	48

Table 4

Control of Botrytis in Tomatoes (Sonato)

Trial at Fairfield Experimental Horticulture Station UK 1977

Chemical	Application	iprodione kg a.i./ha	% infected leaves	Mean No. of ghost spots per fruit
Experiment 1				
iprodione	HV	1.12	18.5	15.5
iprodione	fog	1.12	17.4	6.9
dichlofluanid	HV	1.12	53.3	13.4
dichlofluanid	fog	1.12	33.2	5.3
untreated	-	-	64.6	22.2
Experiment 2				
iprodione	HV	1.12	2.7	4.5
iprodione	fog	1.12	3.7	4.6
dichlofluanid	HV	1.12	2.6	3.2
dichlofluanid	fog	1.12	0.8	2.2

The effect of sequential applications of iprodione for Botrytis control is shown in Table 5 (A.G. Channon: private communication) and in Table 6.

Table 5

Control of Botrytis in Tomatoes treated with iprodione:

Trial at West of Scotland Agricultural College, Auchincruive

Assessment	Application method	Sampling Date			
		11/8	24/8	7/9	21/9
No. stem lesions per 100 plants	HV	1.3	4.3	9.5	10.0
	fog	1.5	1.2	1.2	2.3
No. fruit showing ghost spotting per 100 fruit	HV	14.1	34.6	26.2	6.6
	fog	18.3	19.7	4.8	1.2
No. plants lost per 100 plants	HV	0	0	0	7.5
	fog	0	0	0	3.0

Table 6

Control of Botrytis in Tomatoes: UK 1977

Application	iprodione kg a.i./ha	No. of applications	Botrytis lesions per 100 plants		No. of Botrytis fruit dropped in 10 double rows
			stem	leaf	
HV	1.12	0	3	23	
		2	3	13	(no data)
		4	0	(deleaved)	
fog	1.12	0	2	42	75
		2	3	15	4
		4	0	4	0

Poor control of stem-lesions on horizontally layered stems can occur late in the growing season (Table 7) where it is believed that overlying foliage filters out most of the fog particles. This contrasts with good control of stem lesions (Tables 5&6) at earlier growth stages.

Table 7

Control of Botrytis stem lesions in Tomatoes with iprodione at 1.12 kg a.i./ha:

Trial at Lea Valley Experimental Horticulture Station UK 1978

Tomato variety	Application method	No. of Botrytis lesions on layered stems of 108 plants		Av. % plants removed because stems girdled by Botrytis lesions
		19/9	12/10	
Sarina	fog	10	46	44
	HV	0	0	7.7
Curabel	fog	17	43	39.5
	HV	9	6	10
WW193	fog	29	61	60
	HV	1	7	10.5
Nemato	fog	12	34	39
	HV	2	3	8.5
Virosa	fog	40	56	56.5
	HV	3	4	10.5
Sonatine	fog	21	26	29.5
	HV	0	1	8

In an artificial experiment, leaves were taken from tomato plants subjected to the standard dose of iprodione fog. Botrytis inoculated tampons were placed on the

top or bottom leaf surfaces and subsequent infection noted in a controlled environment. Under this intense disease pressure, 60% control of Botrytis infection was obtained on the upper surfaces of the leaves but only 2% control on lower surfaces. Studies by Jarrett et al (1978) showed that a fog gives negligible underleaf cover in a crop, while MgO slides have shown <1% of droplets impacted on undersurfaces (Griffin, private communication, 1978). It thus seems that underleaf infection of tomato plants by Botrytis is not a normal event or else the control achieved with iprodione fog in trials (Tables 2-6) would not have been possible.

Control of Botrytis on lettuce has also been demonstrated using an iprodione fog. However lettuce tends to be more susceptible to chemical scorch than tomatoes and a careful choice of solvent is needed to ensure crop safety. This is the subject of further studies.

Work has been carried out on ornamentals using a wide range of species at various growth stages. Three iprodione fog applications were made at intervals of three weeks. No phytotoxicity to any of the test species (listed in Table 1 Part B) were found nor any noticeable deposits.

DISCUSSION

Applications of an iprodione fog formulation to tomato crops have given excellent control of Botrytis leaf lesions and ghost spotting. It is believed that deposition of fog almost copies the pattern of natural airborne spore deposition which leads to the disease. In this respect fog treatments give a better control of "ghost spotting" than the typical level of control by HV sprays reported by Soper & Cox (1977).

Late in the crop growth season, poor control of lesions on layered stems by iprodione fog may call for high volume applications to provide successful treatment of this aspect of the disease. In these situations, fogging may best be used in an integrated control programme with conventional sprays.

The present iprodione fogging formulation is not yet recommended for use on lettuce, where further work is required.

Iprodione fog is likely to prove a valuable tool for controlling Botrytis in ornamental crops, while further uses are being tested in cucumbers and in stored cabbage. Iprodione fog has also shown promising control of Rhizoctonia solani when applied to seed potatoes in chitting sheds, and provides a quick, simple method of treating a large tonnage which is otherwise inaccessible in store.

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APPLICATION AND DISTRIBUTION OF *BACILLUS THURINGIENSIS* FOR CONTROL OF TOMATO MOTH IN GLASSHOUSES

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Summary Thermal fogs of *Bacillus thuringiensis* applied at 1, 2 and 4 kg/ha greatly reduced numbers of larvae of the tomato moth, *Lacanobia oleracea* and the damage they caused to the foliage and fruit. The rate of 4 kg/ha gave the best control preventing damage to the fruit with only 8 larvae surviving out of a total of 4000 eggs and young larvae infesting the tomato crops between June and September. Incorporation of a fluorescent dye, saturn yellow, with fogs of bacteria permitted the quantitative measurement of fog droplet distribution throughout the tomato crop. The most important finding was that only 0.5% of the bacteria deposited on the foliage was on the undersurface of the leaves, therefore unless a pesticide has a translaminar, fumigant or systemic action pests present on the underleaf surface will be unharmed. More droplets were deposited by settling than by impingement. Deposition of droplets by impingement was greatest directly in front of the fogging machine but decreased rapidly with distance along the fog flowpath. Droplets settled downwards over all the crop, deposition being greatest in regions where the speed of the fog decreased most. Distribution over upper leaf surfaces depended on the angle of the leaf. Coverage on adjacent 1-cm² areas of a leaf rarely differed by more than x3 on horizontal and often by in the order of x100 on vertical leaves, with the size of droplets rapidly decreasing along the flowpath of the fog. Spore counts gave a better indication of foliar coverage with *B. thuringiensis* than droplet counts. Leaves significantly shaded others only when the intervening distance was 1 cm or less.

INTRODUCTION

With the need to reduce labour input in the production of glasshouse crops, growers are increasingly using labour saving thermal fogging machines for pesticide treatment enabling one man to treat large areas rapidly. Burges and Jarrett (unpubl.) showed that microbial insecticides containing *Bacillus thuringiensis* survived passage through pulse-jet type machines. Consequently the application of a *B. thuringiensis* wettable powder for the control of the tomato moth, *Lacanobia oleracea*, on tomato crops was studied. In an integrated control system *B. thuringiensis* has the advantage that it is active specifically against lepidopterous larvae and can safely be used without harming man or predators and parasites used to control other pests.

As *B. thuringiensis* acts only when ingested by larvae, a good coverage of the crop is essential to obtain efficient larval control. For this reason and because little is known about fog droplet distribution on tomato crops, fog deposits were measured. The methods used may also be applied to studies with chemicals.

METHODS AND MATERIALS

Tomato crops

Experiments were conducted on mature tomato crops (cv. Sonato) grown in double rows by the S-hook layering method.

Application of thermal fogs

For experiments on droplet distribution, fogs were applied in a glasshouse 10 x 9 m with a Pulsfog K10 machine using the largest jet size, 12, with *B. thuringiensis* (Dipel; Abbott Laboratories, North Chicago, Illinois, USA) at the rate of 5% w/v in VK_2 carrier (water, methanol and 2-ethoxyethanol) at the maximum recommended rate (40 ℓ/ha) that avoided phytotoxicity due to VK_2. A fluorescent dye, saturn yellow (H. Haeffrom and Co. Ltd., Chepstow) previously found to have no effect on *B. thuringiensis*, was added to the carrier to enable droplets to be seen under ultra violet (uv) light.

Experiments on the control of the tomato moth were conducted in glasshouses 38 x 13 m. *B. thuringiensis* was applied at 2.5%, 5.0% and 10% in the maximum volume of VK_2 carrier using a Swingfog SNIIP machine with jet number 1.2. A different crop was used for each fogging rate.

During fogging the machine was held horizontally, 0.8 m above the ground, at one end of each pathway aiming the fog between the rows while moving across the glasshouse and back until all the *B. thuringiensis* was dispersed. Fogs were applied only on still days to minimise escape of fog through spaces between glass panes and around ventilators. A fog was allowed to settle for a minimum of 2 h before sampling and addition of larvae.

Sampling techniques to determine fog droplet distribution

Before each fogging, arrangements were made to obtain foliage samples from three sample plants spaced at 2.5-m intervals along one row. At three different heights, 0.4, 1.1 and 1.8 m up each sample plant, leaves were manipulated into four positions, near horizontal, 45°, near vertical facing the direction of the fog and near vertical parallel to the direction of the fog. To measure droplet distribution on both the upper and lower leaf surfaces a small area on each surface was covered with 5-cm wide polyvinyl chloride adhesive tape, so that droplets could settle only on the one (exposed) leaf surface. A section of this covered area on each sample leaf was prevented from sticking to the adhesive tape, by insertion of a 2.5-cm square of paper between the tape and the leaf. Leaf discs could then be easily removed. Four sample leaflets were also laid horizontally along the pathway, with another four positioned horizontally on cards on girders in the space above the row 3.2 m above soil level.

After fogging, sample leaves, also discs 2 cm in diameter cut from each covered area on these leaves, were viewed under u.v. light. Droplets were counted in up to 8 microscope fields at a magnification of x30 to arrive at a total for each disc. Under a lower magnification (x7) the density of droplet cover on the disc was classified subjectively into seven categories and related to droplet counts. To measure droplet distribution over a whole sample leaf a sheet of perspex marked with a grid of centimetre squares was placed over the leaf and the number of squares in each different coverage category was estimated.

The bacterial deposits on the leaf discs were estimated by placing each disc in 10 ml of sterile phosphate buffer, pH 7.2, in a screw capped vial, containing glass balls. This was shaken on a vibratory shaker for 10 min. The washings were diluted (x10 and x100) and the number of viable spores counted by pour plating (Burges and

Thompson, 1971) 1 ml samples in Lab Lemco agar (Oxoid Ltd., London). Bacterial colonies were counted after 24 and 48 h incubation at 30°C.

Control of the tomato moth

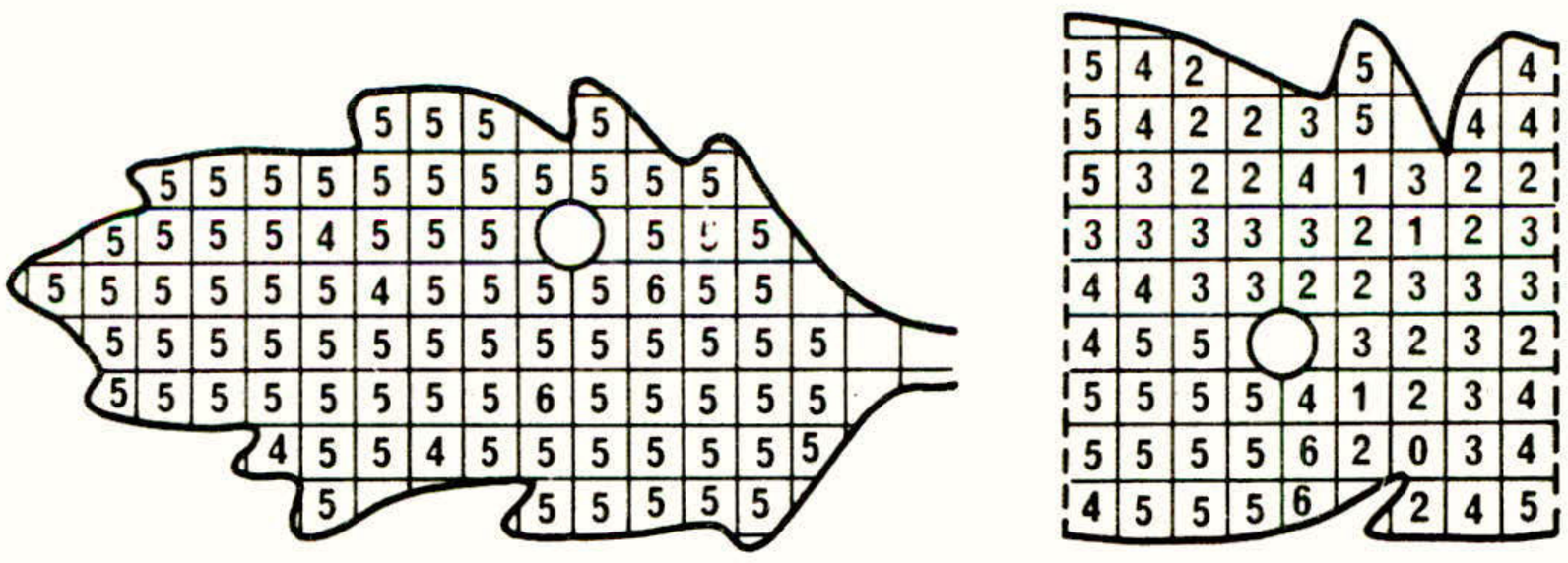
Plants were infested with tomato moth eggs and larvae of different ages which developed from eggs laid by adults which had emerged in the glasshouse 2 weeks before each treatment. Also batches of laboratory-reared larvae were enclosed on entire leaves by muslin bags secured at the leaf petiole just after fogging, at sixteen positions throughout the crops for each treatment. A maximum of 20 larvae were enclosed per bag and moved to a neighbouring fresh leaf every two days to provide fresh food for the larvae and to reduce any effect the shadowing by the muslin may have had in preventing natural loss of activity of *B, thuringiensis*.

RESULTS

Distribution of fog droplets containing *Bacillus thuringiensis*

Fig 1

Distribution of droplets on the upper surfaces of two typical tomato leaves shown as coded droplet ranges judged visually, around the positions of the sampled discs (circles).



Horizontal (a)

Vertical (b)

Code	Mean droplets/cm²
0	0.1
1	3.8
2	32
3	183
4	1017
5	2333
6	4533
7	many extra-large droplets

Subjective visual scans of droplets over whole leaves as well as counts over leaf discs showed that most droplets were deposited on the upper surfaces of the leaves and very few underneath. This was confirmed and quantified by viable spore counts from leaf discs.

Of the viable spores deposited on horizontal and 45° leaves, only 0.5% were deposited on the under-surface. The virtual limitation of spores to the upper surface of these leaves is explained by deposition of droplets by downward settling and impingement. Of the spores deposited on vertical leaves, some 12% were deposited on

the lower surface. This is explained by the curling back of many leaves so that the underside is slightly concave.

Viewing whole sample leaves showed that upper surfaces of horizontal leaves were evenly covered, with the numbers of droplets on adjacent 1-cm^2 areas rarely differing by more than x3, (Fig. 1a). Differences in the order of x100 between neighbouring 1 cm^2 areas were common on vertical leaves, frequently involving patterns like that in Fig. 1b. The surface topography on vertical leaves had an important effect, undulations creating raised areas that tended to be well covered and hollows that sometimes bore virtually no droplets.

The effect of leaf topography explains the wide variation in counts of spores and droplets on disc samples especially on vertical leaves. Examination of the distribution pattern shown by only one leaf disc in a particular posture at each sampling position gave rather fragmentary pictures of distribution of spores over the plants in a row. Examination of mean values, however, gives a reasonably distinct pattern (Fig. 2).

Observation while fogging showed that the fog missed the nearest sample plant (Fig. 2a) and rose slightly around the middle plant. On reaching the end wall it spread mainly upwards, then returned towards the machine, extending into the area above the plants, reaching the end wall behind the fogger where it gently moved downwards, involving a total flow path of 20 m.

Leaf discs taken from the sample plants along the 5-m length of the selected rows showed a reduction in the numbers of spores with distance along the flow path of the fog of about x3.5 on horizontal leaves and x25 on vertical leaves (Fig. 2b, c). The gradient is more extreme if horizontal leaves on the floor and in the roof space are considered as well as those on the plants. The gradient followed the flow path of the fog with the greatest spore coverage on the horizontal leaves on the floor, less on the plants and in the space above the plants and least behind the fogger. The vertical leaves were heavily covered at the lower positions on the middle sample plant and the upper two positions on the end sample plant, consistent with considerable impingement (Fig. 2c). Coverage is also consistent with a downward settling of droplets (Fig. 2b) particularly where the fog lost speed most.

The numbers of droplets per leaf disc (Fig. 2d and e) show the same distribution but less distinctly than the numbers of spores.

Dividing the numbers of droplets into the numbers of spores on the same discs, gives comparative values for average droplet size. Size decreased with distance from the machine along the flow path of the fog. This distribution of droplet size explains why the numbers of droplets give a poorer picture of the distribution of bacteria than spore counts.

The most important factor influencing distribution of bacteria is the orientation of the leaf, with the lower leaf surface receiving 200 times less than the upper on horizontal and 45-degree leaves, which comprised 65.4% of the foliage. Topography of the leaf surface is next in order of importance although playing a significant role only on vertical leaves, which totalled about 9.1% of the foliage. Another factor, shadowing, had a large effect only when the distance between the leaves was 1 cm or less (Table 1). However, only 0.2% were sufficiently shadowed to show this effect.

Table 1

Effect of shadowing by an upper tomato leaf on the deposit of *Bacillus thuringiensis* spores on a lower leaf

Distance (mean) between shadowing and shadowed leaves (cm)	Spores per leaf as % of total on the two leaves*	
	shadowing	shadowed
1	95	5
5	43	57
10	46	54

* Each percentage is a mean of samples from six leaves

Table 2

Larval mortality of 3rd and 5th instar tomato moth larvae 10 days after applying thermal fogs of *Bacillus thuringiensis*

Exp. No.	% concentration (w/v) of *B. thuringiensis*	Mortality (%)	Area (cm^2) of leaf eaten per larvae
1	0	0	21.5
	2.5	77.3	2.25
	10.0	95.6	0.74
2	0	0	17.53
	2.5	80.0	0.78
	10.0	98.0	0.41
3	0	0	18.8
	5.0	91.3	1.1
	10.0	96.8	0.78

Control of the tomato moth

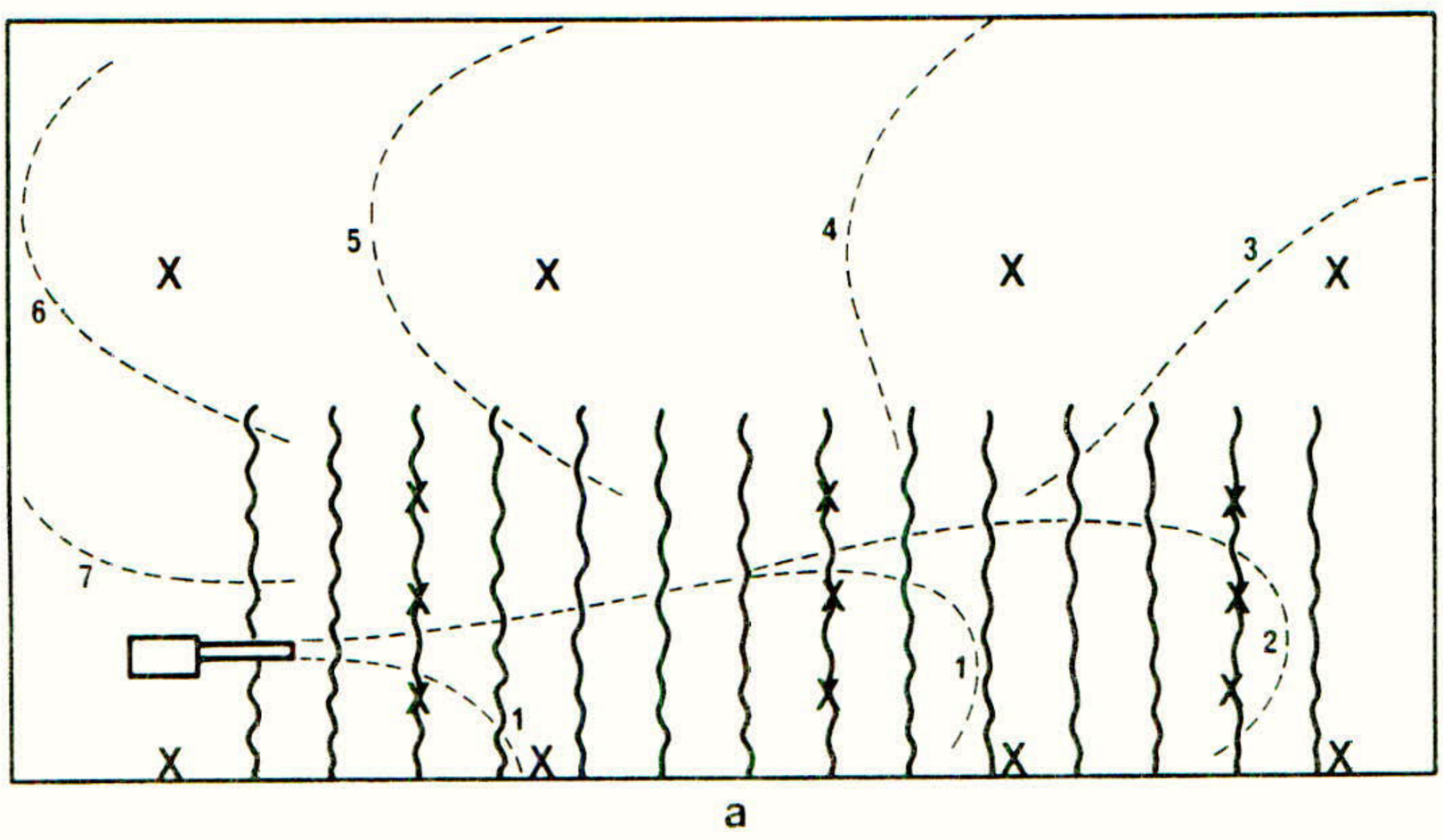
Thermal fogs of *B. thuringiensis* at all three application rates greatly reduced numbers of larvae and consequently the damage they caused to foliage and fruit. *B. thuringiensis* at the highest rate (10% w/v) gave the best control, no fruit being damaged and only 8 surviving larvae being found out of a total of 4000 eggs, first and second instar larvae introduced on the three crops, compared to over 1000 larvae surviving on the untreated control crop after a period of 15 days. Survival of larvae and damage to the crop at the lower concentrations of 2.5 and 5.0% were proportionately greater than those at the 10.0% rate.

Control of the third and fifth instar larvae enclosed by muslin showed the same pattern (Table 2). Whereas survival of control larvae not enclosed was poor that of larvae in the bags was excellent and the bagging technique enabled rapid and accurate larval mortality counts to be made.

Fig 2

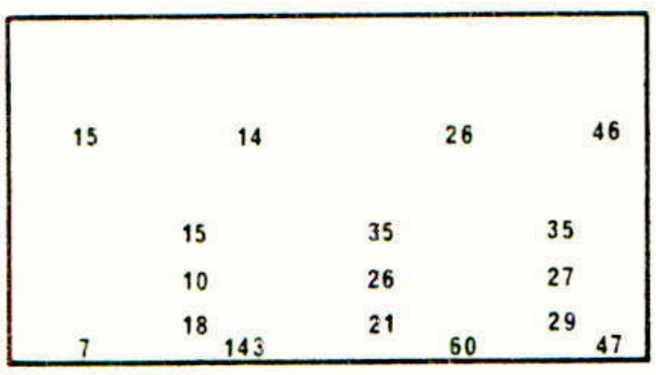
(a) Elevation through row of plants (wavy lines) to show sampling positions (X), position of fogger during fogging and general direction (1 to 7) of distribution of fog (-------).

(b) - (e) Each value at sampling positions on diagrams b-e is based on viable spore counts from four leaf discs taken from leaves at various angles on the plants and two leaf discs from horizontal leaves at positions on the floor and in the space above the plants.



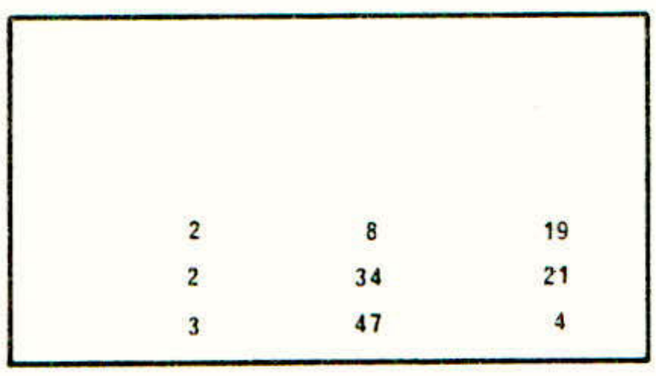
a

Mean of horizontal and 45° leaves

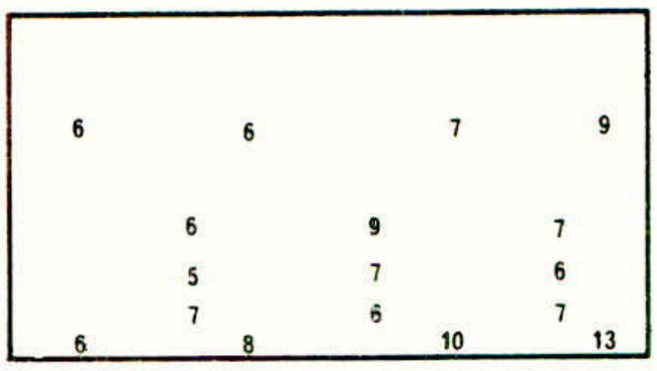


b spores / disc (x 10^4)

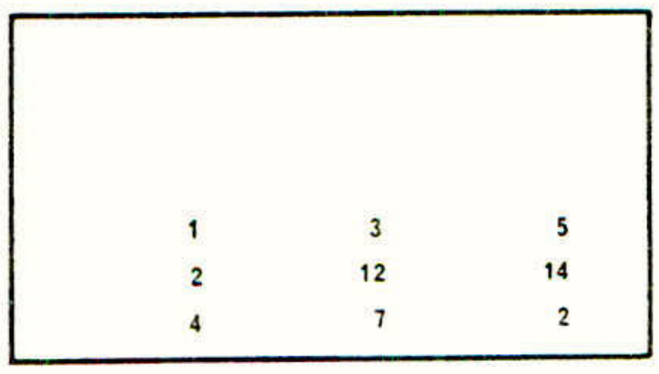
Mean of vertical leaves



c spores / disc (x 10^4)



d droplets / disc (x 10^3)



e droplets / disc (x 10^3)

DISCUSSION

Incorporation of a fluorescent dye (Courshee and Ireson, 1961) with *B. thuringiensis* spores in thermal fogs provided a useful method for quantifying droplet distribution throughout a tomato crop. Such techniques could be used for pesticide distribution on most crops.

The results obtained, which were similar to those found on AYR chrysanthemums (Jarrett *et al.*, 1978), showed that the greatest limitation in the use of thermal fogs for pest control is the very poor underleaf coverage. Thus only chemicals that *slowly* vaporise or have a fumigant, translaminar or systemic action can be effectively used against pests that frequent only the lower surface of the leaf.

This was not a problem for control of the tomato moth using fogs of *B. thuringiensis* as larvae feed for no longer than 5 days, normally only 2 days, on the lower leaf surface, after which time they eat through the leaf, consuming bacteria on its upper surface.

By comparing the distribution of spores over the crop with susceptibility of larvae to *B. thuringiensis*, the proportion of foliage adequately covered to obtain larval control can be obtained. In laboratory bioassay, Burges and Jarrett (unpublished) showed that a coverage of 4×10^5 viable spores per 2-cm leaf disc is required to control larvae at all stages of development. From these results, e.g. at the 5% concentration, it was estimated that at least 74.2% of the foliage was covered with a lethal concentration of bacteria. Larvae feeding on the remaining poorly treated foliage were eventually controlled, as they regularly changed feeding sites, often daily, and in due course ingest a lethal dose of bacteria. For these reasons thermal fogs of *B. thuringiensis* may be recommended for the control of tomato moth larvae at the rate of 4 kg/ha for larvae fourth instar and older, and 2 kg/ha for younger larvae. The degree of control of caterpillars using thermal fogs of *B. thuringiensis* on other protected crops is dependent upon many other factors such as larval behaviour and the susceptibility of the moth species to the bacterium.

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