Studies on *Verticillium lecanii* and *Bacillus thuringiensis* for the control of selected arthropod pests

by

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ABSTRACT

Three projects are presented, each illustrating consecutive stages in the development of a microbial control programme.

The initial project was a survey for fungal pathogens of blackcurrant mites. Samples from various parts of England revealed only *Verticillium lecanii* on dead mites.

In the second project, the potential of *V. lecanii* was studied on a more amenable host, the glasshouse whitefly, *Trialeurodes vaporariorum* (Homoptera), in a more accessible habitat, the foliage of glasshouse crops. Most emphasis was placed on this project. Bioassays of conidia and blastospores showed that the pathogenicity of both was drastically reduced by low ambient humidity. In the glasshouse studies, the two spore types were similar, a single spray giving excellent control of one generation of young whitefly scales at favourable temperatures. Spore concentrations exceeding 10^7 viable spores/ml of spray were unrewarding. A few insects survived due to imperfect spray coverage, because the fungus did not spread from scale to scale. Spraying at two- or four-weekly intervals was necessary for continuous pest control, which would be uneconomical. The fungus was unaffected by the species of crop plant. It did not curb other biocontrol agents, the parasitic wasp, *Encarsia formosa*, and the predatory mite, *Phytoseiulus persimilis*, in the glasshouse. Control of whitefly by the fungus early in the season proved

economically feasible before seasonal conditions allowed effective control by E. formosa. The storage of blastospores and the effect of temperature on fungal growth were studied in the laboratory. Survival of blastospores at 2°C was poor in eighteen suspending media. At -20°C, half-lives exceeded one year in some media. Freeze-drying killed most blastospores, except in a few media, in which about half survived.

In the third project, isolates of Bacillus thuringiensis were screened against the wax moth, Galleria mellonella, as a part of an international programme to find better bacterial strains. The well refined technique was precise and reproducible. The activities of isolates fell into three distinct arbitrary categories. No isolates were significantly better for practical pest control than the best commercial product. The relationship of this work to the programme as a whole is described.

The perspectives of these three projects in integrated pest control are discussed.

I. CONTENTS

II GENERAL INTRODUCTION

Pest control relies heavily on the use of chemical pesticides. Increasing difficulties are being encountered due to the appearance and expanding dominance of pesticide resistant strains of pests. Pesticides create problems in the environment because they are poisons, many potent against non-target life forms. One of the promising alternatives to chemical pesticides is the use of pathogens, a developing, but still limited method (Burges and Hussey, 1971).

The logical steps in the development of the use of pathogens against a particular pest are:

1. A survey for the existence of pathogens.
2. Study of the biology, epizootiology, production and application of potentially suitable pathogens.
3. Development of the technology of standardisation and improvement of suitable pathogens.

This thesis presents three projects, one at each of these three stages. Different pests, crops and environments were involved. A survey was made for pathogens of the blackcurrant mite. The feasibility of using Verticillium lecanii on glasshouse whitefly was examined. Finally work is presented which forms a small part of a large advanced programme on the standardisation of a commercialised pathogen, Bacillus thuringiensis. Although different organisms were involved, they each illustrate a stage of the development of a microbial agent for use in an integrated control programme. Most

emphasis was placed on *V. lecanii* against whitefly, because it presented the best opportunity of developing a varied research programme on a pest problem of current importance.

III EFFECT OF FUNGI ON THE BLACKCURRANT MITE

A. INTRODUCTION

The first step towards the development of the use of pathogens in an integrated pest control system is the identification of naturally occurring pathogens. The importance of pathogens in the natural control of mites has been demonstrated by several authors. In general, however, our knowledge of micro-organisms associated with mites is very incomplete compared with that of insect pathogens. For example, no specific bacterial diseases of mites have been recorded (Lipa, 1971). Few viral diseases of mites are known and the viruses have not been described taxonomically. Although more than 250 species of microsporidians are known in insects, only three have been recorded in mites (Lipa, 1971). Parasitic fungi are the commonest pathogens known to infect various species of mites and are major factors in the natural control of many species of phytophagous mites. Research involving myco-acaricides as possible alternatives to chemicals has increased in recent years.

Thus in the present work, during 1977 and 1978, a survey was conducted for fungal pathogens associated with the blackcurrant gall mite, Cecidophyopsis ribis Westwood in England. Also infectivity tests were carried out in the laboratory, using fungal pathogens on the mites.

1. Literature review

Few mycoses of eriophyid mites have been described. The occurrence of Verticillium lecanii (cited as Botrytis eriophyes Masse) in populations of blackcurrant gall mites was reported by Taylor (1909) from England. Nalepa (1910) made a vague mention of a fungus infecting unspecified eriophyids. In 1911, Del Guercio briefly described a fungus, probably V. lecanii from Phytoptus avellanae, the mite associated with big bud of hazel. Fisher (1950b) described Hirsutella thompsonii from an eriophyid, the citrus rust mite, Phyllocoptruta oleivora, in Florida, where the presence of the fungus was mentioned first by Speare and Yothers (1924). It is the most important natural enemy attacking the citrus rust mite in Florida causing dramatic epizootics during summer in citrus groves (Muma, 1955; Muma et al., 1961; McCoy et al., 1976a, b). It also infects P. oleivora in Texas, China, Surinam and Cuba (Villalon and Dean, 1974; Yen, 1974; van Brussel, 1975; Cabrera, 1977). Also it infects the blue berry bud mite, Acalitus vaccinii, the citrus bud mite, Eriophyes sheldoni, and other Eriophyes spp. (Baker and Neunzig, 1968; McCoy and Selhime, 1977). An undetermined species of Cephalosporium was described by Pesante (1962) from Phytoptus avellanae in Italy. Charles (1941) gave no records of fungi associated with eriophyid or other mites in her North American check list and Leatherdale (1958) added no further data in his British list. In 1963, a fungus which had the gross characteristics of the Masse's species, was present on some of the mites in the galls of plum leaves. This was named as Paecilomyces eriophytis by Leatherdale (1965). He found that Paecilomyces eriophytis, originally described

from an eriophyid, Cecidophyopsis ribis, was infective to Eriophyes padi, Aceria hippocastani and Panonychus ulmi. Though P. eriophytis described by Leatherdale (1965) appears to be V. lecanii it differs from the latter due to the presence of conidial chains which are mostly dry. He mentioned that this fungus was recorded from Berkshire, Gloucestershire and Kent, all in the southern half of England.

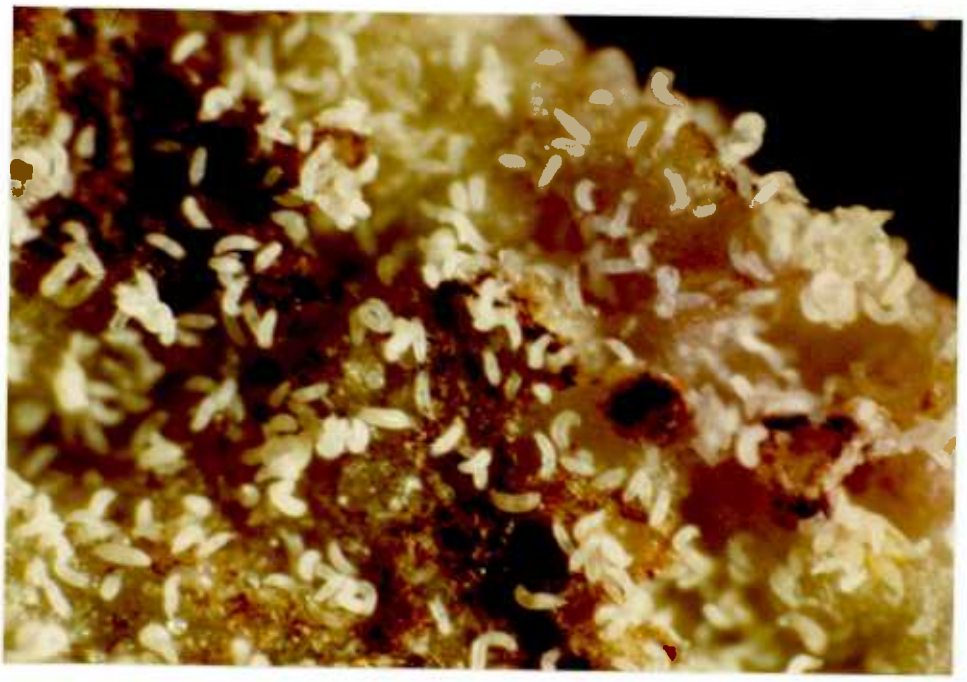
2. Blackcurrant gall mite, Cecidophyopsis ribis

The blackcurrant gall mite has appeared in literature under various generic names, including Phytoptus and Eriophyes. It is a pest of blackcurrants in Europe and British Columbia. The gall mites are elongate in shape, pearly white in colour and less than 0.25 mm long. They breed in the buds of blackcurrant (Fig. III - 1 and 2). All the main varieties of blackcurrants are susceptible (ADAS, MAFF, 1977a). The dispersal of mites from old galls to young buds starts usually in April and may continue into June or even July. However, most of the dispersal occurs between the first open flower and early fruit swell and appears to be closely associated with warm, humid periods during flowering. Dispersing mites swarm on to the outside of the galls and then crawl or leap off, some eventually reaching nearby branches. Dispersal is assisted by rain and wind and by insects such as aphids and capsid bugs. During the migration period the mites live unprotected and feed on the outer bud scales, shoots and leaves. Except for the brief interval between leaving a gall and entering a new bud, a mite lives entirely within a bud. The mites can resist

0·12 mm

Fig. III - 1

Eriophyid mites in a blackcurrant "big bud" cut lengthwise to expose interior



0·4 mm

Fig. III - 2

A closer view of Fig. III - 1

temperatures of -7° to -2°C and become active when the temperature rises (Jeppson et al., 1975). Thousands of mites and eggs may develop in a single bud. The eggs hatch in about 3 days and many generations of mites develop each year.

Newly infested buds gradually change shape during the summer and by autumn they are recognizable as galls or big buds, usually swollen and rounded. In early spring the galled buds swell further and begin to break, but they fail to open normally and do not produce flowers or leaves.

The blackcurrant mite is a vector of the virus disease known as 'reversion' which has been recognized for many years as a widespread and prevalent disease of blackcurrants. The infected plants have to be destroyed as their cropping ability becomes seriously impaired and they serve as a source of infection to the healthy plants.

B. SURVEY FOR NATURAL PATHOGENS

1. Materials and methods

Parcels of big buds were received from various parts of England by post. From each parcel 20-30 big buds were removed from the branches and, under sterile conditions, cut lengthwise into two. These were kept on moist filter papers with the cut end upwards in petri dishes. Small pieces from freshly cut surfaces of eight buds were inoculated on to plates of Sabouraud Dextrose Agar containing 100 µg streptomycin/ml to avoid bacterial contamination. The buds were examined under a binocular microscope for signs of fungal growth and for the presence of active mites. Then the buds and the

inoculated agar plates were incubated at 20°C for about one week. The buds and the agar plates were examined on days 3 and 7 after setting up the experiment, under a binocular microscope for fungal growth. Mites with fungus were mounted on a glass slide with a drop of cotton blue in lactophenol and examined under a light microscope immediately and after one week, when hyphal growth inside the mites could be seen easily.

2. Results

All the mites from a few buds from four of 39 localities in 1977 and one of 15 localities in 1978 were found dead and associated with V. lecanii. In all the other big buds there were active mites. No other fungal pathogens were found. Since none of the infected mites were found alive it is not certain whether the fungus invaded live or dead mites.

V. lecanii was isolated from one sample received from Challock, Kent and the culture was maintained on Sabouraud Dextrose Agar for future studies.

C. LABORATORY STUDIES WITH FUNGAL PATHOGENS

1. Materials and methods

Potted blackcurrant plants bearing big buds were stored at 2°C in the laboratory. Also many more potted plants were stored outdoors for use in the infectivity tests. Large numbers of mites and eggs were found inside the big buds collected from the plants in the laboratory throughout the year and outdoors until April.

Mites could be kept alive on half buds in the laboratory for about one week. The buds remained green and the mites did not migrate from the buds to filter paper. The eggs hatched on the half-buds and the nymphs were active.

The big buds were cut lengthwise into two under sterile conditions and examined under a binocular microscope for the presence of active mites. Spores from cultures on agar plates were checked for viability before use in the infectivity studies. The viable spores scraped from the surface of the following fungal cultures were applied to groups of four half-buds/culture. Each group was kept on moist filter paper in a petri dish and incubated at 20°C;

Verticillium lecanii, isolate C-3, GCRI, and an isolate from C. ribis in the present survey.

Hirsutella thompsonii

Beauveria bassiana

Metarhizium anisopliae

Paecilomyces farinosus

The infectivity experiments were repeated eight times. The buds were examined on or near days 1, 2, 3, 4 and 7 after inoculation.

2. Results

V. lecanii (both isolates) killed all the mites within 2-4 days after treating the buds with spores. It grew and sporulated on individual dead mites and then all over the bud surface. No live mites could be seen with fungal growth. In the control buds mites were alive and active. The mites from control buds had normal body shape, while those from *V. lecanii*-treated buds were often distorted and collapsed. Also hyphal growth was seen inside the dead mites stained with cotton blue in lactophenol (Fig. III - 3). The fungus grew profusely and saprophytically on the decayed buds. *H. thompsonii* grew on two to three mites only once, though it was tested on mites in 32 half buds in eight experiments. *M. anisopliae* grew on some mites (Fig. III - 4) in two of eight experiments. This was confirmed by microscopic examination. *B. bassiana* and *P. farinosus* grew on no mites in the eight experiments.

To test whether *V. lecanii* can persist in the environment, the outdoor blackcurrant plants bearing big buds were sprayed once at the beginning of April between 19-19.30 hrs with 2×10^7/ml viable blastospores. No diseased mites were seen in fifteen buds collected before spraying but, three months after spraying, *V. lecanii* developed on mites in seven of 17 buds during incubation for one week at high humidity in the laboratory.

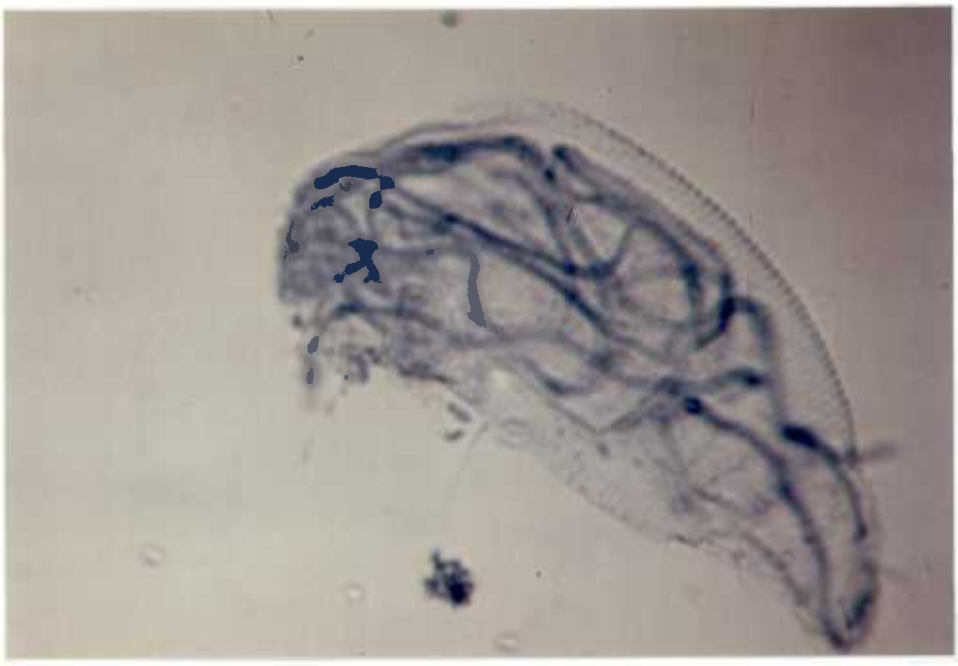


Fig. III - 3

A blackcurrant mite with Verticillium lecanii inside its body, stained with cotton blue in lactophenol

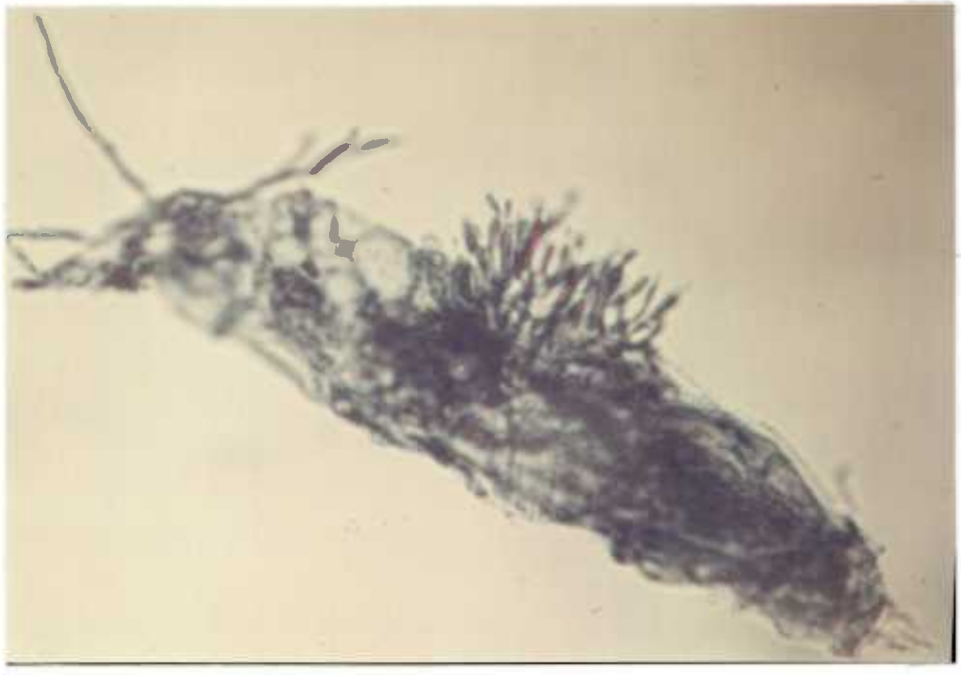


Fig. III - 4

A blackcurrant mite bearing Metarhizium anisopliae, stained with cotton blue in lactophenol

D. DISCUSSION

The incidence of V. lecanii in big buds of blackcurrant was very low, indicating that it does not spread efficiently under natural conditions. Perhaps aphids and other such insects infesting the blackcurrants may have carried the fungus to the big buds. The incidence of V. lecanii on another pest in a different habitat was also low under natural conditions. Barson (1976) reported that, from 1972 to 1974, estimates of the natural larval mortality (> second instar) of elm bark beetles caused by pathogenic organisms were always below 7.5% of the beetle population in England. V. lecanii was frequently isolated from field-collected dead larvae in his studies.

V. lecanii is a facultative parasite. When hosts are not available it can survive by growing on decaying plant residue. Taylor (1909) reported that for six months of the year the fungus is saprophytic on the decaying tissues of the big bud in which it had previously led a parasitic existence on mites. It was shown in the present study that the fungus survived in the outdoor environment from April to July. Most probably the fungus was growing on decaying portions of big buds. This was encouraging and further studies may produce fruitful results especially if the fungus can survive the winter on big buds so that when temperature rises in the spring the fungus can kill the mites. However, it is unlikely that the fungus can gain access to the mites through the densely adhering scale-leaves in a closed big bud, so infection may be late in mite-breeding season.

In the present infectivity studies carried out in the laboratory with various fungal species, though no live mites were found bearing V. lecanii or the other fungal pathogens, it is probable that V. lecanii infected and killed the mites because all the mites in the treated buds were dead within 2-4 days in contrast to the control buds which had live mites. Both control and treated buds remained green, thus the mites did not die due to starvation. Taylor (1909) reported that blackcurrant mites were parasitized by V. lecanii (cited as Botrytis sp.). He found that the fungus was virulent and the infection rapid, killing all the mites and their eggs. The present study confirms his finding.

Taylor (1909) also reported that at an early stage of infection, a mite, while still alive, may have conidiophores and hyphae emerging from the skin and in most cases subcutaneous hyphae could be distinguished. The internal tissues contracted from the cuticle and the mite became distorted in shape with its interior a mass of interwoven hyphae.

It is unlikely that fungal pathogens will replace chemical control of blackcurrant mites because, as well as the difficulty caused by the hidden habit of the mites, planting certified stock, careful timing of chemical sprays such as endosulfan, and intensive roguing, keep most of the commercial plantations virtually free from mite infestation. In most areas, endosulfan is now used almost exclusively to control gall mite (ADAS, MAFF, 1977a) None of the available chemicals or V. lecanii kills mites within galled buds.

It is therefore necessary to protect the new growth during

spring when mites are leaving the galls. However, to avoid any hazard from residues on the fruit at harvest, the number and timing of endosulfan applications to fruiting bushes are strictly limited; a minimum interval of 6 weeks must elapse between the last application and harvesting. V. lecanii would have an advantage during this period since it has not been recorded as a harmful agent to human beings though it is being widely used in experiments in many parts of the world. V. lecanii is preferable to the other fungi tested in the present study because it was the most virulent pathogen. Brady (1979) reported that V. lecanii parasitises all developmental stages of insects of all groups and of Arachnida. This fungus may even infect other pests of blackcurrants. Thus the possibility of using this fungus as an alternative to chemicals may be worth evaluating.

A few high volume sprays of spore suspensions of V. lecanii in the evenings to avoid sunshine in April and May, when the big buds partly open and the mites migrate from old buds to the new buds, may be useful. The spray can penetrate inside old buds, spores can be carried by migrating mites to new buds and the fungus can grow saprophytically on decaying buds and sporulate to provide more spore inoculum. During April and May the temperatures in the day time reach about 15°-20°C, which is adequate for the growth of V. lecanii (Section IV, B, 1). Also there is sufficient rainfall to keep the decaying plant matter moist and hasten the growth and development of this fungus. Inside the big bud the mites and V. lecanii can survive the low temperatures of the winter. V. lecanii survived at -20°C for long periods in the laboratory (Section IV, B, 2).

In studies with the coconut eriophyid mite, Eriophyes guerreronis, Hall et al. (in press) reported that the natural incidence of H. thompsonii was low, but when present on a nut, the fungus assumed epizootic proportions beneath the bracts of coconuts, except in one sample in which the disease was incipient. They also tested against E. guerreronis the strain of V. lecanii isolated from C. ribis in the big buds received from Challock in the present study. Though they could see invading hyphae of H. thompsonii inside live mites mounted and cleared in Hoyer's fluid, they could not discover live mites bearing internal growth of V. lecanii. However, the strain of V. lecanii grew and sporulated profusely on mites in 3-4 days, apparently killing them. McCoy and Selhime (1977) found no anatomical deformation until after death of P. oleivora caused by H. thompsonii. They suggested that for positive identification of infected mites, dead mites should be cleared, mounted and examined with a phase contrast microscope.

Gerson et al. (1979) reported that H. thompsonii grew well on cadavers of diverse insects and mites. They also found that H. thompsonii was highly pathogenic to the carmine spider mite, Tetranychus cinnabarinus, and to the oriental spider mite, Eutetranychus orientalis. McCoy and Couch (1979) reported that H. thompsonii is infectious at the conidial stage and attacks a number of species of mites, primarily eriophyids inhabiting citrus. They suggested that the fungus required 90-100% R.H. or free water to be infectious. In the present study the strain of H. thompsonii obtained from McCoy did not kill blackcurrant mites. Perhaps further studies using other strains may give fruitful results.

IV. VERTICILLIUM LECANII ON GLASSHOUSE WHITEFLY

A. INTRODUCTION

Glasshouse whitefly, Trialeurodes vaporariorum Westw. is a serious pest of many species of plant grown in glasshouses in the U.K. (ADAS, MAFF, 1978) and in many other countries, e.g. the Netherlands, Sweden, U.S.A., Canada and Japan. Crops subject to severe attacks are cucumber, tomato, French bean and a large number of ornamentals, among which may be mentioned fuchsia, Gerbera, Pelargonium, poinsettia, Solanum and occasionally chrysanthemum.

1. Biology of the whitefly

Adult whiteflies infest the uppermost leaves and lay eggs on the ventral leaf surface. Mobile larvae ("crawlers") emerge from the eggs but soon become firmly anchored to the leaf where they pass through the larval and pupal instars as "scales", terminating in the emergence of the new adults. During the pupal instar the scales become thicker, heavily coated with wax and bear long waxy filaments. Adult whiteflies are 1 mm long and are snowy white due to a covering of white, mealy wax. Meanwhile the plant has grown so that the various developmental stages are found progressively lower down the plant, all on the ventral leaf surface. Both the adults and young scales suck sap from the foliage through their stylets. When present in large numbers they weaken the plant. The principal injury, however, is due to the excretion, by adults and scales, of "honey dew", a sticky substance on foliage and fruit. This encourages extensive growth of sooty mould, Cladosporium sphaerospermum (Fig. IV - 1). In severe infestations this fungal mat interferes

Fig. IV - 1

Sooty mould damage on tomatoes due to heavy infestation of glasshouse whitefly

with photosynthesis. If an infestation is allowed to increase unchecked much foliage may be killed and the crop seriously reduced. Tomato and cucumber fruits have to be cleaned before marketing.

The life-span of the female adults is about three to six weeks during which time each lays about 200 eggs. The duration of the life-cycle and rate of egg production varies with temperature which is illustrated in detail in Table IV - 1, for correlation with data on biocontrol agents presented later.

2. Chemical control

Many chemical insecticides give some control. Repeated treatments at 3-5 day intervals are essential for several weeks before control can be achieved. This may cause phytotoxicity. Strains of whitefly resistant to one or more of these compounds have become established (Wardlow *et al.*, 1975; Moreton and Wardlow, 1974).

Biological control was introduced to overcome these problems. No chemical used for whitefly control is tolerated by the predator, *Phytoseiulus persimilis* (Scopes and Ledieu, 1979).

3. Biological control by a parasitic insect

Whiteflies are parasitised by certain minute Chalcid wasps, one of which, *Encarsia formosa* Gahan has been used successfully in the U.K. for many years to control whitefly infestations on a variety of glasshouse plants (ADAS, MAFF, 1978). Females insert eggs into third instar whitefly scales (Hussey *et al.*, 1969). At 18°C each adult lays at least 60 eggs (as many as 400 have been recorded) during its adult life of 18-20 days (Scopes and Ledieu, 1979). The complete life-cycle takes place within the scale. Its duration is temperature-dependant as illustrated in Table IV - 2 for the design of an

Table IV - 1

Effects of temperature on approximate rates of whitefly development

(Agricultural Training Board; 1975a)

Temperature, °C	Incubation period of eggs (days)	Development period (days)		Total	Adult longevity (days)	Eggs/ female/ day
		Larval instar (scales)	Pupa			
15.6	15	27	21	63	50	4
18.3	12	17	11	40	40	5
21.0	8	10	7	25	32	5.5
23.9	6	8	6	20	20	7

Table IV - 2

Development of Encarsia formosa at different temperatures

(Agricultural Training Board; 1975b)

Temperature °C	Oviposition to black scale (days)	Black scale to adult (days)	Total life-cycle (days)
12.8	33	57	90
18.3	17	13	30
23.9	9	8	17

integrated control trial. Wyatt (1972) stressed the importance of the relative rates of host and parasite population increases in biological control strategies. At 27°C, E. formosa develops twice as fast as whiteflies but fecundity of both is about equal; at 18°C, development rates are equal but whiteflies lay ten times more eggs (Anon, 1976). Biological control by E. formosa is best above 21°C (Hussey et al., 1969; Hussey and Bravenboer, 1971). Burnett (1960a, 1960b, 1967) studied pest-parasite interactions and noted that when there was a shortage of third instar scales the younger scales were repeatedly probed and killed. There was no oviposition in second instar scales. Adult parasites normally feed on honeydew (Hussey et al., 1969).

The use of parasitic insects and predatory mites for biological control greatly limits the range of chemicals that can be used against the other pests which may attack the crop. Invasion by aphids, for instance, may necessitate the abandonment of biological control. Also at some seasons and in some circumstances biological control by predators and parasitic insects fails. An alternative was, therefore, sought for E. formosa against the whitefly.

4. Pathogens of whiteflies and occurrence of Verticillium lecanii

Glasshouse whiteflies were found naturally infected and killed by three fungi, Penicillium sp., Cladosporium sphaerospermum and Verticillium lecanii Zimm. (cited as Cephalosporium aphidicola Petch) by Hussey (1958). He found that V. lecanii was a true pathogen of whitefly scales and adults but not of eggs and crawlers. Whiteflies on centropogon at Wisley were infected with V. lecanii (cited as Cephalosporium lefroyi) (Horne, 1915). In Sweden, V. lecanii

naturally infected whiteflies, aphids and thrips on glasshouse crops (Ekbom, 1979a).

Species of Aschersonia were reported as parasitic fungi of scale insects (Coccidae) and whiteflies (Aleyrodidae) in North America (Mains, 1959). Primak and Chizhik (1975) studied the possibility of utilization of Aschersonia aleyrodis to control T. vaporariorum. Aschersonia has been used in the past few years in the U.S.S.R. (Osokina and Izhevskij, 1976). However, these fungi are less common than V. lecanii.

The nomenclature of the Verticillium-Cephalosporium group of fungi was reviewed by Hall (1977) who favoured the system of Gams (1971), which has also been followed in this thesis. V. lecanii is an insect pathogen with a fairly wide host range and a broad geographical range. Evlachova (1938) reported that it was widespread on scale insects in the sub-tropical regions of the Union of Socialist Soviet Republics (U.S.S.R.). Samsinakova and Kalalova (1975) recommended the use of V. lecanii for control of the scale insect, Coccus hesperidum. Easwaramoorthy and Jayaraj (1978) found that this fungus was highly effective in the field against Coccus viridis, the coffee green bug in India. The fungus was recorded from Ceylon by Nieter in 1861 (Petch, 1925) as a parasite of the scale Lecanium coffeae and also of aphids (Petch, 1932) and later recorded on Capitophorus fragariae on strawberry in Scotland (Petch, 1942). Among the parasitic fungi observed during a survey for natural enemies of aphids in India, V. lecanii (cited as Cephalosporium aphidicola Petch) took a heavy toll of Aphis spiraecola, Brevicoryne brassicae, Lipaphis erysimi and Myzus persicae (Ramaseshiah and Dharmadhikari, 1968). In Britain, Leatherdale (1970) listed

Homoptera as frequent hosts of the fungus. Hall (1977) and Hall and Burges (1979) reported their successful attempts to control some aphid pests of chrysanthemum in glasshouses in the U.K. V. lecanii has also been recorded from other insect groups including Coleoptera (Leatherdale, 1970; Barson, 1976; Santharam et al., 1978) and mites (Leatherdale, 1965). Glen and Milsom (1978) found in the U.K. in 1975, 1.1% of larvae of codling moth, Cydia pomonella constructing cocoons on the soil was covered with fungi, notably V. lecanii. Mancharan and Jayaraj (1979) and Balasubramanian (1979) reported V. lecanii as a promising biological control agent of Nilaparvata lugens, the brown plant hopper of rice in India. Spencer (1980) recorded parasitism of carnation rust (Uromyces dianthi), a phytopathogenic fungus, by V. lecanii in England.

5. Biology and potential of V. lecanii for controlling whiteflies

A mixed method involving submerged and surface cultures has been developed by several workers, mostly in the Ukrainian Institute for Plant Protection in Kiev, for the production of conidia of Beauveria bassiana, Metarhizium anisopliae and Verticillium lecanii (Goral, 1971; Goral and Lappa, 1973; Samsinakova and Kalalova, 1976). This technique has enabled the production of conidia of B. bassiana in an experimental preparation called "Beauverin" or "Boverin" with 2×10^9 conidia/g in inert Kaolin in the U.S.S.R. In Brazil M. anisopliae conidia grown on boiled rice for two to three weeks was dried at 25°C and 35% R.H. for three days and ground into a powder named "Metaquino". This preparation was stored at 7°C (Aquino et al., 1975;

Guagliumi et al., 1974; Moura Costa and Magalhaes, 1974; Moura Costa et al., 1974). Evlakhova (1966) has cultivated V. lecanii (cited as Cephalosporium lecanii) on media based on corn, wheat, potato, pumpkin and other vegetables. A wettable powder formulation of conidia of V. lecanii, cultured on inexpensive solid substrate by Tate and Lyle Ltd., Reading, England, has been used successfully to control aphids on chrysanthemum in commercial trials in England (Hall and Burges, personal communication). This fungus can also be produced on a large scale for experimental purposes in liquid media (Hall, 1977 and section IV, D, 2). Hall (1977) suggested that it had potential for commercial exploitation for pest control in glasshouses.

Though some insecticides and fungicides were found to affect growth and germination of spores of this fungus in the laboratory (Olmert and Kenneth, 1974; Wilding, 1972b; Easwaramoorthy and Jayaraj, 1977), it is possible to control the pests in field and glasshouse when the chemicals were used with care (Hall, 1975; Easwaramoorthy et al., 1978).

V. lecanii has no ability to spread on its own among pests on crops because the spores are not air-borne but covered by slime at the tips of conidiophores, in contrast to the dry powdery spores of two other entomopathogenic fungi, Beauveria bassiana and Metarhizium anisopliae.

The most important feature of V. lecanii is its ability to infect and kill insects in the glasshouse environment (Hall and Burges, 1979) and in the field (Easwaramoorthy and Jayaraj, 1978). Laboratory studies show that it grows and sporulates well on

artificial media and is highly infective against insects at temperatures between 20 and 25°C (Barson, 1976; Hall, 1977).

The storage ability of conidia and blastospores has been only partially investigated. Though blastospores of B. bassiana and M. anisopliae could be produced on a large scale, their rapid death caused large scale production to be abandoned in some countries (Ferron, 1978).

The aim of the present study is to find out whether V. lecanii can be used to control whiteflies in glasshouses and to investigate outstanding problems, such as the storage of spores.

In the laboratory two aspects in the biology of V. lecanii were studied. One was the effect of different temperatures on growth on a good artificial medium so that its temperature requirements could be correlated with those of the whitefly. The second aspect was the preservation of blastospores by a variety of methods. In parallel with these studies, the susceptibilities of different stages of the insect and the effect of spore concentrations on whitefly scale mortality at dry, humid and wet conditions were investigated.

In glasshouses, on cucumbers infested with whiteflies, the effects of a single spray of conidia, a double spray of conidia and blastospores at different concentrations and finally repeated sprays of blastospores at different frequencies and concentrations were tested. Initially, synchronised populations of scales were used at different temperatures. Then multi-aged populations were used under conditions simulating commercial cultivation.

In both laboratory and glasshouse, the compatibilities of V. lecanii with the two commonly used biological control agents,

Phytoseiulus persimilis and E. formosa, were studied to assess its potential for integration into biological control programmes. Finally an integrated glasshouse trial was conducted. Investigations included the effect of plant species on spore survival on leaves and pathogenicity of fungus on scales on various plant species.

B. BIOLOGY OF VERTICILLIUM LECANII

1. Effect of temperature on growth

For use of Verticillium lecanii in laboratory and glasshouse experiments it was necessary to know the temperatures favourable for its growth. As effect of temperature on growth of the strain C-3 (GCRI) of V. lecanii has been investigated only once (Hall, 1977) it was considered necessary to repeat the experiment in order to confirm his findings.

(a) Materials and methods

Treatments at all temperatures were identical using strain C-3 (GCRI) on Sabouraud Dextrose Agar (SDA) of standard strength, (65 g/litre of distilled water) in 9 cm-petri dishes. As growth is influenced by agar depth (Chaudhuri, 1923), the agar (25 ml/dish) was allowed to solidify with the dishes on a horizontal surface. Inoculum plugs of 12 mm diameter, cut from a 10-day uniform confluent growth of V. lecanii on SDA, were placed one per petri dish in a central hole of matching size cut in the new agar. Four replicate dishes per temperature were incubated at 10 temperatures between 2.0°C and 31.5°C (Fig. IV - 2). Sometimes temperature

fluctuations of about ± 0.5°C occurred. Growth was estimated by measuring twice weekly the diameter of colonies growing outwards from the inoculum plugs until the fungus reached the margins of the dishes.

(b) Results

Growth was negligible at 2°C until four weeks after inoculation, then slight, with the growth medium appearing reddish underneath the colony. At 11.5°C to 25°C the colonies were white. Growth increased with temperature up to an optimum near 25°C, then decreased rapidly (Fig. IV - 2 and Fig. IV - 3). The growth-temperature curve in Fig. IV - 2 is skewed towards high temperature. There were very small irregular discoloured (grey) colonies at 30°C and no growth at 31.5°C. Selected growth rates are given in Table IV - 3 between 10-25 days while growth was even as shown by the straightness of the curves.

(c) Discussion

Hall (1977) found that the growth rate of V. lecanii was optimal at 23.5°C and above 25°C the rate fell steeply. Growth ceased above 30°C. At temperatures below 20°C growth slowed but was still detectable at 2°C. His findings agree with the results of the present study, probably because the same isolate of the fungus and growth medium were used in both studies.

The shape of the curves in Fig. IV - 2 are typical of temperature-growth curves of living organisms (Cochrane, 1958). Growth at temperatures near the minimum is often so slow that it is undetectable at a time when cultures at more favourable temperatures

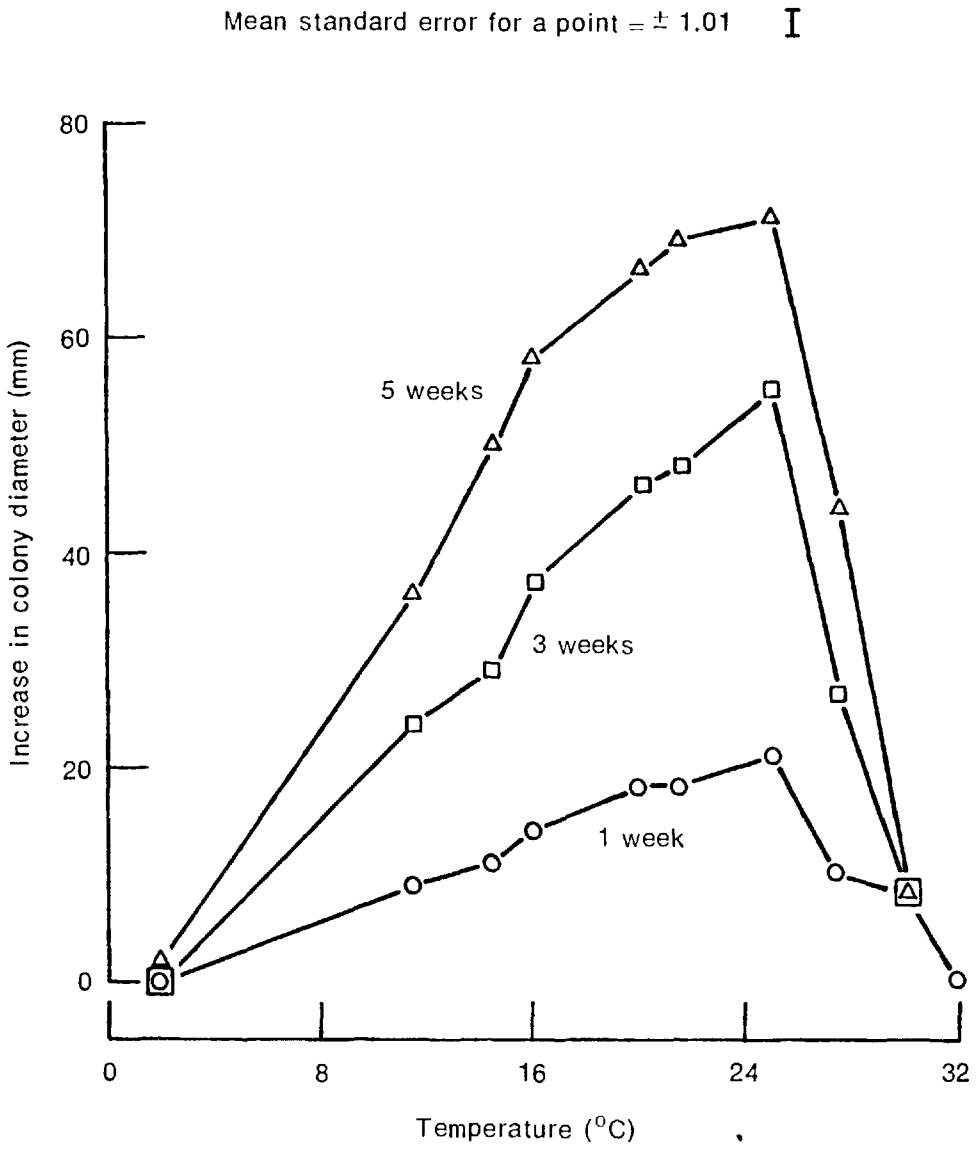


Fig. IV – 2

Effect of temperature on radial growth of *Verticillium lecanii* on Sabouraud Dextrose Agar when incubated for 1, 3 and 5 weeks after inoculation. Increase in colony diameter is the colony diameter less the initial plug size (12 mm).

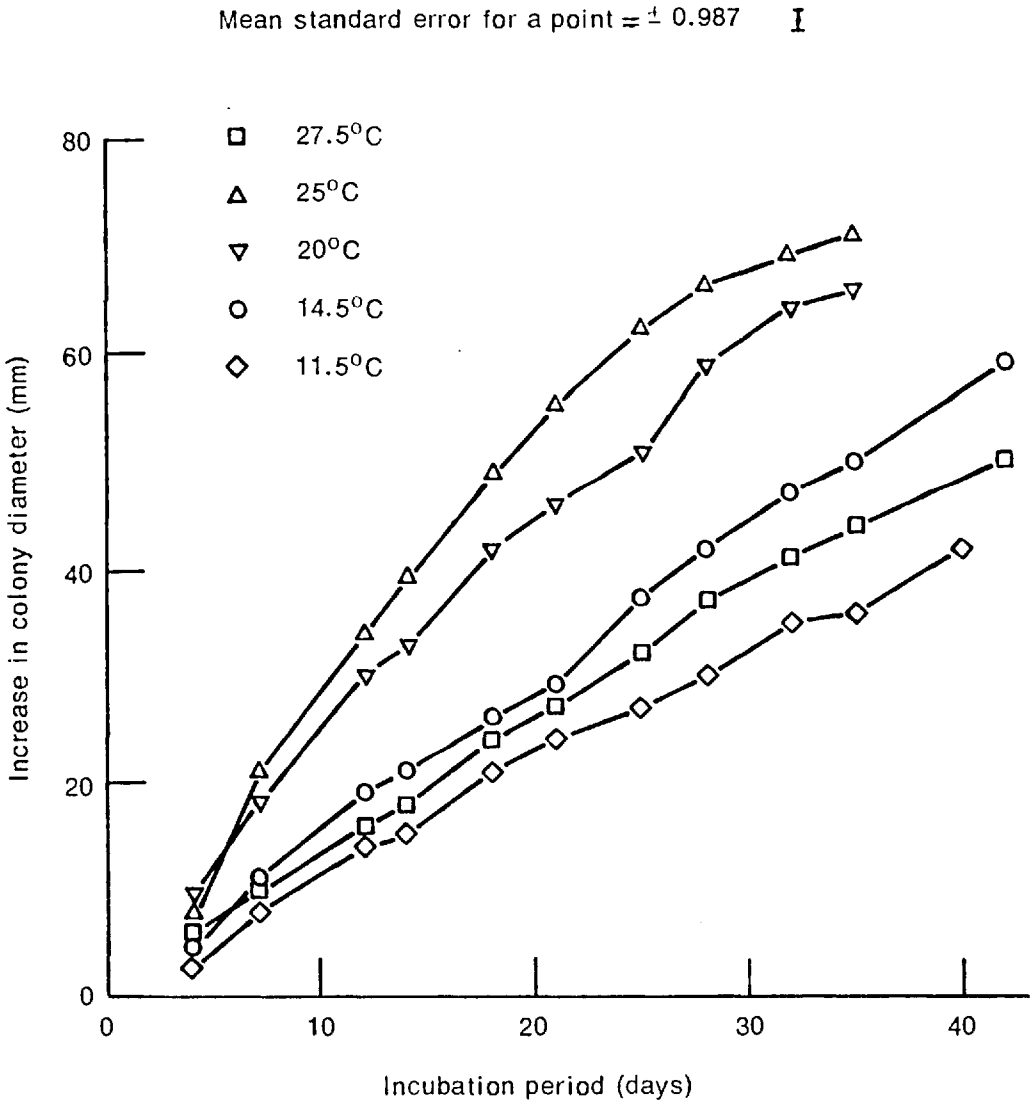


Fig. IV – 3

Effect of temperature on radial growth of *Verticillium lecanii* on Sabouraud Dextrose Agar when incubated for up to 6 weeks. Increase in colony diameter is the colony diameter less the initial plug size (12 mm).

Table IV - 3

Rate of growth of Verticillium lecanii at five selected temperatures on Sabouraud Dextrose Agar given as slopes of fitted straight line graphs (Fig. IV - 2) between days 10 and 25

Temperature, °C	Growth, mm/day
11.5	0.6
14.5	1.4
20.0	1.8
25.0	2.5
27.5	1.1

are growing vigorously. Growth at supra optimal temperature may begin rapidly but slow down or cease entirely after a period of time (Brooks and Cooley, 1921; Norman, 1930; Stoll, 1954). Thus use of only one incubation period may introduce error. Hence growth curves at 1, 3 and 5 weeks after incubation were given in Fig. IV - 2.

Easwaramoorthy and Jayaraj (1977) found that the growth was best at 25°C to 30°C, the maximum growth occurring at 25°C. They used Czapekdox agar medium and a different isolate of the fungus, probably from an area with a warm climate in India. However, only one observation was taken, i.e. at 6 days after seeding. This is inadequate to draw any reasonable conclusion. In contrast, in the present study colonies were measured twice weekly for up to 6 weeks. Ekbom (1979b) obtained results very similar to those of the present study, using an isolate from the temperate climate of Sweden. Barson (1976) found a rather lower temperature range with the maximum mean linear growth rate of an isolate from the temperate U.K. from Scolytus scolytus on nutrient agar at about 20°C, very slow growth at 30°C, which almost stopped after 18 days, while at 5°C — although slow — the growth was still increasing after 18 days. Ganhao (1956) reported a temperature optimum for total growth at 23°C and a steep decline in growth above 25°C. Thus the results of the present study are close to those of the other workers except the one in India.

The optimum temperatures for different geographical isolates of the same species may vary: sub-tropical strains of a species may have a higher temperature optimum than strains from temperate regions (Brown and Wood, 1953).

The upper temperature limits for different strains of V. lecanii

vary greatly (Hall, unpublished). Several strains grew well at 31°C, four grew at 34°C and one grew very slightly at 36°C. Different species within a genus differ widely. Optimum growth rates for several plant pathogenic species of the genus Verticillium are lower than those of V. lecanii (Isaac, 1953).

In the present study the optimum temperatures were found by measurement of radial growth. Chaudhuri (1923) found excellent correlation of radial growth on agar with dry weight and germ tube growth in temperature studies on Verticillium albo-atrum. Domsch (1955) has confirmed the correlation in temperature response.

The optimum temperature for growth can be altered by nutritional factors. The response of Phycomyces blakesleeanus to temperature (Robbins and Kavanagh, 1944) illustrates the principal ambiguities in the concept of optimum temperature. First, growth is normally most rapid at 20-25°C, but the maximum total amount of growth at limiting levels of thiamine is greatest at 10°C. Second, the effect of temperature on final dry weight can be almost wiped out if the nitrogen supply is made limiting. Another example of the dependence of temperature characteristics on other factors has been elucidated by Fries (1953). Coprinus fimentarius grows poorly at 44°C because of the failure of methionine biosynthesis to keep pace with other processes; if exogenous methionine is supplied, growth at the elevated temperature is normal. The apparent optimum temperature for radial growth of Sclerotinia fructicola is affected by pH (Tilford, 1936). Thus, in the present study, the range of optimum temperatures reported for V. lecanii (C-3) are specific to the conditions of the experiment described but probably representative of

the organism's typical ability to grow because Sabouraud Dextrose Agar medium is near-optimal for growth.

The results of the present study probably reflect the likely performance of the fungus on an insect. In glasshouse experiments the control of whitefly scales by V. lecanii was higher in the compartments with favourable temperatures than in those with unfavourable high temperatures (section IV, D, 2 and 3). The relation between a fungus-incited plant disease and temperature is complex. In general, storage and transit rot diseases are most severe at the temperature which favours mycelial growth of the pathogen (Lauritzen and Harter, 1925; Weimer and Harter, 1923; Lauritzen, 1929; Wellman, 1932).

There are some plant diseases in which the observed action of temperature appears to be exerted on the pathogen, but on spore germination or sporulation rather than on mycelial growth (Felton and Walker, 1946; Jones, 1924; Jones et al., 1926). It is conceivable of course that pathogenicity, as distinct from growth, is affected by temperature.

2. Preservation of blastospores

Blastospores died within a few weeks when stored at 2°C for use in various experiments. Thus it was necessary to culture fresh blastospores for laboratory and glasshouse experiments, which was not only difficult but also inconvenient. Therefore experiments were carried out in the hope of prolonging viability during storage. First the effect of the initial age of spores on survival was studied, followed by the effects of various suspending media on survival at 2°C,

at approximately -20°C and during freeze drying.

(a) General methods and materials

(i) Culture of blastospores. About 150 ml of a solution of Sabouraud Liquid Medium (SLM) in distilled water (D.W.) at 30 g/litre was dispensed into each of six to eight 250 ml conical flasks. Two to three drops of polypropylene glycol 2025 (BDH Chemicals Ltd., England) were added to each flask which was stoppered by a rubber bung with a glass tube plugged with cotton wool passing through the middle to provide ventilation. After autoclaving and cooling, the flasks were inoculated with aliquots of conidia harvested in sterile distilled water from cultures on Sabouraud Dextrose Agar (SDA) in petri dishes. Harvesting and inoculation were carried out aseptically in a sterile cabinet previously irradiated with ultra violet light for 45 mins. and flushed with a continuous flow of sterile filtered air.

The flasks were incubated on a wrist action flask shaker (Gallenkamp, England) at 20 to 21°C for 5 to 8 days except for the 'age' experiment. After checking for contamination the blastospores were centrifuged at 3500 r.p.m. for about 30 min. The pellet was thoroughly mixed with sterile distilled water (SDW) and centrifuged again to remove traces of the spent culture medium. The pellet was resuspended in about 25 ml SDW and dispensed into sterile McCartney bottles containing various media for use at 2°C, -20°C and for freeze drying. The same spore suspension was used for all three treatments whenever treatments were set up on the same day.

(ii) Media for blastospore suspension. Sterile media were used (Table IV - 6). D.W. was used for dilution. Glucose was autoclaved before mixing with horse serum purchased sterile. Skimmed milk (10% w/v) was sterilized by autoclaving for only five mins. to avoid caramalization of lactose. All the other media were autoclaved normally (15 p.s.i. for 20 min. at 121°C).

Hank's solution was prepared by autoclaving parts A and B (Oxoid Ltd.) separately, cooling, mixing together, diluting and reautoclaving. To 400 ml of this solution after cooling, 3.2 ml of 4.4% sodium bicarbonate was added to form a balanced salt solution.

The constituents were:

Hank's Solution

Part 'A'	Percent in solution	Part 'B'	Percent in solution
KCl	0.04	$CaCl_2$	0.014
Na_3PO_4	0.006	$MgSO_4$	0.01
KH_2PO_4	0.006	$MgCl_2$	0.01
NaCl	0.4358	Dextrose	0.1
Phenol red	0.002 (q.s.)	NaCl	0.3642

Sabouraud Liquid Medium

Formula	Percent in solution
Pancreatic digest of casein (Oxoid L42)	0.5
Peptic digest of fresh meat (Oxoid L49)	0.5
Dextrose	2.0

pH 5.7 (approx.)

Dissolved 30 g in 1 litre of distilled water and autoclaved.

Nutrient Broth (Oxoid Ltd.)

Formula	Percent in solution
'Lab-Lemco' Beef Extract	0.1
Yeast Extract (Oxoid L20)	0.2
Peptone (Oxoid L37)	0.5
Sodium chloride	0.5

pH 7.4 (approx.)

Dissolved 13 g in one litre of distilled water and autoclaved.

Honey (Prickly-pear honey of Queensland) Strong (1952)

Formula	Percent
Water	20
Cane sugar	0.95
Invert sugar	77.70
Dextrin	0.23
Minerals	0.11
Acid (formic acid)	0.14
Others	0.87

Skimmed Milk Powder (Oxoid Ltd.)

Average analysis:	Percent
Moisture	5.0
Ash	8.0
Total Nitrogen (includes Casein and lactalbumin)	5.3
Reducing sugars (as lactose monohydrate)	48.0
Ether soluble extract	0.25

Horse Serum

Seralbumin, a simple protein soluble in water, and globulin.

Spent SLM

Blastospores in the SLM in which they were grown.

(iii) Total Spore Count (T.S.C.). After thorough mixing using a whirlimixer, a spore suspension was suitably diluted with D.W. The coverslip of an 'Improved Neubauer Counting Chamber' (Gallenkamp Ltd., England) was fixed by pressing firmly down on to the base moistened by breathing on it. When colour rings appeared under the cover slip, the spore suspension was shaken well and applied with a Pasteur pipette to the counting chamber without flooding. The spores were allowed to settle for 2 mins. and counted under phase contrast microscope.

(iv) Viable Spore Count (V.S.C.). Molten Sabouraud Dextrose Agar (SDA) was uniformly spread on a clean horizontal glass slide and allowed to solidify. About 20 to 25 ml distilled water was used to dilute three to four drops of spore suspension (10^6 to 10^8 spores/ml) in a McCartney bottle and mixed well. With a Pasteur pipette, one or two drops were added to the agar-slide near the two ends and in the middle. Then the slide was incubated on a moist filter paper in a petri dish at $20^{\circ}C$ for about 15 h before counting live and dead spores under phase contrast illumination at a magnification of X400. Ungerminated spores and a few atrophied hyphae, non-uniform in appearance were counted as dead. The live spores germinated and even produced short branches. These appeared smooth in outline and

uniform in thickness. The whole length of the slide was examined for uniformity in distribution of live and dead spores, taking counts at different positions to ensure that a true mortality pattern was determined. When one day's observation was inconsistent with previous observations, the V.S.C. was repeated by setting up a new agar slide.

(b) Effect of blastospore age on survival at 2°C

(i) Materials and Methods. Conidia from a single petri dish culture were inoculated into six conical flasks with SLM to culture blastospores (section IV, B, 2, (a), (i)). After days 2, 3, 7, and 11 of inoculation, one flask was centrifuged, the pellet washed and divided into two portions. One portion was thoroughly mixed with sterile distilled water and the other with sterile fresh SLM, in two separate McCartney bottles per medium and stored at 2°C with loose lids. Viable spore counts were made on or near days 0, 1, 2, 4, 8, 16 and 32 with diluted samples from the two replicate bottles, inoculated one at each end of the same slide. This experiment was repeated once, extending the storage period to 64 days.

(ii) Results. Analyses of probit percent spore mortality against log time (Table IV - 4) for the blastospores of each age suspended in both media showed that the median lethal times, LT_{50}s, were all less than a month.

The older the spores, the longer the survival in both media (Fig. IV - 4). The statistical significance of this effect was examined by a linear regression analysis of survival against age for each storage medium using

Table IV - 4 **

Effect of age of blastospores of Verticillium lecanii on survival when suspended in two liquid media and stored at 2°C

Age in days	Medium	No. of experiments	Log LT_{50} ± S.E in log days	Natural LT_{50} & 95% limits in days	Sign of difference in Log LT_{50} SLM-D.W.
2	SLM*	1	0.729 ± 0.028	5 (5, 6)	+
	D.W.	1	0.343 ± 0.159	2 (1, 4)	
3	SLM	2	0.924 ± 0.213	8 (3, 22)	+
	D.W.	2	0.827 ± 0.175	7 (3, 15)	
5	SLM	1	1.195 ± 0.024	16 (14, 17)	+
	D.W.	1	0.989 ± 0.074	10 (6, 13)	
7	SLM	2	1.256 ± 0.178	18 (8, 41)	+
	D.W.	2	1.088 ± 0.145	12 (6, 24)	
9	SLM	1	1.348 ± 0.013	22 (21, 24)	+
	D.W.	1	1.141 ± 0.0201	14 (13, 15)	
11	SLM	1	1.380 ± 0.0203	24 (22, 26)	+
	D.W.	1	1.265 ± 0.030	18 (16, 21)	

* SLM is fresh Sabouraud Liquid Medium and

D.W. is Distilled Water

** Details in Appendices IV-1 and IV-2

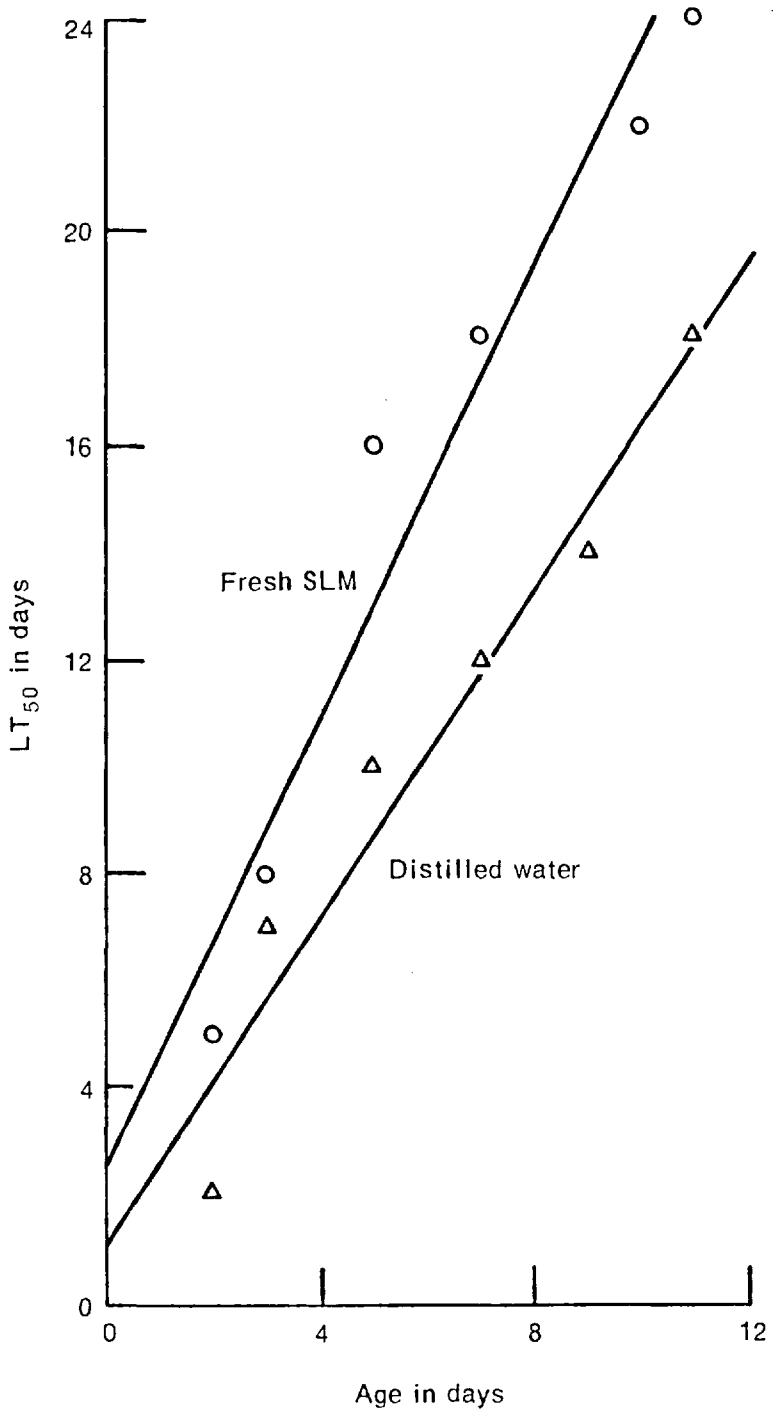


Fig. IV – 4

Effect of age of blastospores of *Verticillium lecanii* on spore survival at 2°C in two storage media.

the following equation:

$$y = a + bx$$

with age as x and LT_{50} as y so that a = the intercept, and b = slope which is a measure of the effect of spore age on survival. A straight line graph was fitted for each medium (Fig. IV - 4). In both regression analyses t = 8.2 with df = 4 giving a significance level, P, of 0.001 that the slope is greater than zero and the effect real.

Spores of all the ages tested survived longer in fresh SLM than in distilled water (Table IV - 4 and Appendices IV - 1 and 2). The "sign test" (Siegel, 1956) was used to test the significance of this effect because each pair of Log LT_{50}s for the two media involved spores of different age, hence from different populations. In applying the sign test the difference between Log LT_{50} for fresh SLM and Log LT_{50} for D.W. was scored as plus or minus for each of the six age groups. The null hypothesis tested is that the median difference is zero, i.e. that there will be equal numbers of plus and minus signs. This hypothesis is rejected if too few differences of one sign occur. All six signs are plus (+). A two-tailed test rejected this hypothesis at a level of P = 0.032 (Table D of Siegel, 1956) i.e. the effect of the media was significant at this level.

(c) Effect of suspending media on survival at $2^{\circ}C$ and at $-20^{\circ}C$

Four experiments were carried out at $-20^{\circ}C$ and seven at both $2^{\circ}C$ and at $-20^{\circ}C$ using 18 (1 D.W.) liquid media for suspending blastospores.

(i) Materials and Methods. Freshly grown 5-8 days old blastospores were used in all experiments (section IV, B, 2, (a)). The spores were centrifuged, washed and suspended in about 25 ml distilled water as stock. About 2 ml of stock suspension was added to about 20 ml aliquots of 17 media in 25 ml screw capped glass bottles, allocated between eleven starting dates to spread the work load (Table IV - 6), and stored at 2°C and -20°C. At 2°C blastospores in two replicate lots of each medium were sampled at geometrically increasing intervals up to about two months for viable spore counts and once for total spore count. At -20°C, to avoid frequent thawing and re-freezing, the contents of each McCartney bottle ($\simeq$ 20 ml) were divided into five aliquot s ($\simeq$ 4 ml) in small 7 ml screw capped bottles, a fresh aliquot being used for each of the first five counts in the order no. 1 to 5. Further counts were done by thawing again in the same order. These were always refrozen, however. The first sample (day 0) at -20°C was taken one hour after freezing was complete to establish the effect of the freezing and thawing process. After it had been established that repeated slow ($\simeq$ 1°C/min.) thawing and slow freezing was not harmful only one bottle per medium was used on the last four starting dates. The viable spore counts were conducted as described in section IV, B, 2, (a).

The probit mortalities for each bottle of suspension against log time were analysed separately to obtain a median lethal time (Log LT_{50}) for each LT_{50} for repeat bottles were combined as follows.

To combine j estimates of Log LT_{50}, M, each with variance V^1, the variation of the points about each line was first examined using a χ^2 test. If significant at the 0.05 level, the estimated variance was corrected by a factor of $\chi^2/(k-2)$.

The homogeneity of the j estimates was then checked by another χ^2 test where

$$\chi^2_{j-1} = \Sigma(M^2/V) - [\Sigma(M/V)]^2 / [\Sigma(1/V)]$$

If the values for j were homogeneous i.e. $P(\chi^2_{j-1}) > 0.05$, the variance between estimates U was assumed to be zero. Then a weighted mean $\bar{M}$ was calculated as,

$$\bar{M} = [\Sigma(M/V)] / [\Sigma(1/V)]$$

and variance $(\bar{M}) = 1/\Sigma(1/V)$

If the values for j were heterogeneous i.e. $P(\chi^2_{j-1}) \leq 0.05$, the variance between estimates (u) was found,

$$u = [\Sigma(M^2) - (\Sigma M)^2/j] / [(j-1) - (\Sigma V)/j]$$

This was used to calculate a semi-weighted mean,

$$\bar{M} = [\Sigma(M/V+u)] / [\Sigma(1/V+u)]$$

and variance $(\bar{M}) = 1/\Sigma[1/V+U]$

Confidence limits were calculated assuming a normal distribution if $P(\chi^2_{j-1}) > 0.05$, or otherwise a t distribution with f degrees of freedom.

$$f = [\bar{n}(\mathcal{E}V)^2] / \mathcal{E}(V^2) ,$$

where $\bar{n}$ is the mean number of spores counted/suspension medium over all j lines.

(ii) Results. As an example, the results of the probit analysis before and after combination are given for spores suspended in one medium, 3% sucrose and 3% gelatin (Table IV - 5).

The combined data for all (18) suspension media stored at 2°C (CR) and -20°C (DF) are given in Table IV - 6.

For storage at 2°C, the LT_{50}s ranged between 7 and 39 days, with only two values exceeding one month. Survival in distilled water and horse serum was low, and it was higher in media containing nutrients.

For storage at -20°C, the LT_{50}s ranged between one day and 970 days, the lowest being in Hank's balanced salt solution and the highest in 10% skimmed milk.

The blastospores had a half-life of more than one year in the following five media:-

10% skimmed milk.

7.5% glucose in horse serum.

3% gelatin + 3% sucrose.

Distilled water.

3% gelatin + 3% dextrose.

Of these, survival in only 10% skimmed milk was significantly ($P = 0.01$) longer than in distilled water. The spore survivals in the other thirteen media were lower than in distilled water. In

Table IV - 5

Survival of blastospores of Verticillium lecanii in 3% sucrose and 3% gelatin at 2°C and -20°C. Example of combination of probit analysis for several experiments.

Date experiment started	2°C		-20°C	
	Log ± S.E. LT_{50}	Natural LT_{50} & 95% limits (days)	Log ± S.E. LT_{50}	Natural LT_{50} & 95% limits (days)
3.11.78	1.313 ± 1.315	21 (-, -)	2.580 ± 0.043	380 (327, 506)
7.12.78	1.402 ± 0.048	25 (20, 31)	3.100 ± 0.403	1258 (490, -)
14.12.78	- -	-	2.685 ± 0.063	484 (380, 691)
20.12.78	- -	-	4.313 ± 0.949	- (1984, -)
28.12.78	- -	-	2.840 ± 0.147	691 (440, 2673)
Combined	1.402 ± 0.0480	25 (20, 31)	2.632 ± 0.034	428 (367, 500)

Hank's solution the spores died very quickly. Repeated thawing and freezing blastospore suspensions in distilled water did not apparently harm the survival of spores in the above experiments.

From the combined data for each medium, the effects of storage at 2°C and -20°C were compared by calculating a t value by using the formula,

$$t \text{ (approx.)} = \frac{m_1 - m_2}{\sqrt{s_1^2 + s_2^2}}$$

where m_1 and m_2 are the mean log LT_{50} values for the two temperatures. s_1 and s_2 are the S.E.s for their respective means. For each t value approximate P value was obtained from the table of student's t distribution.

The results (Table IV - 6) show that the temperature effects were not significantly different when spores were suspended in the following seven media:-

7% peptone + 7% sucrose.

10% glycerol.

10% honey.

5% sodium glutamate.

Fresh Sabouraud Liquid Medium.

Spent Sabouraud Liquid Medium.

Hank's solution.

In the other eleven media (including distilled water) spores survived significantly longer at -20°C than at 2°C.

Table IV - 6

Survival of blastospores of Verticillium lecanii in liquid media stored at 2°C and -20°C

Medium	Treatment	No. of experiments	Survival (Log days) Log LT_{50} ± S.E.	Survival Natural LT_{50} & 95% limits (days)	t	P
10% skimmed milk	2°C	2	1.108±0.047	13(10,16)	20	0.001
	-20°C	4	2.987±0.083	970(668,1409)		
7.5% glucose in horse serum	-20°C	4	2.673±0.055	471(367,605)	-	-
3% gelatin + 3% sucrose	2°C	2	1.402±0.048	25(20,31)	21	0.001
	-20°C	5	2.632±0.034	428(367,500)		
Distilled water	2°C	3	0.885±0.019	8(7,8)	14	0.001
	-20°C	5	2.582±0.124	382(216,676)		
3% gelatin + 3% dextrose	2°C	3	1.266±0.334	18 (4,86)	4	0.001
	-20°C	5	2.577±0.130	377(207,687)		
Horse serum	2°C	1	0.820±0.251	7(1,16)	5	0.001
	-20°C	4	2.111±0.032	129(111,149)		
KH_2PO_4 0.0425 g/litre, pH7.2	2°C	2	1.084±0.211	12(5,32)	4	0.001
	-20°C	2	2.028±0.051	107(85,134)		
5% lactose in 10% glycerol	2°C	2	1.415±0.057	26(20,34)	6	0.001
	-20°C	3	1.914±0.067	82(61,111)		
7.5% glucose in nutrient broth	2°C	1	1.510±0.038	32(21,36)	3	0.01
	-20°C	5	1.809±0.087	64(43,96)		

Continued/...

Table IV - 6 (Continued)

Medium	Treatment	No. of experiments	Survival (Log days) Log LT_{50} ± S.E.	Survival Natural LT_{50} & 95% limits (days)	t	P
5% sodium glutamate	2°C	2	1.590±0.059	39(30,51)	0.45	0.7
	-20°C	3	1.702±0.244	50(16,155)		
5% inositol	2°C	2	1.089±0.070	12(9,17)	5	0.001
	-20°C	2	1.557±0.073	36(26,50)		
Fresh Sabouraud Liquid Medium	2°C	2	1.125±0.235	13(5,39)	1	0.2
	-20°C	4	1.457±0.086	29(19,42)		
7% peptone + 7% sucrose	2°C	3	1.328±0.058	21(16,28)	1	0.2
	-20°C	6	1.428±0.064	27(20,36)		
10% honey	2°C	2	1.465±0.202	29(12,74)	0.15	0.9
	-20°C	3	1.420±0.228	26(9,75)		
7% peptone + 7% dextrose	2°C	2	1.232±0.075	17(12,24)	2	0.05
	-20°C	6	1.403±0.022	25(23,28)		
10% glycerol	2°C	3	1.319±0.132	21(11,38)	0.5	0.6
	-20°C	4	1.209±0.173	16(7,36)		
Spent Sabouraud Liquid Medium	2°C	2	0.976±0.027	9(8,11)	0.93	0.4
	-20°C	2	0.761±0.227	6(2,16)		
Hank's solution	2°C	2	1.175±0.192	15(6,36)	1	0.2
	-20°C	2	-0.056±0.804	1(0,36)		

(d) Effect of suspending liquid media on spore survival during freeze-drying

Six experiments were carried out on a small scale using 14 liquid media as suspending fluids.

(i) Materials and Methods. Some of the spore suspensions used at 2°C and -20°C were also freeze dried. Glass ampoules of 6 mm diameter were washed with distilled water, dried and autoclaved. Typed labels (5 x 30 mm) of chromatographic paper were placed in the ampoules. The freeze-drying machine held thirty ampoules which were divided between eight to fifteen media. The spore suspensions were pipetted into the ampoules without touching the sides, which causes charring later while heating to constrict the ampoules. Ten drops were added into each ampoule and stored at 2°C until freeze-dried, to avoid germination and growth of spores.

A centrifugal freeze drier (Edwards High Vacuum Ltd., model 5PS) with phosphorus pentoxide as desiccant, was used. During 7 minutes centrifugation under vacuum the spore suspensions in the ampoules were slant frozen. The vacuum was maintained for about 4 hours and then released. This was the primary drying. The ampoules were constricted individually by a gas flame and dried under vacuum for a further 16 to 17 hours when the vacuum level was about 4 microns Hg. The ampoules were sealed while maintaining the vacuum. A sample of 50 ampoules was checked for maintenance of vacuum with a high frequency tester. All the tested ampoules retained vacuum. The rest were not checked as the electron beam in this test may kill some spores.

The ampoules were stored at 2°C in darkness. Prior to the viable spore count as described in section IV, B, 2, (a), (iv), each ampoule was broken and soaked in about 25 ml distilled water for about 30 min. in a screw capped bottle, then thoroughly shaken.

(ii) Results. Table IV - 7 shows the survival of blastospores freeze dried on separate dates. Since the moisture content of the final freeze dried product probably varied on the different dates, which would be expected to influence the survival of spores, no statistical analysis was carried out. Instead results for each suspending medium were averaged.

Freeze drying killed many spores. However, the average percent survival in 10% honey, 7.5% glucose serum and 7.5% glucose in nutrient broth ranged between 43% and 55%. In 7.5% glucose serum the survival in different batches ranged between 40% and 72%. In five other media the average percent survival was about 20% or more and still lower in four media. In 10% glycerol and 5% lactose in 10% glycerol no spores survived.

(e) Discussion

(i) Age of spores in culture. The older the blastospore culture, the longer the spores survived when stored at 2°C. Since at 2°C there is slow metabolism in the spores during storage, this suggests that the blastospores accumulate a nutrient reserve and mature with time during culture. The blastospores survived longer in

Table IV - 7

Percent survival of blastospores of Verticillium lecanii suspended in liquid media and freeze dried. One to three ampoules/medium in each batch were tested for viability.

Medium	Batch freeze-dried						Total no. of ampoules tested	Average percent survival
	1	2	3	4	5	6		
10% honey	-	-	57	53	-	-	3	55
7.5% glucose in horse serum	-	59	-	72	40	43	9	54
7.5% glucose in nutrient broth	-	44	-	48	37	42	9	43
5% sodium glutamate	59	6	-	22	-	-	7	29
7% peptone + 7% sucrose	39	-	21	-	29	23	10	28
7% peptone + 7% dextrose	26	-	24	22	32	18	11	24
3% gelatin + 3% dextrose	44	-	14	5	27	15	11	21
3% gelatin + 3% sucrose	23	-	10	7	28	30	12	20
Fresh Sabouraud Liquid Medium	-	-	11	19	-	-	4	15
5% inositol	3	17	-	2	21	22	11	13
10% skimmed milk	34	2	-	1	4	5	11	9
Horse serum	8	0.1	-	0.5	-	-	7	3
10% glycerol	-	-	-	0	-	-	2	0
5% lactose in 10% glycerol	-	-	0	0	-	-	4	0
Fresh blastospores before freeze-drying	96	92	97	98	94	85		

the fresh Sabouraud Liquid Medium than in distilled water, so presumably they can absorb and use nutrients at 2°C. Hall (1977) found that blastospore viabilities decreased with storage time. The LT_{50} of his blastospores cultured for 48 h and 72 h, harvested and stored in distilled water at 2°C was more than 50 days, some surviving more than 208 days. In the present study under similar conditions to his experiment, the blastospores from 48 h and 72 h cultures had LT_{50}s of 2 and 7 days, respectively. No reason can be given for this difference.

(ii) Storage of blastospores at 2 and - 20°C. Roberts and Campbell (1977) suggested that spore viability is lost more slowly at low than at high temperature. This was usually so for V. lecanii on comparing 2 and -20°C. The longest LT_{50} in eighteen suspension media at 2°C was 39 days (5% sodium glutamate) and at -20°C the LT_{50}s in five media exceeded 1 year the longest being 970 days in 10% skimmed milk (extrapolated value).

Suspending media had an important influence, the half life being only 7 days in the poorest medium (horse serum) at 2°C and only one day in the poorest medium (Hank's solution) at -20°C. In eleven out of eighteen media survival was significantly better at -20°C than at 2°C, in the other seven media the difference was less to virtually nil. There may be a number of reasons for these effects of media. The survival in distilled water and horse serum at 2°C was very poor probably due to lack of nutrients. Spore survival was higher in media containing sugars, minerals and

nitrogen sources (amino acids, peptones etc.). This supports the earlier suggestion that the blastospores can absorb and use nutrients at 2°C. The very low rate of metabolism at -20° explains why survival was so much longer there in many media. (Metabolism is considered to be at a standstill below -130°C.)

At -20°C in distilled water, there was apparently no freezing and thawing injury to blastospores in many repeated experiments. However, there was great mortality in some media such as Hank's solution and spent Sabouraud Liquid Medium. This was probably due to slow cooling to freezing at approximately 0.5 to 1°C/min. On slow cooling, ice crystals separate out extracellularly and the concentration of solutes increases causing harm by gradual dehydration of spores. Micro-organisms are sometimes frozen in distilled water but they are usually suspended in two types of aqueous solutions (Mazur, 1966). One type includes more or less physiological media such as buffer, physiological saline, peptone broth and growth medium. These media are not protective relative to distilled water and, as in the present work, are often deleterious. The other type of media contain substances such as glycerol, sugars and skimmed milk are added for the specific purpose of preventing or reducing freezing injury. In the present study it was found that an additive was not necessary to protect against freezing and thawing injury.

After freezing death results from long term exposure to residual concentrated solutions after extracellular ice crystals are formed. An additive could reduce the injury due to concentrated

solutes either by acting as an innocuous diluent of the toxic solutes or by actually blocking or slowing their deleterious action. The number of viable cells usually decreases with storage time in the frozen state at temperatures above -70°C. The rate of this decrease is greatly influenced by the suspending medium. Even distilled water contains solutes which will concentrate during freezing (Mazur, 1966). There are a number of additives that decrease storage death rates. These include colloidal or high molecular weight compounds such as milk protein (Ulrich and Halvorson, 1946-47; Arpai, 1962; Nakamura and Dawson, 1962; Moss and Speck, 1963), gelatin (Squires and Hartsell, 1955) and other complex mixtures such as serum (Clement, 1961), and a boiled water extract of Escherichia coli (Bretz and Basa, 1960). Some such compounds protected V. lecanii blastospores.

Survival in 10% skimmed milk was better than in any other medium including distilled water. This agrees with Moore and Carlson (1975). They noted that in experiments with plant-pathogenic bacteria, survival of cells was generally enhanced by suspending the cells in 10% skimmed milk prior to freezing and storage. Milk protein (casein) and perhaps calcium ions (Ca^{++}) may give protection to the spores. Further, lactose in milk too can protect.

Many of the protective compounds are low molecular weight substances including glycerol (Squires and Hartsell, 1955; Clement, 1961; Nakamura et al., 1962), sucrose (Sato, 1954), glucose (Devik and Ulrich, 1948-49) and lactose (Mead et al., 1960). However, the sugars are not always protective. Clement (1961) found a greater

storage death rate of E. coli when stored in 7.5% glucose compared with McFarlane (1941), who found that sucrose could either protect or destroy E. coli depending on its concentration. Sato (1954) reported survivals of 74, 91, 99 and 82 per cent for E. coli cells suspended in water, 2% sucrose, 0.5% gelatin and 50% serum cooled slowly to -30°C. Postgate and Hunter (1961) found that the viability of Aerobactor aerogenes dropped rapidly with storage time at -20°C when the cells were suspended in 10% solutions of sucrose and of glucose, but not of glycerol. With V. lecanii blastospores, in gelatin, sucrose and glucose at the 3% level in distilled water the survival was good but not significantly different from that in distilled water alone. This suggests that these additives are not harmful at these concentrations.

In the media which had a deleterious effect on V. lecanii blastospores at -20°C when compared with distilled water, some had 7% or more sugars and hence may be too concentrated to improve survival. Others had peptone, minerals or were growth media. Sabouraud Liquid Medium itself has peptone and dextrose as does glucose broth. Inositol and glycerol too did not protect the spores. Honey was suggested as a better adjuvant for frozen storage than glycerol (Yamasato et al., 1973). In the present study in 10% honey and in 10% glycerol the blastospores lost viability quickly. Thus concentration is probably important and requires further investigation.

Daily and Higgens (1973) have shown that inclusion of 10% glycerol with 5% of lactose increased the viability of spores,

vegetative cells and Streptomycete mycelial fragments. With V. lecanii in this study too 5% lactose in 10% glycerol was a better medium (LT_{50} = 82 days) than 10% glycerol (LT_{50} = 16 days). Horse serum alone was not protective but in 7.5% glucose serum the survival was very good. In Hank's solution, a complex mixture of mainly inorganic chemicals, the spores died fast, perhaps due to toxic high concentration of salts when extracellular ice crystals formed.

It can be concluded about the chemistry of preservation of suspensions from the findings in the present study that:

(1) at 2°C, irrespective of the type of suspending fluids the blastospores loose viability within approximately two months,

(2) at -20°C, the blastospores are not injured by freezing and thawing in distilled water,

(3) at -20°C, the washed blastospores survive well in distilled water (LT_{50} = 382 days),

(4) at -20°C the suspending media tested were mostly deleterious rather than protective except for four media which were not harmful,

(5) at -20°C, 10% skimmed milk prolonged the survival (extrapolated LT_{50} = 970 days) in comparison to distilled water.

Thus it is possible to store for about a year at -20°C blastospores cultured for about one week, washed thoroughly with distilled water to remove culture medium and suspended in distilled

water. Additives are not necessary though survival can be enhanced by skimmed milk. The concentration of blastospores between approximately 10^6 to 10^8 spores/ml appears to be less important as various concentrations were used in each medium when experiments were repeated (Appendices IV - 3 to IV - 13). However, such suspensions are dilute and work is needed on higher concentrations forming slurries to conserve space. This method of storage has the disadvantage of requiring expensive low temperature appliances particularly for long distance transport. For such purposes alternative methods are desirable. Also as physiological changes may occur in stored frozen cells, pathogenicity studies should be undertaken.

(iii) Freeze-drying. Freeze-drying has expanded in the pharmaceutical and food industries and it is now on the verge of widespread acceptance in the chemical industry (Rey, 1975). It is the most widely used method for culture preservation (Haynes et al., 1955). Freeze-drying of micro-organisms is used for long-term preservation at ambient temperatures though it is generally less flexible, more costly and time consuming. However, as the product does not have to be kept cool in transit and is of light weight, the blastospores could be transported at less cost. Thus freeze-drying of blastospores was investigated in various media using the same batches of spores as used in some freezing experiments. Generally, freeze-drying caused high immediate mortality, so ampoules were not stored for studies on survival during prolonged storage.

For many micro-organisms the commonly used suspending fluids

give a very high percentage survival and recovery following freeze-drying (Lapage et al., 1970). Fry and Greaves (1951) found that addition of 7.5% glucose to nutrient broth improved viability after freeze-drying. They also found that when broth was substituted by serum there was a further marked improvement. This agrees with the finding for blastospores of V. lecanii, that an average of 43% survived in 7.5% glucose broth in comparison to 54% in 7.5% glucose serum. Survival in honey was the highest. In honey too about 95% to 99.5% of total solids are sugars and about 0.2 to 1% comprise colloids.

Fry and Greaves (1951) found that overdrying was injurious to bacteria. Some organisms could survive initial freezing and initial drying but were totally destroyed by continued drying (Greaves, 1962a). Overdrying has to be avoided since in many instances a certain amount of residual water is needed to maintain viablity in bacteria, yeasts, viruses and fungi (Rey, 1975). This is a matter of experience and varies from one organism to another. The residual moisture is one of the most critical factors affecting long-term storage. Moisture content of the product must be less than 5%, preferably between 3 to 5%, to ensure maximum survival (Ignoffo, personal communication). Perhaps the V. lecanii blastospores were overdried in some media during secondary drying. Variations in the residual moisture content are probably responsible for variable results in the present studies.

Holm-Hansen (1963) successfully freeze-dried a variety of algae. Storage of bacteria following freeze-drying requires the maintenance of about 1% residual moisture (Scott, 1960). Glucose

and sucrose in the medium retain about 1% moisture content which is adequate for bacteria. A slightly higher moisture content, 3 to 5% may be more suitable for blastospores of V. lecanii. Perhaps about 16 hours of secondary drying may be too severe for blastospores of V. lecanii, since at the end of the secondary freeze-drying the vacuum was at 4 microns Hg. Lapage et al. (1970) recommended 4 hours each for primary and secondary drying. It is suggested that the relationship of the length of secondary drying time and the moisture content of the final product be investigated for improving viability.

Further useful comments can be made about media. Horse serum serves as a protective colloid and is better than gelatin, but both on their own are inadequate (Lapage et al., 1970). Horse serum with glucose served as a better protectant for blastospores of V. lecanii. Skimmed milk too is regarded as providing a protective colloid (Fry, 1966). It was less effective than glucose-serum, but it can be heat-sterilized and gives a powdery product. Nutrient broth, sodium glutamate and other similar compounds protect by the amino group neutralizing toxic carbonyl radicals in the glucose and in the cells themselves (Lapage et al., 1970). As total desiccation lead to total death it is assumed that 10% glycerol and 5% lactose in 10% glycerol did not retain moisture during secondary drying for the survival of blastospores. As 7.5% glucose serum is expensive it is not suitable for large scale freeze-drying. Further studies with 10% honey and its constituent ingredients are necessary as it is less expensive and gave more than 50% survival when tested twice.

The freeze-dried ampoules of V. lecanii were stored for various periods up to about 2.5 months at 2°C until viability was tested. The higher the temperature of storage the lower the survival rate is likely to be. However, possibly with some suspending fluids and drying techniques the blastospores will survive at a higher temperature for a limited time. M-inositol gave poor results though it is a commonly used suspending fluid. Semenov (1973) used 185 strains of fungi suspended in horse serum, sucrose with gelatin, and skimmed milk and noted a high percentage survival when the vials were stored for 2 to 3 years at 4 to 10°C.

Freeze-drying has not replaced other means of preserving highly decomposable products (Rey, 1975). When considering the evolution of freeze-drying in the recent past it is seen that it has been restricted to those products which cannot be stabilized in any other way (e.g. plasma, vaccines and sera) or which show improved quality for a rather high average cost (coffee, mushrooms, diced chicken, etc.).

C. WHITEFLY-VERTICILLIUM LECANII RELATIONSHIP

1. Bioassay of conidia and blastospores on scales

For both glasshouse and laboratory studies, it is necessary to develop a bioassay technique to measure the effect of concentration of the two spore types. This was investigated initially by single assays, using only one spore type (conidia), and later by paired assays for comparing both spore types in the laboratory.

(a) Materials and Methods

Whitefly scales on leaf discs were dipped in spore suspensions, incubated for a week and their mortality assessed.

(i) Conidia and Blastospore Suspensions. Fresh spore suspensions were prepared for each assay. Blastospores were cultured as described in section IV, B,2. Conidia were obtained from cultures on Sabouraud Dextrose Agar in 9 cm petri dishes, which had been incubated for 2 to 3 weeks at 23°C and then stored at 2°C until used. The conidia were harvested in distilled water by scraping with smooth edged bent glass rods. The suspensions of conidia and blastospores were filtered through a cheese cloth to remove hyphae, centrifuged, washed with distilled water and finally suspended in 0.02% Triton X100 in potassium dihydrogen phosphate-buffer (0.0425 g/ litre), pH 7.2 (standard buffer). The concentrations and viability of the spore suspensions were determined as described in section IV, B, 2.

(ii) Production of Whitefly Pupae. Whiteflies were reared on tobacco plants, Nicotiana tabacum Cultivar 'White Burley', in three glasshouses at 24°C. Clean plants were in one house. The other two houses were used for different stages of whitefly production. In the first of these two houses adult whiteflies were produced. Periodically two tobacco plants were kept in this house for about 8 h for oviposition by a vast number of adult whiteflies. The plants were gently agitated a few times to ensure an even distribution of adults and hence eggs on the leaves. At the end of the 8-h period

the plants were shaken to remove most whiteflies and fumigated with dichlorvos (DDVP)-slow release strip to kill the remainder overnight in a covered hand truck, also used as a plant transporter. The terminal buds of these plants were then removed to encourage leaf expansion and the plants were kept in the second house, a scale-development house. After young flat scales turned into pupae, i.e. plump scales with a convex upper surface, 32 mm leaf discs each bearing about 30 pupae or more were cut from the plants, for bioassays in the laboratory. Only once leaf discs with 5-day old larval scales were used in a bioassay.

(iii) Assay Procedure. Leaf discs were dipped in spore suspensions in standard buffer, which wetted the dorsal and ventral leaf surfaces thoroughly, and the excess liquid was drained off. For controls the spores were omitted. Groups of four or more leaf discs were placed on moist filter papers, in 9-cm plastic petri dishes covered with lids. The dishes were kept in a perspex cage resting in a tray of water at 20°C. At least four leaf discs were used per concentration of spores. The assays were assembled in the evening. Next morning and then daily for one week, excess waterdroplets condensing on the scales and lids were blotted off by paper tissues to prevent drowning. These conditions will be termed "humid" (Tables IV - 8 & 9). In the eighth and later assays blotting was avoided by leaving the lid off, so that evaporation occurred. However, filter papers and leaf discs dried up occasionally, necessitating frequent wetting of the filter paper so the conditions were called "dry". Later drying was reduced by covering the door to

the cage with polythene sheet, conditions also called "humid" (Table IV - 9). In one assay the free water was left on the leaf discs with the dishes covered by lids, termed, "wet" condition.

Mortality was assessed after one week under a binocular microscope. Young flat scales were found unsuitable for experiments as it was extremely difficult to distinguish live from dead. Pupae obtained three weeks after egg laying, before the beginning of adult emergence were best. Clear, cream coloured, translucent, plump pupae with red eyes were regarded as live and those covered with profuse white growth of fungus as dead. Sometimes dead pupae were flat due to desiccation.

(b) Results

Control mortality was low in most assays and ranged from 1.0 to 9.0%, except 27.40% in the assay in wet conditions. Distinct profuse growth of V. lecanii was seen covering V. lecanii-treated scales, individually. No cross infections of pupae by spreading hyphal growth were noted on the leaf discs. The data from each assay were individually subjected to probit analysis (Finney, 1952). The slopes within the ten paired assays did not differ significantly, i.e.,

$$\frac{\text{Difference of slopes of conidia and blastospores}}{\text{Standard error of difference}} < 1.96$$

Therefore the data were reanalysed using parallel probit lines within each pair (Table IV - 8).

The Log LC_{50} values varied between assays for both spore types,

Table IV - 8

Bioassay of conidia of Verticillium lecanii on pupae of Trialeurodes vaporariorum: slopes of probit lines fitted individually to assays

Assay batch	Log LC_{50} ± S.E.	LC_{50} & 95% fiducial limits x 10^5 spores/ml	Slope ± S.E.	Moisture availability
1	5.62 ± 0.13	4.1 (2.1-7.4)	0.69 ± 0.09	Humid
2	5.85 ± 0.24	7.1 (1.7-20.0)	0.66 ± 0.16	Humid

depending mainly on the humidity. They were about 6 in "dry" conditions, 5 in "humid" and 4 in "wet" conditions. In the wet condition, the control mortality was high, averaging 27.40% in four replicate dishes (range 7% to 43%). Penicillium grew on most of the dead scales in the control but not on scales treated with V. lecanii, perhaps due to interspecific competition between the two fungi and due to larger amounts of honey dew secreted by control (live) scales than the infected fungus-treated scales.

As the two slopes of probit lines, one for conidia and the other for blastospores, within each of the ten assays (Appendix IV - 14) were not significantly different it is concluded that the spores responded similarly to the different experimental conditions in each assay.

Analysis of the differences between spore types indicated that blastospores were significantly more potent than conidia at the 0.01% level (Table IV - 9). However, variation between assays were observed, conidia appearing slightly more potent in some assays.

The log LC_{50} values for conidia ranged from 4.17 ± 0.33 to 7.00 ± 0.37 and for blastospores from 3.30 ± 0.47 to 6.84 ± 0.13. The slopes of the parallel lines for both spore types in the ten assays ranged from 0.47 ± 0.09 to 2.09 ± 0.50.

(c) Discussion

Pathogenicity of V. lecanii spores to whitefly pupae at 20°C increased with humidity and the presence of free water may also have had an effect. This agrees with the results in glasshouse experiments (Sections IV, D, 2, 3 and 4 and IV, F, 4).

Table IV - 9

Bioassays of conidia and blastospores of *Verticillium lecanii* on pupae of *Trialeurodes vaporariorum*: parallel probit lines were fitted to the two assays in each batch

Assay batch	Spore type	Log LC_{50} ± S.E.	Differences in Log LC_{50}s ± S.E.	Slope ± S.E.	LC_{50} & 95% limits x 10^5 viable spores/ml	Moisture availability
1+	Conid.	6.34±0.25	0.81±0.32	0.65±0.10	21.8(7.9,85.0)	Humid
	Blast.	5.53±0.20			3.4(1.4,8.9)	
2	Conid.	4.98±0.13	-0.08±0.20	0.88±0.10	0.96(0.52,1.7)	Humid
	Blast.	5.07±0.16			1.2(0.54,2.3)	
3	Conid.	4.59±0.40	0.31±0.53	0.66±0.16	0.39(0.03,1.9)	Humid
	Blast.	4.28±0.36			0.19(0.01,0.71)	
4	Conid.	5.09±0.22	0.69±0.33	0.77±0.13	1.2(0.37,3.1)	Humid
	Blast.	4.40±0.24			0.25(0.06,0.6)	
5	Conid.	5.19±0.14	0.64±0.20	1.02±0.12	1.5(0.77,2.8)	Humid
	Blast.	4.55±0.14			0.35(0.17,0.65)	
6	Conid.	5.91±0.06	-0.20±0.08	1.02±0.05	8.07(6.2,10.1)	Dry
	Blast.	6.11±0.06			12.8(9.9,16.7)	
7	Conid.	6.66±0.14	-0.18±0.19	2.09±0.50	46.0(21.7,91.1)	Dry
	Blast.	6.84±0.13			69.0(36.1,140.0)	
8*	Conid.	7.00±0.37	0.92±0.46	0.69±0.16	99.2(21.7,1020.0)	Dry
	Blast.	6.08±0.26			11.8(3.5,50.7)	
9	Conid.	6.47±0.37	0.04±0.49	0.69±0.18	29.7(6.0,305.0)	Dry
	Blast.	6.44±0.32			27.4(6.1,175.0)	
10	Conid.	4.17±0.33	0.87±0.57	0.47±0.09	0.15(0.019,0.52)	Wet
	Blast.	3.30±0.47			0.02(0.0008,0.1)	
	Mean difference		0.47			
	Standard deviation (σ)		0.35			
	d.f.		9			
	t		$\frac{0.47}{0.11} = 4.27$			
	P		> 0.01			

$$t = \frac{\text{Mean Log } LC_{50}\text{(conidia)} - \text{Log } LC_{50}\text{(blastospores)}}{\text{Standard deviation of the differences}}$$

$$\text{Standard deviation of the differences} = \frac{\sigma}{\sqrt{10}} = \frac{0.35}{3.16} = 0.11$$

* Fully grown pupae, about to emerge as adults were used

\+ Larval scales obtained 13 days after egg laying were used

The low values of slopes of probit lines (Tables IV - 8 & 9) and Appendix IV - 14) are probably due to non-toxic infection by V. lecanii causing mortality of pupae. Heterogeneity of the insect population used may be another factor causing low values of slopes. The toxin, bassianolide produced by (one strain of) V. lecanii (Kanaoka et al., 1978) is perhaps less important than infection in causing mortality. However, the effect of environmental conditions on the values of slopes of probit lines, cannot be neglected because in the assay done under wet conditions, the slope value was the lowest (Table IV - 9).

Furthermore it is not easy to detect death in whitefly scales, while the adults are fragile and difficult to handle (Wardlow et al., 1972). Examination of each scale under a microscope can only improve the observation. Thus it is known that very precise bioassays with whitefly scales are difficult in comparison to mobile insects where live and dead insects can easily be recognised.

Though on average conidia had significantly higher LC_{50} than blastospores in the ten bioassays in the laboratory, in the glasshouse experiment (section IV, D, 3) they were similar.

It is necessary to standardise all factors likely to influence the potency of the V. lecanii spores to the insect when comparisons need to be made between the results of assays undertaken at different times. But the knowledge of how factors such as relative humidity and temperature affect the susceptibility of an insect to the pathogen and the efficiency of the pathogen in killing the insect, will help in forecasting the efficacy of the pathogen in

different climatic conditions.

In the present study, as far as possible, most factors likely to influence the assay results were standardized except relative humidity of the air around the treated insect. However, relative humidity is an important factor as explained by the following examples. Laboratory studies have shown that saturated or near saturated humidity conditions are necessary for conidia of Entomophthora species to germinate (Yendol, 1968; Newman and Carner, 1975a, 1975b; Shimazu, 1977a). Insects were usually infected by Entomophthora species in the laboratory only when they were in moist conditions (Klein and Coppel, 1973; Hartman and Wasti,1974; Carner, 1976). Missonier et al. (1970) postulate that a minimum of 8-h per day of R.H. above 90% is required to maintain enzootic infection of aphids by Entomophthora spp. All Entomophthora species require saturated moisture conditions in which to complete their life cycle (Wilding, in press).

The effect of humidity is also found striking with the chemical insecticide, dinitro ortho-cresol DNOC at 0.2% caused 0% kill at 50% R.H. but 100% kill at 84% R.H. Even 0.5% DNOC killed only 2% of individuals at 50% R.H. (Way, personal communication). However, the mode of action of humidity is probably different to its action with a fungus.

Standardisation of humidity is difficult, often only with the aid of expensive and sophisticated equipment. However, in a small scale, using saturated salt solutions constant humidites can be maintained in small containers such as desiccators in which dishes of treated insects could be stacked. Bioassays carried out at 100% R.H.

may give more consistent results (Hall, 1979) but those with different accurately controlled levels of R.H. may be crucially important in view of their relevance to practicality.

2. Effect of V. lecanii on eggs and adults

(a) Eggs

(i) Materials and Methods. In two experiments freshly laid eggs and in one experiment 5 day-old eggs on tomato leaf discs, 32 mm in diameter, were dipped in spore suspensions to thoroughly wet both sides, drained and incubated at 20°C at approximately 100% relative humidity. The spores were suspended in 0.02% Triton X100 in KH_2PO_4 buffer, pH 7.2, omitting the spores for a control treatment. Concentration of spore suspension and viability of spores were determined as in section IV, B, 2.

Nine and twelve days after setting up the experiments 1 and 2 respectively, the leaf discs were examined under a binocular microscope for growth of fungus on eggs, crawlers or scales and for unhatched and hatched eggs. In the first experiment no quantitative observation was taken. In the second experiment the number of eggs and scales were counted.

(ii) Results and Discussion. V. lecanii did not kill whitefly eggs in both experiments. The eggs hatched and the crawlers settled as scales in control and fungus treated leaf discs alike (Table IV - 10). From old eggs crawlers emerged shortly (a few days) after dipping in spore suspension and the resultant scales became infected. With young eggs, the crawlers at observation, were still emerging or the scales had recently developed so there was

Table IV - 10

Effect of conidia and blastospores of *Verticillium lecanii* on eggs of *Trialeurodes vaporariorum*

Age: eggs	Viable spores/ml	Days after dipping	Total no. of eggs	Percent eggs unhatched	Presence of fungus
		Experiment - 1			
5 days	0 (control)	9	ca. 60	Low	None on eggs and scales
	5.9×10^7 conidia	9	ca. 60	Low	None on eggs: present on new scales
	4.3×10^6 blastospores	9	ca. 60	Low	
Few hours	0 (control)	9	ca. 60	High	None on eggs and active crawlers
	5.9×10^7 conidia	9	ca. 60	High	
	4.3×10^6 blastospores	9	ca. 60	High	
		Experiment - 2			
Few hours	0 (control)	12	111	2.7	
	1.7×10^7 conidia	12	113	0.9	None on eggs
	1.9×10^7 blastospores	12	169	0.6	

insufficient time post hatching for infection to become visible.

Since the fungus did not kill eggs in the laboratory under very moist conditions, it is unlikely to kill eggs in less moist conditions prevailing in glasshouses. Also spores sprayed on to eggs in glasshouses are likely to die before eggs hatch. Thus repeated spore sprays are necessary to control whitefly populations in glasshouses.

(b) Adults

(i) Materials and Methods. Three experiments were carried out to find whether whitefly adults released on fungus-sprayed plants become infected and killed. About 50-100 whitefly adults (Table IV - 11) were released 2 h after spraying on 2-4 control and 2-4 fungus-sprayed French bean plants in separate, closed, illuminated perspex cages, resting on trays with standing water at 20°C. The spores were suspended in 0.02% Triton X100 in KH_2PO_4 buffer, pH 7.2, omitting the spores for a control treatment and sprayed thoroughly to wet the ventral leaf surfaces.

One week after spraying, mortality of adults and growth and sporulation of V. lecanii were assessed.

(ii) Results and Discussion. V. lecanii killed a large proportion of adults on fungus-sprayed plants (Table IV - 11). The control mortality was low. However, as the blastospore spray was viscous, the leaf surfaces may have been wet when adults settled, sticking down the wings and causing rapid death of some adults. Other adults were infected from wet leaf surfaces and killed later. These (adults) were seen with V. lecanii growth.

Table IV - 11

Mortality of adult whiteflies released on French bean plants sprayed with blastospores of Verticillium lecanii at 20°C and approximately 100% R.H. in the laboratory

Experiment	Concentration of spray: viable spores/ml	Total adults	Percent dead
1	0 (control)	110	9.1
	9.0×10^7 (blastospores)	104	100
2	0 (control)	54	20.4
	5.6×10^7 (blastospores)	53	84.9
3	0 (control)	117	25.6
	4.5×10^7 (blastospores)	126	96.8

In glasshouses drier conditions dry spray rapidly and spores soon die (section IV, D, 4). Only adults alighting on the wet leaves will become infected and those emerging later from pupae will escape infection.

D. CONTROL OF WHITEFLY IN GLASSHOUSE

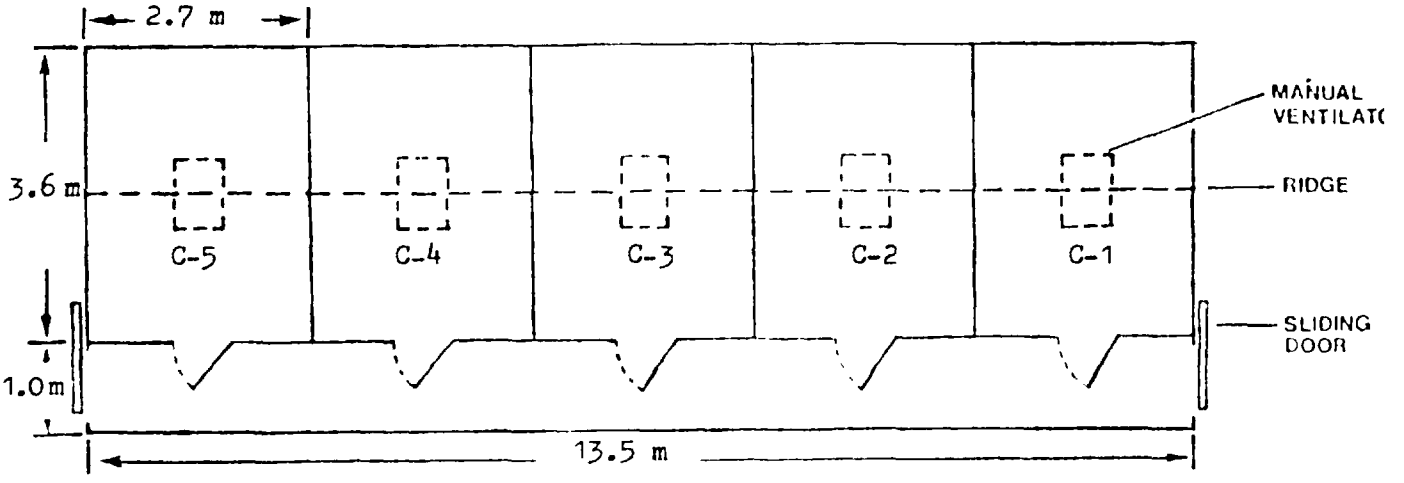
Three glasshouse experiments were carried out to study the feasibility of using V. lecanii for the control of whiteflies on cucumbers, grown in autumn 1977, winter 1977-78 and spring-summer 1978. Only conidia were tested against the scales during Expt. 1, both conidia and blastospores in Expt. 2, and blastospores alone in Expt. 3.

1. General methods and materials

These are common for all three experiments unless otherwise specified.

(a) Structure of glasshouse

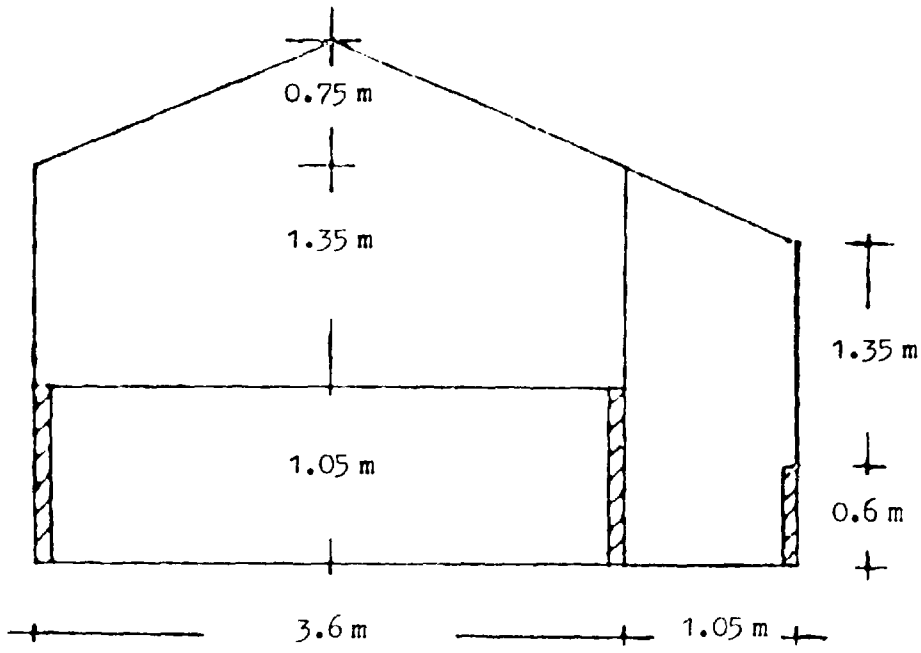
The three experiments were carried out in a compartmented glasshouse shown in plan in Fig. IV - 5 and elevation in Fig. IV - 6. The walls, including partition walls, were of concrete. The glass, including that of the doors, was fixed in the cedarwood with putty without overlap of glass sheets to ensure that the compartments were as air-tight as possible. One ventilator, 46 cm x 23 cm, in the outer wall of each compartment was centrally positioned half way up the wall. Each compartment had two hand-operated roof ventilators (0.9 m x 0.6 m).



Scale, 3 mm to 0.3 m

Fig. IV - 5

Plan of the compartmented glasshouse used in the three experiments on control of whitefly on cucumber by *Verticillium lecanii*



Scale, 6 mm to 0.3 m

Fig. IV - 6

Cross sectional elevation of compartmented glasshouse shown in Fig. IV - 5

(b) Preparation of glasshouse before planting

Individual compartments were fumigated overnight with DDT/ Lindane smokes to kill all residual pests from previous experiments and then ventilated to remove the fumigant before planting the cucumbers.

(c) Plants

The variety 'Farbio' was used in Expts. 1 and 3 and 'Butchers Disease Resister' in Expt. 2. Pest- and disease-free plants, about 40 cm to 45 cm in height, in whalehide pots were installed twelve per compartment, with six on either side. By about a week it was observed that the plants were established and healthy in the new environment.

(d) Introduction of whitefly

Whitefly adults collected from a clean culture on tobacco plants were released into each compartment. In Expts. 1 and 2 the adults were killed by fumigation with dichlorvos ('Vapona', Shell Chemical Company), an organophosphate insecticide, one day after release to ensure that only sufficient eggs were laid on the cucumber leaves, while for Expt. 3 the adults were not killed but allowed to lay eggs continuously.

(e) Production of spores

Conidia and blastospores used as inocula for mass production were obtained as described in sections IV, C, 1 and IV, B, 2 respectively. Conidia were mass produced on Sabouraud Dextrose Agar (SDA) in stainless steel trays (28 cm x 33 cm and 23 cm x 28 cm),

incubated for 2 to 3 weeks at 20°C to 25°C and stored at 2°C for a few days until the date of spraying. The conidia were harvested in distilled water by scraping with smooth edged bent glass rods, filtered through double layered cheese cloth to remove large particles and hyphae, centrifuged and resuspended in a little distilled water as a stock suspension for dilution and spraying.

Blastospores were cultured in Sabouraud Liquid Medium (SLM) in a 20-litre glass fermentor (Fig. IV - 7). Polypropylene glycol-2025 (BDH Chemicals Ltd., Poole, England) was added at the rate of 1 ml/ 1000 ml SLM. In addition, a few drops of 'Antifoam A' (Sigma Chemicals Ltd., London) were added before autoclaving to prevent foam development during agitation of the medium while culturing the spores. Further additions of a few ml. of autoclaved antifoam were made if foam developed while culturing. The SLM in the larger fermentor was normally inoculated with about 2 litres of blastospores. Sometimes in addition to this,conidia harvested in distilled water from a pure culture in a stainless steel tray were added to obtain greater yields in a short time. On average a yield of about 5×10^7 blastospores/ml was obtained in five days. Always the fresh blastospores were almost totally viable. These spores were harvested in a continuous rotor centrifuge (MSE 18000, Crawley, Sussex) at 8500 to 9000 r.p.m. The pellet was suspended in about one litre of distilled water and mixed thoroughly by agitation with a magnetic stirrer to obtain a uniform suspension before dilutions were made for spore count of the spray suspension.

Fig. IV - 7

A 20-litre glass fermentor vessel with Sabouraud Liquid Medium on a magnetic stirrer for the culture of blastospores of Verticillium lecanii

(f) Spraying spores

Spores of known viability were diluted with standard buffer solution, to the required concentrations. Pneumatic hand sprayers of 1-litre capacity (The Master No. 5P/T, The Philip B. Waldron Co., Birmingham 11, England) were used in Expts. 1 and 2 as the plants were small and for Expt. 3 a 5-litre pneumatic hand sprayer (A.S.L. Killaspray) was used.

Leaves bearing developing whitefly scales were sprayed at high volume to thoroughly wet the ventral surfaces. Control plants were sprayed with buffer containing no spores. Spraying was always done after 6 p.m. in the hope of avoiding any possible harmful effect of sunshine.

(g) Temperature and relative humidity

Thermohygrographs (Negretti and Zambra Ltd., London and C.F. Casella and Co. Ltd., London) were maintained in each glass-house compartment. These were calibrated before use and checked frequently during the experiments using a whirling hygrometer and thermometer.

(h) Humidification

Some compartments were artificially humidified with DW from 6 p.m. to 6 a.m. using a humidifier (Defensor 505, A.G. Zurich, Switzerland) during the Expts. 1 and 2 but not in Expt. 3.

(i) Cultivation practices

The plants were illuminated artificially whenever needed to obtain good growth. Normal commercial plant cultivation practices

were followed. When the plants reached a height of 2 metres, the terminal buds were removed to encourage development of side shoots to obtain an "umbrella system" of training in which the three top most branches were allowed to grow downwards. For the control of powdery mildew caused by the fungus, Sphaerotheca fuliginea, dimethirimol, (PP675 - ICI, Plant Protection Division) a systemic fungicide, was applied as a diluted soil drench. This chemical has no significant effect on insect pests, red spider mites and their predators (Worthing, 1979) or V. lecanii (section IV,D, 5).

(j) Sampling and observation

About two weeks after spraying when dead scales become dry and flat, leaf discs of 32 mm diameter were collected from the leaves bearing scales, three from each plant, unless otherwise specified. The discs were kept on moist filter paper in petri dishes to prevent wilting before examination under a binocular microscope for live and dead scales; and empty pupal skins. Scales killed by V. lecanii were easily distinguished from healthy scales by their white fluffy appearance (Figs. IV - 8 and IV - 9).

In Expts. 1 and 2 contagion of V. lecanii from dead whitefly scales to newly emerged adults was assessed by counting live and dead adults on the ventral surface of 15 leaves per compartment per stage of leaf maturity, graded as young, medium and old (Figs. IV - 10 and IV - 11). In Expt. 3 the sampling method differed.

Fig. IV - 8

Healthy whitefly scales on the ventral surface of a cucumber leaf

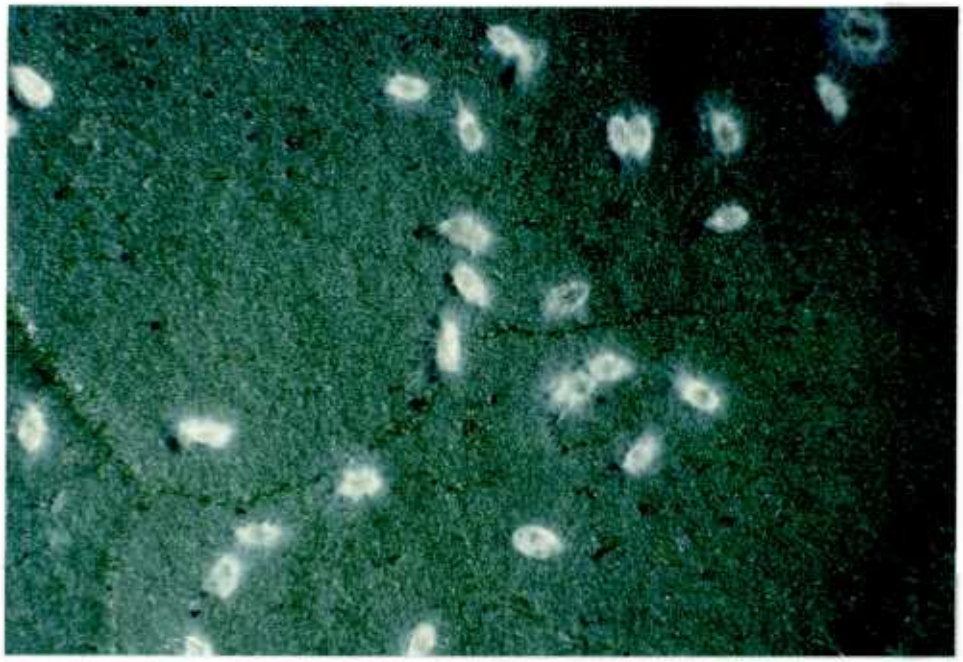


Fig. IV - 9

Dead whitefly scales infected with Verticillium lecanii on cucumber leaf

Fig. IV - 10

Healthy whitefly adults on the ventral surface of a cucumber leaf

Fig. IV - 11

Dead whitefly adult infected with Verticillium lecanii

2. Single spray of conidia (Expt. 1)

Initially the power of V. lecanii to kill whitefly scales was assessed on a small scale by spraying a suspension of conidia.

(a) Methods

Each of two compartments of the glasshouse (Fig. IV - 5) had control plants on one side and treated plants on the opposite side (Fig IV - 12). A few thousand whitefly adults were released into each compartment. Eggs were laid over 2 days, September 14 and 15, 1977 and then the adults were killed. Two weeks later developing scales were sprayed with a suspension of conidia having 2.8×10^7 viable spores/ml. After spraying, compartment C-5 was artificially humidified and the other not. Leaf discs were collected 10, 12, 14, 18 and 20 days after spraying and adults examined on days 35 and 42.

(b) Results

Mortality of scales (Table IV - 12) in the control plants was low compared with the high mortality on the treated plants in both compartments. The mortality of treated scales was lower in the end compartment, C-5, with lower relative humidity (R.H.) and higher temperature (Table IV - 29), probably due to more solar heating through the glass end walls in day time than in the inner compartment, C-4, which was shaded by compartments on either side, resulting in high R.H. and optimum temperature for growth of the pathogen.

On treated plants many adults were dead on sample leaves in both compartments. Both the numbers of adults and percent mortality increased with the age of the leaves (Table IV - 13). The dead

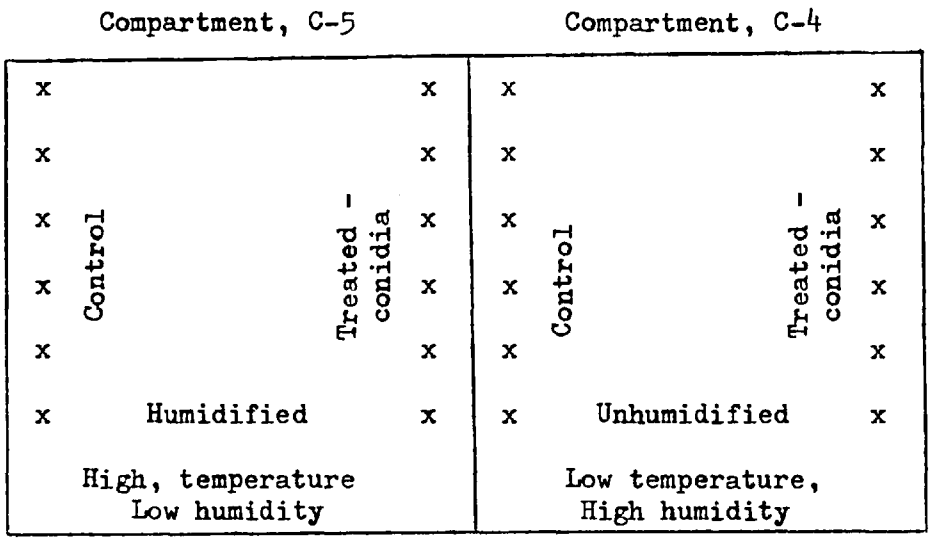


Fig. IV - 12

Layout of the experiment on single spray of conidia of *Verticillium lecanii* for the control of whitefly on glasshouse cucumber

x, denotes the plant position

Table IV - 12*

Effect of spraying conidia of Verticillium lecanii on whitefly scales on cucumbers in glasshouses at low and high humidities (Exp. 1)

Days after spraying	Control mortality		Treated mortality		P
	Compartment C-4 High R.H., low temperature M ± S.E.	Compartment C-5 Low R.H., high temperature M ± S.E.	Compartment C-4 High R.H., low temperature M ± S.E.	Compartment C-5 Low R.H., high temperature M ± S.E.	
10	5.8 ± 1.7	13.3 ± 2.7	89.9 ± 2.2	61.0 ± 3.5	< 0.001
12	9.6 ± 1.5	9.7 ± 2.0	84.9 ± 2.1	67.9 ± 3.2	0.001
14	6.2 ± 1.4	5.8 ± 1.6	91.5 ± 3.2	80.6 ± 3.3	0.05
18	6.2 ± 1.5	11.2 ± 2.0	93.5 ± 1.4	81.1 ± 2.4	0.001
20	16.2 ± 3.2	30.7 ± 3.5	96.3 ± 0.8	81.7 ± 3.1	0.001

M is the mean of the six percentage mortalities from six plants.

* Details in Appendix IV - 15

Table IV - 13

Mortality of whitefly adults due to Verticillium lecanii on 15 leaves in each of three stages of maturity on cucumber plants in the two compartments

(Exp. 1)

Days after spraying	Maturity of leaf	Compartment C-4. High R.H., low temperature		Compartment C-5. Low R.H., high temperature	
		Total adults	% dead	Total adults	% dead
35	Young	130	70	150	11
	Medium	360	80	400	56
	Old	460	81	640	84
42	Young	200	4	180	14
	Medium	690	35	330	40
	Old	1340	61	500	82

adults adhered to the ventral surface of the leaves and were covered with sporulating V. lecanii, easily seen as a white fluffy mass, Fig. IV - 11.

On the control plants much "honey dew" was excreted by the scales and adults whiteflies and the leaf surfaces were shiny in appearance at the initial stages and were later covered with sooty mould (Fig. IV - 13). On the treated plants the leaves appeared normal. A few scales on the control plants bore fungi other than V. lecanii at the last observation, perhaps due to an excess of honey dew but on the treated plants V. lecanii was the only fungus seen on the scales.

3. Double spray of conidia and blastospores at two concentrations (Expt. 2)

Large scale conidia production for spraying is expensive and time consuming. Blastospores can be cultured quickly and more cheaply. However, as blastospores are produced in liquid media, it was feared that these would dehydrate and die in the glasshouse environment quicker than the conidia, which are produced on the agar surface. The aim of this experiment was to discover whether blastospores could equal the control achieved by conidia and to examine the effect of two spore concentrations.

(a) Methods

Conidia were obtained from a 3-week old culture and blastospores from a five-day old culture. The concentrations used and the times of two sprayings are given in Table IV - 14.

One control and two treated compartments had six plants on each

40 mm

Fig. IV - 13

"Honey dew" and "Sooty mould" on the dorsal side of cucumber leaves

Table IV - 14

Concentrations of conidia and blastospores of *Verticillium lecanii* sprayed on 23.12.77 and on 20.1.78

Date sprayed	Days after release of adults	Spore type	Concentration of spray in spores per ml		% viability
23.12.77	15	Conidia	High	1.8×10^7	not available (Fresh)
			Low	3.6×10^6	
		Blastospores	High	1.8×10^7	"
			Low	3.5×10^6	
20.1.78	43	Conidia	High	3.8×10^7	73
			Low	7.6×10^6	
		Blastospores	High	3.6×10^7	95
			Low	7.2×10^6	

side. In the treated compartments each group of three adjacent plants was regarded for statistical purposes as a plot in which all plants were treated alike. The allocation of treatments is given in Fig. IV - 14. Each compartment was regarded as a block in which there was no replication of plots. In a completely randomized design the variation between the compartments is included in the residual variance, making the usual significance tests less sensitive. Thus the experiment was treated as a randomized block design. The compartments were fairly uniform though differences between them existed during the first part of the experiment.

The whitefly infestation was started with eggs laid over 1 day (8.12.77). Two sprays were applied 15 and 43 days later.

At the first spraying (23.12.77) one of the treated compartments, C-4, was artificially humidified and the other, C-5, was not. At the second spraying (20.1.78) the humidifier was moved to the other compartment, C-5. As the temperature in C-5 was unfavourably high to V. lecanii, it was reduced to an optimum level before the second spraying.

Leaf discs were cut for mortality assessment on days 14, 17 and 21 after the first spraying and 14, 17, 21 and 24 days after the second spraying. On the day of the second spraying mortality of scales was also assessed in a similar manner. Further leaf discs were collected on days 28, 31 and 35 after the second spraying to study whether infection spread to the new generation of scales developing from survivors of the spray.

Spread of V. lecanii to whitefly adults was assessed on days 13, 20, 27 and 34 after the second spraying.

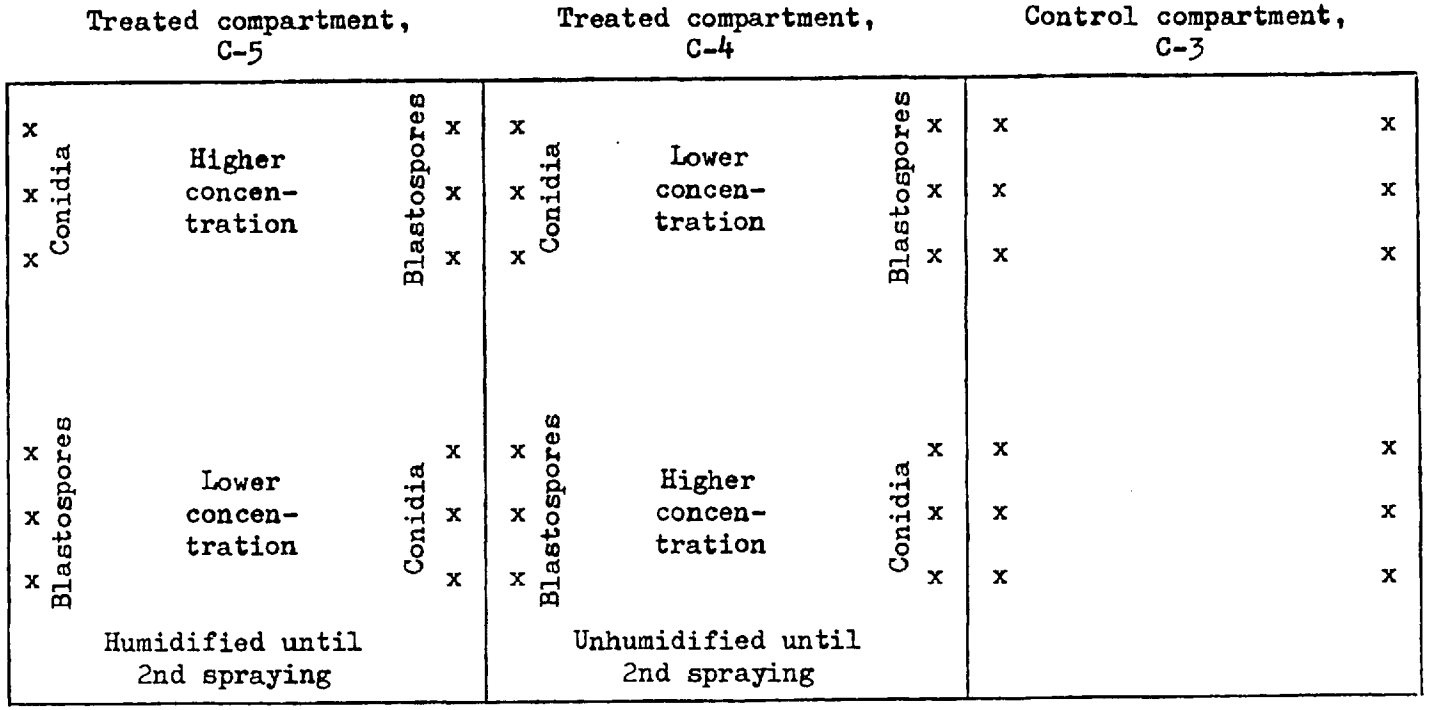


Fig. IV - 14

Layout of the experiment on double spray of conidia and blastospores of Verticillium lecanii at two concentrations for the control of whitefly on glasshouse cucumber

x, denotes the plant position

(b) Results

Mortalities of whitefly scales caused by the first spray of conidia and blastospores of V. lecanii at two concentrations are given in Table IV - 15. Whitefly adults emerged from the survivors and laid eggs on young leaves at higher positions on the main stem. From these a new generation of scales of variable age developed. Thus during the second spraying adults, eggs and scales were exposed to the spray. Observations after the second spraying are given in Table IV - 16.

Mortality in the control compartment, C-3, was negligible compared with the high mortality (36 to 99%) in the two treated compartments, C-4 and C-5. However, the mortality patterns in the two treated compartments appeared to differ from each other after the first spraying but were similar after the second spraying. Possibly the increase in mortality in compartment C-5 after the second spraying was due to reducing its temperature to the optimum level, 20°C to 25°C for V. lecanii.

To distinguish the specific effects of spore type and concentration from the effects of compartment and background variation (error) in the observations, the results were subjected to analyses of variance. In analyses for each date of observation after the two sprayings, the interaction between spore-type and concentration was not significant. Hence the interaction (1 d.f.) and the error (3 d.f.) terms were summed and the variance ratios for compartment, spore type and concentration calculated with 1 d.f. and 4 d.f. (Table IV - 19). At all three obsevations after the first spray the compartment effect was significant at the 5% level, spore

Table IV - 15*

Mortality (%) of whitefly scales after spraying spores of *Verticillium lecanii* on 23.12.77

Control (C-3)**	Spore type	Spores per ml	Treated	
			High R.H., low temperature (C-4)**	Low R.H., high temperature (C-5)**
		OBSERVED 14 DAYS AFTER SPRAYING		
0.7	Conidia	1.8×10^7	87.8	36.3
		3.6×10^6	42.0	38.6
	Blastospores	1.76×10^7	88.7	60.3
		3.52×10^6	63.8	39.1
		17 DAYS AFTER SPRAYING		
1.5	Conidia	1.8×10^7	87.4	37.6
		3.6×10^6	59.3	45.4
	Blastospores	1.76×10^7	88.9	64.5
		3.52×10^6	71.1	44.6
		21 DAYS AFTER SPRAYING		
3.9	Conidia	1.8×10^7	93.2	43.5
		3.6×10^6	62.4	36.5
	Blastospores	1.76×10^7	85.4	70.8
		3.52×10^6	74.9	52.4

* Details in Appendix IV - 16

** Control, mean of 12 plants; treated mean of three plants

Table IV - 16*

Mortality (%) of whitefly scales after spraying spores of *Verticillium lecanii* on 20.1.78

Control** (C-3)	Spore type	Spores/ml	Treated**	
			Humidified (C-4)	Unhumidified (C-5)
		OBSERVED 14 DAYS AFTER SPRAYING		
0.5	Conidia	3.8×10^7	95.1	97.4
		7.6×10^6	87.2	88.6
	Blastospores	3.6×10^7	86.9	91.5
		7.2×10^6	91.4	84.8
		17 DAYS AFTER SPRAYING		
1.5	Conidia	3.8×10^7	98.7	95.8
		7.6×10^6	94.9	90.8
	Blastospores	3.6×10^7	93.6	95.9
		7.2×10^6	91.5	85.9
		21 DAYS AFTER SPRAYING		
2.0	Conidia	3.8×10^7	95.8	96.4
		7.6×10^6	88.7	90.0
	Blastospores	3.6×10^7	88.6	93.3
		7.2×10^6	85.6	87.9
		OBSERVED 24 DAYS AFTER SPRAYING		
6.4	Conidia	3.8×10^7	94.8	99.4
		7.6×10^6	94.0	90.2
	Blastospores	3.6×10^7	90.0	98.8
		7.2×10^6	92.3	93.3

* Details in Appendix IV - 17

** Control, mean of 10-12 plants; treated mean of 3 plants

type was not and concentration was close to the 5% level of significance in two out of the three observations. After the second spraying, however, the compartment effect was insignificant, the spore type effect very insignificant again (except in one of the four observations) and the concentration was insignificant at two observations and highly significant at the other two.

To obtain a clearer understanding of these results the mean mortalities for the three or four observations were averaged for each of the two sprayings (Table IV - 20) and reanalysed (Table IV - 21).

The compartment effect appeared highly significant after the first spraying but negligible after the second. The concentration of spores was almost significant at the 5% level in the first and significant in the second spraying. Spore type was significant in neither spraying.

A new generation of adults emerged from the scales which survived the second spraying (Fig.IV - 15a,b) and laid eggs on young leaves on new branches. The scales developing from these eggs were examined for spread of infection from the leaves sprayed earlier. Most were live (Table IV - 17). The few adults seen with V. lecanii were dead (Table IV - 18). It was possible to distinguish V. lecanii from other fungi which rarely existed on the dead adults. There was no spread of infection from treated compartments to the control. The control plants were sticky with "honey dew". Sooty mould had covered most of the leaves which had "honey dew" in the initial stages (Fig. IV - 16 and IV - 17). Later, some of these leaves

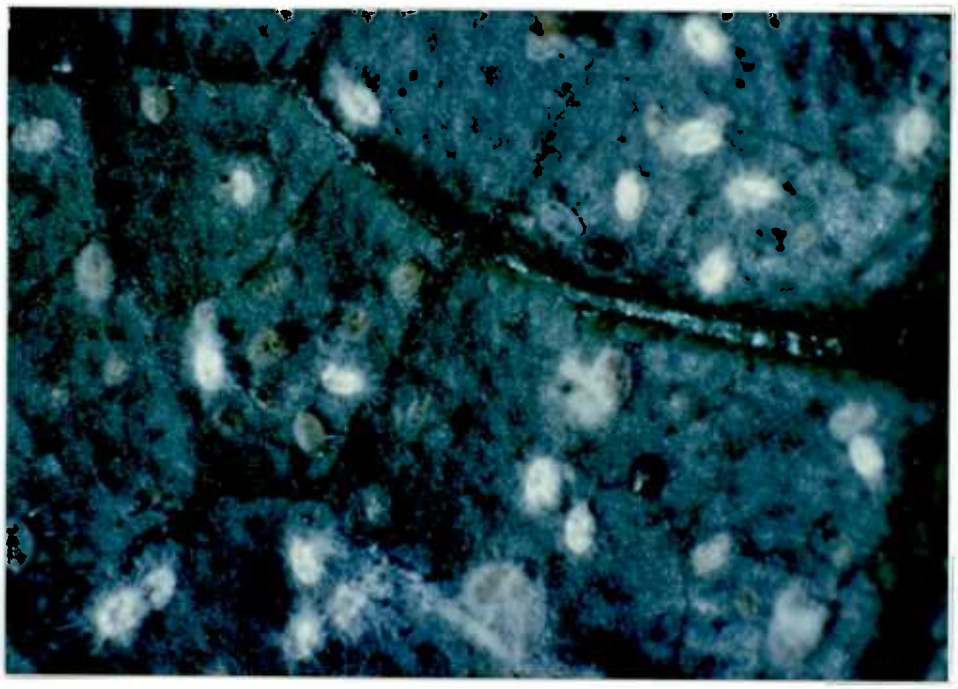


Fig. IV - 15a

Some live whitefly scales among many dead scales infected with Verticillium lecanii. A few scales were parasitised by Encarsia formosa

Fig. IV - 15b

A closer view of Fig. IV - 15a. On a Verticillium lecanii-sprayed leaf, a healthy whitefly scale adjacent to a dead scale, and one black parasitised scale with emergence hole of Encarsia formosa

Table IV - 17*

Mortality (%) of whitefly scales on newly developed unsprayed leaves on plants sprayed with Verticillium lecanii on 23.12.77 (a) and on 20.1.78 (b)

Control** (C-3)	Spore type	Spores/ml	Treated** Humidified (C-4)	Treated** Unhumidified (C-5)
		OBSERVED 28 DAYS AFTER 2ND SPRAYING		
1.6	Conidia	a 1.8×10^7	9.8	1.4
		b 3.8×10^7		
		a 3.6×10^6	2.4	15.6
		b 7.6×10^6		
	Blastospores	a 1.76×10^7	32.8	14.9
		b 3.6×10^7		
		a 3.52×10^6	19.0	25.1
		b 7.2×10^6		
		31 DAYS AFTER 2ND SPRAYING		
Not available	Conidia	a 1.8×10^7	2.7	6.2
		b 3.8×10^7		
		a 3.6×10^6	0	15.7
		b 7.6×10^6		
	Blastospores	a 1.76×10^7	22.8	11.7
		b 3.6×10^7		
		a 3.52×10^6	1.0	5.4
		b 7.2×10^6		
		OBSERVED 35 DAYS AFTER 2ND SPRAYING		
2.5	Conidia	a 1.8×10^7	18.4	4.9
		b 3.8×10^7		
		a 3.6×10^6	0.7	0
		b 7.6×10^6		
	Blastospores	a 1.76×10^7	0.6	19.7
		b 3.6×10^7		
		a 3.52×10^6	10.5	0.7
		b 7.2×10^6		

* Details in Appendix IV - 18

** Control, mean of 10 plants; treated, mean of 3 plants

Table IV - 18

Mortality of whitefly adults due to V. lecanii on 15 leaves in each of three stages of maturity on cucumber plants sprayed on 23.12.77 and 20.1.78

Days after second spraying	Stage of maturity of leaf	Control (C-3)		Treated (C-4)		Treated (C-5)	
		No.	Dead %	No.	Dead %	No.	Dead %
13	Young	820	0	510	0	280	0
	Medium	1380	0	230	4	240	0
	Old	390	0	200	1	100	0
20	Young	2220	0	700	0	320	0
	Medium	3120	0	900	1	1320	0
	Old	2890	0	290	1	670	0
27	Young	2910	0	880	1	1240	0
	Medium	5050	0	600	13	600	0
	Old	5140	0	240	8	320	0
34	Young	Too many to count	-	710	5	780	0
	Medium		-	870	27	730	1
	Old		-	740	12	650	1

Table IV - 19

Variance-Ratio (F) for each effect after spraying spores of *Verticillium lecanii* (Expt. 2). At 5% significance level, with 1 and 4 d.f., F = 7.7

Date sprayed	Source of variation	Days after spraying			
		14	17	21	24
23.12.77	Compartment	10.0	17.2	18.0	-
	Spore type	1.9	2.03	3.3	-
	Concentration	6.8	4.4	6.3	-
20.1.78	Compartment	0.02	2.8	4.9	1.2
	Spore type	1.5	4.6	14.9	0.2
	Concentration	2.9	11.5	29.7	1.8

Unsprayed new leaves on plants sprayed twice earlier		Days after second spraying		
		28	31	35
	Compartment	0.1	0.2	0.04
	Spore type	6.4	0.4	0.1
	Concentration	0.02	0.7	1.5

Table IV - 20

Mean percentage mortalities based on three or four observations for plots of three plants after spraying spores of *Verticillium lecanii* (Expt. 2)

Date sprayed	Spore type		Spores/ml	Mortality % Compartment 4	Mortality % Compartment 5
23.12.77	Conidia		1.8×10^7	89	39
			3.6×10^6	55	40
	Blastospores		1.8×10^7	88	65
			3.5×10^6	70	45
20.1.78	Conidia		3.8×10^7	96	97
			7.6×10^6	91	90
	Blastospores		3.6×10^7	90	95
			7.2×10^6	90	88
Unsprayed new leaves on plants sprayed twice earlier on 23.12.77 (a) and 20.1.78 (b)	Conidia	(a)	1.8×10^7	10	4
		(b)	3.8×10^7		
		(a)	3.6×10^6	1	10
		(b)	7.6×10^6		
	Blasto-spores	(a)	1.8×10^7	19	15
		(b)	3.6×10^7		
		(a)	3.5×10^6	10	10
		(b)	7.2×10^6		

Table IV - 21

Analysis of variance for Table IV - 20. At 5% significance level with 1 and 4 degrees of freedom (d.f.), F = 7.7

Date sprayed	Source of variation	Sum of squares	d.f.	Mean square	F
23.12.77	Compartment	1561.57	1	1561.57	17.2
	Spore type	251.22	1	251.22	2.8
	Concentration	637.78	1	637.78	7.0
	Interaction + error	367.94	4 (1+3)	91.23	-
	Total	2815.50	7		
20.1.78	Compartment	0.93	1	0.93	0.2
	Spore type	16.85	1	16.85	3.3
	Concentration	43.85	1	43.85	8.7
	Interaction + error	20.20	4 (1+3)	5.05	-
	Total	81.84	7		
New unsprayed leaves	Compartment	0.01	1	0.01	0.0003
	Spore type	103.90	1	103.90	5.02
	Concentration	34.24	1	34.24	1.7
	Interaction + error	82.77	4 (1+3)	20.69	-
	Total	220.91	7		

Fig. IV - 16

"Sooty mould" on cucumber plants in the control glasshouse compartment

Fig. IV - 17

A closer view of Fig. IV - 16

wilted and died prematurely. In contrast the plants in the two treated compartments appeared healthy with only a few leaves bearing honey dew and sooty mould (Fig. IV - 18 and IV - 19).

4. Different frequencies of spraying blastospores at different concentrations (Expt. 3)

In Expt. 2 conidia did not kill significantly more whitefly scales than blastospores so only blastospores were used in Expt. 3. Since the adults which emerged from survivors of the spore sprays in the two previous experiments continued to multiply on the new leaves, in Expt. 3 blastospores were sprayed at different frequencies and concentrations mainly to find out whether the pest population could be kept continuously under control when cucumbers were grown under conditions similar to commercial practice and when all stages of the pest were present simultaneously, instead of in discreet generations as in Expts. 1 and 2.

(a) Methods

The experiment was carried out in five compartments of the glass-house (Fig. IV - 5), one control and four treated. The layout of the experiment is given in Fig. IV - 20. The compartments C-2 and C-4 were sprayed monthly and C-3 and C-5 fortnightly, making this a split-plot experiment with latin square arrangement in each compartment. Each latin square was formed by four plots, two on each side of a compartment. Each plot comprised three adjacent plants treated alike. At each spraying two concentrations of blastospores were used in each compartment so that the latin square was a 2 x 2 design.

Fig. IV - 18

Healthy cucumber plants in Verticillium lecanii-treated glasshouse compartment

Fig. IV - 19

A closer view of Fig. IV - 18

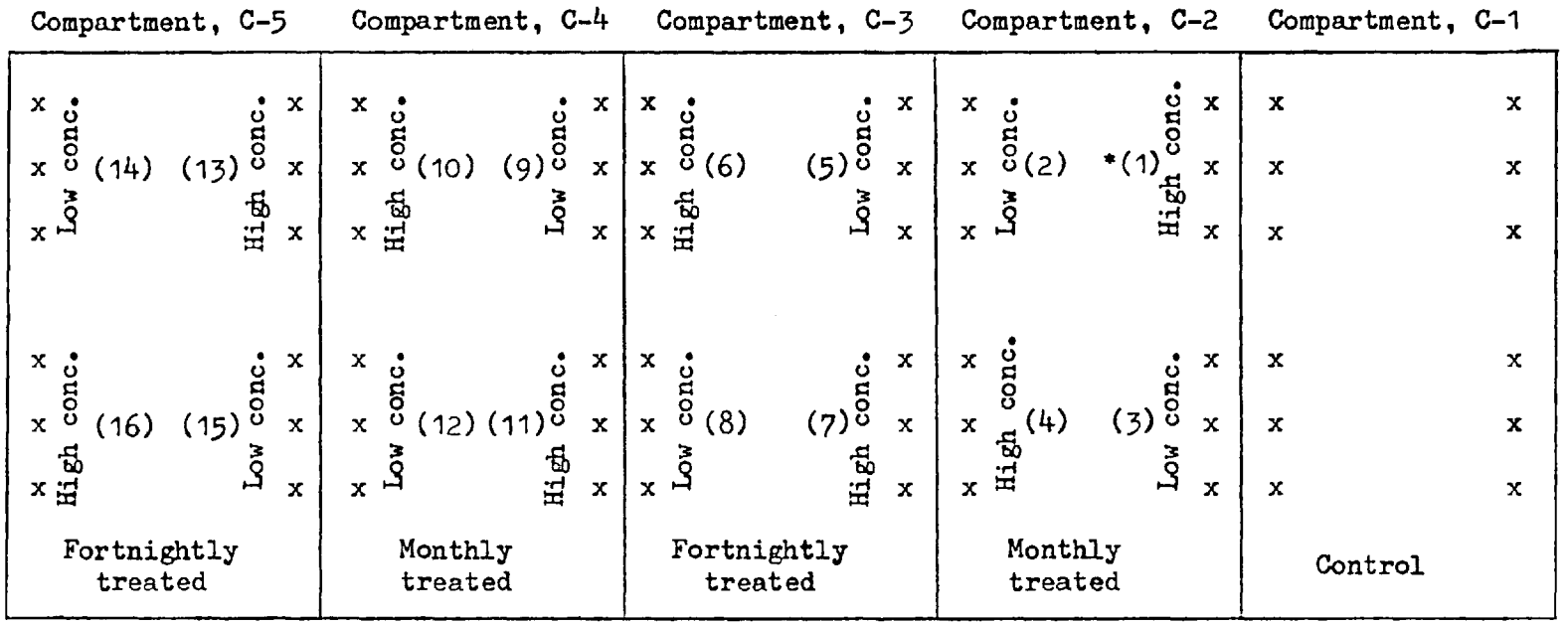


Fig. IV - 20

Layout of the experiment on effect of different frequencies of spraying blastospores of *Verticillium lecanii* at different concentrations

x, denotes the plant position;

* Numbers in brackets are the plot numbers for statistical analysis (Table IV - 21)

In the control compartment all twelve plants were treated alike. In all five compartments 75 whitefly adults per plant (900 adults per compartment) were released on 23.3.78. The adults were allowed to lay eggs continuously in contrast to the previous two experiments. The first spray was timed 13 days later when the scales were developing. Subsequent sprays fortnightly made a total of five sprays in compartments C-2 and C-4. Monthly sprays in compartments C-3 and C-5 totalled three. Viable spore concentrations sprayed on different dates are given in Table IV - 22. As far as possible a complete coverage of spray to thoroughly wet the ventral surface of all leaves was attempted.

Sample leaf discs for mortality assessment were cut about two weeks after each spraying. After the first spray (5.4.78), i.e. 27 days after release of adults, sample leaves were selected at three heights above the bases of the plants, which at this time were still young enough to have only the main stem, so that scales of different ages were sampled. Leaf 16 above the base bore the most susceptible stage of the pest (young scales). Leaf 12 had some advanced pupae which would emerge before V. lecanii could grow and kill them. Leaf 20 had some unhatched eggs which are not susceptible to this pathogen. One disc was cut from each leaf at each height on each of the three plants in a plot so that replication was threefold. By sampling time after the later sprayings, the plants had grown extensively. As the whiteflies lay eggs on young leaves, upper leaves were selected for sampling. After the main stem was stopped at 2 metres the uppermost pendant branch was chosen for sampling. By this time the leaf canopy was dense. As another measurement of

Table IV - 22

Concentrations of blastospores of Verticillium lecanii sprayed on different dates (Expt. 3)

Date sprayed	Concentration of sprays in viable spores/ml	
5.4.78	High	1.0×10^8
	Low	1.7×10^7
21.4.78	High	1.8×10^7
	Low	3.6×10^6
5.5.78	High	3.0×10^7
	Low	1.5×10^7
17.5.78	High	2.5×10^7
	Low	5.0×10^6
1.6.78	High	2.0×10^7
	Low	4.0×10^6

the effectiveness of control, the sizes of the surviving whitefly populations in all five compartments were compared by counting adults on all the leaves of the top-most branch of each plant. The top-most branch was chosen as it received more sunlight than the others and hence attracted most adults. Even very young leaves hidden in the middle of the canopy do not attract whitefly adults. Some older leaves exposed to sunlight and in high positions bore more adults than the shaded young leaves. Therefore, in addition to above, the four top-most whole leaves on the main stem of each plant in all five compartments were selected for approximate estimation of live whitefly scales and live and dead adults.

At the end of the experiment when the compartments were fumigated, all the live adults were killed. These fell from the leaves but those adults which had already been killed by V. lecanii still adhered to the ventral surface of the leaves. These bodies were counted on five leaves per plant (60 leaves/compartment) in the twelve plants in the control compartment to find out whether infection had spread from adjacent treated compartments.

(b) Results

In all observations taken after each of the five sprayings the control mortality was very low in contrast to the very high mortality of the treated scales (Table IV - 23).

The effects of different concentrations of blastospores and of sprayings at different frequencies were analysed by analyses of variance. The results from the control compartment were not included in the analysis. The first four observations after the

Table IV - 23+

Percentage mortality of whitefly scales after spraying different concentrations of blastospores of Verticillium lecanii at different frequencies on cucumbers in glasshouses (Expt. 3)

Compartment	Plot	Frequency of spray	Spray date and leaf no. sampled								Fresh** scales on new unsprayed leaves
			Main stem					Uppermost branch			
			5.4.78				21.4.78	5.5.78	17.5.78	1.6.78	
			12*	16*	20*	16	22	1	4	8	
2	1	4 weeks	97	98	77	96	96	85	84	71	26
	2		83	94	57	94	62	91	78	61	
	3		90	97	64	95	82	77	83	42	
	4		91	96	80	89	97	85	85	74	
3	5	2 weeks	84	93	68	90	83	86	94	91	12
	6		98	99	95	98	93	84	98	80	
	7		99	98	99	98	99	88	92	94	
	8		95	87	81	93	93	92	94	84	
4	9	4 weeks	94	74	39	74	47	82	95	92	11
	10		98	97	94	98	95	92	96	79	
	11		99	100	85	100	76	98	97	93	
	12		83	85	78	89	52	79	94	85	
5	13	2 weeks	100	94	81	99	73	93	99	100	20
	14		89	84	48	94	87	96	100	81	
	15		96	85	37	92	79	93	99	95	
	16		100	99	86	100	100	95	100	96	
1		No spores	9	1	1	1	2	2	9	15	5

+ Details in Appendices IV - 19 and 20

* One disc per leaf was examined. Others, three discs per leaf.

**Samples from 12, 6, 4, 9 and 12 plants from compartments 2, 3, 4, 5 and 1 respectively were examined according to availability of scales

first spray (columns 4-7 in Table IV - 23) were analysed to find the effect of concentration of spray. Later observations after the second to fifth sprayings (columns 8-11 in Table IV - 23) were analysed to find the effect of both spray concentration and frequency. As examples, the analyses of variance of columns 7 and 8 are given in Tables IV - 24 and IV - 25. The interaction of frequency and concentration of spray was not significant. Therefore tables of means for concentration of spray (Table IV - 26) and for frequency of spray (Table IV - 27) were prepared separately. The effect of different concentrations of the first spray on scales on the leaf 16 on the main stem as observed on 2.5.78 (27 days after spraying) was not significant (Tables IV - 24 and IV - 26). The concentration of spray had a significant effect on scales on the other leaves sampled after the first spray (Table IV - 24) and on scales examined after the second and fourth sprayings but not in the other two (Table IV - 24). From these results it appears that about 10^7 viable spores/ml gives very high mortality of scales and further increase in concentration is unnecessary. The effect of spraying at different frequencies was never significant (Table IV - 27).

From Table IV - 28 it is clear that the population of scales and adult whiteflies was consistently very much higher in the control compartment than in the treated compartments. However, compartment C-2 had a higher population than the other three treated compartments. In compartment C-2 it was observed earlier that a few leaves with several hundred scales escaped spraying by chance in the initial stages of the experiment. The adults emerged from these multiplied on new leaves. High proportions of live scales and adults were

Table IV - 24

Analysis of variance for mortality in column no. 7 in Table IV - 23

Source of variation	Degrees of freedom	Sum of squares	Mean square	F calculated	F at 5% level
Between compartments (blocks)	3	73.1	24.37	0.38	9.3
Between rows	4	71.6	17.90	0.28	9.1
Between columns	4	65.8	16.44	0.26	9.1
Concentration	1	210.2	210.25	3.3	10.1
Residual	3	192.4	64.15	-	-
Total	15	613.2	-	-	-

Table IV - 25

Analysis of variance for mortality in column no. 8

in Table IV - 23

Source of variation	Degrees of freedom	Sum of squares	Mean square	F calculated	F at 5% level
BETWEEN COMPARTMENTS					
Frequency	1	627.5	627.5	2	18.5
Residual	2	659.1	329.6		
Total	3	1286.6			
WITHIN COMPARTMENTS					
Rows	4	307.5	76.9	2	19.3
Columns	4	555.5	138.9	4	19.3
Concentration	1	1288.8	1288.8	35	18.5
Frequency x concentration	1	612.6	612.6	17	18.5
Residual	2	73.9	36.9	-	
Total	12	2838.3		-	
GRAND TOTAL	15	4124.9	-		

Table IV - 26

Mortality of whitefly scales after spraying different concentrations of blastospores of Verticillium lecanii on cucumbers in glasshouse (Expt. 3)

Spray no. and dilution ratio	compartments sprayed	Leaf no.	Mean % mortality for 8 plots, each of 3 plants. Concentration+		SED*	d.f.	P
			High	Low			
		Main stem					
1 (1:5)	4	12	98	89	0.55	3	0.001
		16	97	87	3.5	3	0.1
		16 (repeated)	97	90	4.0	3	0.2
		20	87	59	5.3	3	0.01
2 (1:5)	2	22	91	73	3.0	2	0.02
		Uppermost branch					
3 (1:2)	4	1	90	87	3.3	2	0.5
4 (1:5)	2	4	94	92	0.58	2	0.05
5 (1:5)	4	8	86	79	6.5	2	0.5

+ See Table IV - 22

* SED = Standard Error of Differences of Means

Table IV - 27

Mortality of whitefly scales caused by spraying blastospores of *Verticillium lecanii* at different frequencies on cucumbers in glasshouse (Expt. 3)

Spray no.	Leaf no.	No. of Compartments sprayed	Mean % mortality for 8 plots, each of 3 plants		SED*	d.f.	P
			Monthly sprayed	Fortnightly sprayed			
	Main stem						
1	-	4	-	-	-	-	-
2	22	2	76	88	9.1	2	0.3
	Uppermost branch						
3	1	4	86	91	3.7	2	0.3
4	4	2	89	97	6.9	2	0.4
5	8	4	75	90	12.8	2	0.4

* SED = Standard Errors of Differences of Means

Table IV - 28

Number of live whiteflies on cucumbers after spraying blastospores of Verticillium lecanii at different frequencies (Expt. 3)

Spray no.	Days after previous spray	Control compartment, C-1	Treated compartments			
			C-2 4-weekly	C-3 2-weekly	C-4 4-weekly	C-5 2-weekly
		Adults on 12 uppermost branches (one branch per plant)				
1	16	1740	790	130	220	650
2	6	2830	2560	400	400	760
3	14	4160	1800	520	700	770
4	8	3770	1230	390	260	260
5	7	38400	4260	410	590	640
	15	40800	4540	300	310	760
	8	33900	3700	90	140	190
		Adults on 48 uppermost leaves on main stem (4 leaves per plant)				
	8	1140	880	410	330	140
		Scales on 48 uppermost leaves on main stem (4 leaves per plant)				
	5	9970	3450	860	850	760

Table IV - 29[+]

Temperature and relative humidity in the compartments of the glasshouse (Fig. IV - 5) for Expts. 1, 2 and 3 on the use of Verticillium lecanii for the control of whiteflies on cucumbers

Spray date	Observed date	Compartment no.	°C			% R.H.		
			Max.	Min.	Mean	Max.	Min.	Mean
			Experiment 1					
30.9.77	30.9-17.10	4	29	22	24	95	66	80
		5	29.5	23	26.5	89	40	70
			Experiment 2					
23.12.77	23.12.77-	3	24	18	21	70	56	63
	9.1.78	4	29	20.5	23.5	86	54	72
		5	31	25	28	70	43	54
20.1.78	16.1-6.2	3	28	20	22	90	78	83
		4	29	21	23	88	51	67
		5	25	20.5	23	89	41	63
			Experiment 3					
5.4.78	4.4-17.4	1	29	20	22.5	94	50	60
		2	26	19	21.5	94	42	70
		3	28	20	22	94	52	72
		4	28	20	22.5	94	46	70
		5	30	19	23	96	36	67
21.4.78	17.4-1.5	1	28	20	22.5	94	45	67
		2	30	20	22.5	92	48	72
		3	31	21	23	94	50	78
		4	31	22	23	96	46	77
		5	33	20	22.5	96	46	73
5.5.78	1.5-15.5	1	26	20	22	92	40	69
		2	26	20	22	90	42	77
		3	26	22	22.5	94	52	80
		4	25	21	22.5	94	46	78
		5	27	19	22.5	96	44	75
17.5.78	15.5-29.5	1	29	20	23	92	40	70
		2	30	20	23	92	42	74
		3	27	21	22.5	94	50	72
		4	26	23	23	92	50	74
		5	31	20	23.5	96	50	74
1.6.78	29.5-15.6	1	31	17	22	94	38	69
		2	31	15	22	98	42	73
		3	30	17	23	92	48	76
		4	28	19	23	98	50	77
		5	32	15	23	98	52	76

[+] Details in Appendix IV - 21

present on new leaves in all four compartments treated with V. lecanii.

There was no apparent spread of infection from treated to control compartment. The plants in the control compartment were covered with honey dew and sooty mould and were wilting in contrast to the clean and healthy plants in the treated compartments.

5. Discussion

In all three experiments the treated plants remained in good condition whereas dense whitefly scale and adult populations on the control plants allowed sooty mould to grow and spoil the plants.

A single spray of conidia was sufficient to kill most whitefly scales on cucumber plants grown in glasshouses. Mortality of scales was higher in the compartment with the higher relative humidity (Expt. 1) and optimum temperature for the fungus (approximate mean, 24°C). High relative humidity resulted mainly from a lower temperature in the compartment, C-4, and the humidifier overnight was not effective enough to over-ride the drying effect of the higher temperature, approximate mean, 27°C, in C-5 (Table IV - 29). The lower mortality observed in the drier, warmer compartment was probably due to:

(1) Slow growth of V. lecanii above 25°C,

(2) warmth speeding the development of the pupae so that some emerged before the fungus could kill them,

(3) insufficient moisture for high degree of spore germination and infection.

While the temperatures differed substantially in the compartments in Expt. 2 (Table IV - 29) the compartment effect was significant after the first spraying. However, when the temperature (approximate mean, 27°C) in the compartment C-5 was reduced to the optimum level (approximate mean, 23°C) for the fungus, the compartment effect was not significant, indicating that the high temperature and its accompanying effects in C-5 were responsible for the lower mortality than in C-4. The strain used, (C-3, GCRI, IMI 179172) grows well at temperatures from 20°C to 25°C. At temperatures above 25°C growth was very slow and above 30°C there was no growth on an agar medium (section IV, B, 1). Thus the laboratory results agree with the glasshouse results.

In small glasshouse experiments in Sweden a high degree of infection and mortality of even one generation of whiteflies on cucumbers and tomatoes was not obtained by artificial application of V. lecanii (Ekbom, 1979b). Her failure could be due to the following reasons:-

(1) Viability of spores sprayed may have been low.

(2) Temperatures in the glasshouses often exceeded 30°C. This is unsuitable for V. lecanii (section IV, B, 1).

(3) The first spraying was several weeks after establishing whiteflies. Thus it is possible that pupal skins, which were already present at the time of spraying, were scored in her samples as survivors together with live scales.

(4) Other serious pests severely damaged the experimental plants and the experiment was discontinued prematurely.

She recommends the storage of blastospores cultured in liquid medium in the refrigerator for several weeks. In the present study (section IV, B, 2) the LT_{50} of blastospores in the culture medium (spent Sabouraud Liquid Medium) stored at 2°C was 9 days. At 2°C in all eighteen liquid media used for suspension, the blastospores lost viability quickly, the longest LT_{50} being approximately equal to one month. These results indicate the necessity of checking the viability of the spores before spraying on pests.

In Expts. 2 and 3, though the control compartments were adjacent to treated compartments, V. lecanii infection did not spread into the control compartments. Control mortality was low on the plants grown in the same compartment on the opposite side to that of the treated plants (Expt. 1). This indicates that the fungus does not spread from plant to plant even in the same compartment. The increased control mortality on day 20 after spraying (Expt. 1) was due to fungi other than V. lecanii. These could be Penicillium sp., recognizable by the long white spore chains growing on branched conidiophores, and Cladosporium sphaerospermum Penzig., with erect brown hyphal tufts. These two fungi were found associated with dead whitefly scales by Hussey (1958) and probably grew on "honey dew" excreted by live scales and adults. There was no "honey dew" apparent on the treated leaves as most of the scales were killed by V. lecanii. On sprayed leaves, some scales survived very close to others that died and became festooned with spores (Fig. IV - 11).

The three or five repeated sprayings in Expt. 3 kept the

whitefly population under control in a dense mature cucumber crop. In all three experiments there were dead adults covered with white cotton-like mycelium. Some of these would have emerged from infected pupae and the others might have been infected by alighting on dead scales bearing sporulating V. lecanii. However, there were some live adults and scales on the newly developing leaves on treated plants. Thus complete control could not be achieved even after five sprayings at fortnightly intervals. Susceptibility of whitefly adults to this fungus was also found by Hussey (1958) and Ekbom (1979b).

As the few scales which survived the sprayings in all three experiments (Figs. IV - 15a and IV - 15b) produced a new generation that escaped infection, high volume sprayings on developing young scales are necessary to keep the pest under control. V. lecanii cannot be introduced into a whitefly population in the expectation of natural spread. In Sweden adults contaminated or infected by exposure to cultures of V. lecanii for 8 h to 24 h, then released onto cucumbers infested with whitefly in a glasshouse failed to give satisfactory control by spread of infection (Ekbom, 1979b).

As the plants in Expt. 3 were large with a dense canopy as in a commercial glasshouse, it was extremely difficult to get a complete spray coverage at this stage of the crop. Thus alternative methods of pest control have to be used on a dense mature crop. The hymenopterous parasite, Encarsia formosa, invaded the glasshouse naturally and parasitised some of those scales which survived the fungal spray (Fig. IV - 15a and IV - 15b) and some of the new generation of scales, which developed after the treatment with

blastospores of V. lecanii. This suggests that E. formosa could be used as soon as temperatures are high enough to allow it to reproduce fast enough (Table IV - 2). V. lecanii could be used earlier when temperatures are low. This was investigated in another experiment (section IV, F, 4).

Conidia and blastospores did not differ significantly in killing scales in Expt. 2. Higher concentrations of spores caused higher mortality than the lower concentrations. However,more than 10^7 viable spores per ml would be unrewarding.

In all three experiments the two varieties of cucumber plants 'Farbio' and 'Butcher's Disease Resister' treated with V. lecanii were healthy and normal in growth, as was the variety 'Landora' in Ekbom's work (Ekbom, 1979b). There was no evidence that V. lecanii attacked the plants.

The systemic fungicide, dimethirimol, was used in all three experiments for control of cucumber mildew. Although no plants not treated with fungicide could be used for comparison, the high degree of control of whitefly scales obtained on V. lecanii-treated plants indicated that the fungicide did not harm V. lecanii. Development of V. lecanii (cited as Cephalosporium aphidicola) was not inhibited by dimethirimol in in vitro experiments by Wilding (1972b). He also found that neither triarimol nor dimethirimol inhibited the fungus in individuals of the aphid, Aphis gossypii, that fed on cucumber plants treated with these fungicides. Other agrochemicals already in use for the protection of cucumbers in glasshouses should be tested for adverse effects on V. lecanii for use in integrated control of whiteflies.

E. EFFECT OF PLANT SPECIES ON VERTICILLIUM LECANII

Plant species and their varieties differ in susceptibility to phytopathogenic fungi. This is partly due to varying morphological and/or physiological characteristics affecting the spore survival and germination on the leaf surface and due to environmental factors.

In the studies on use of Verticillium lecanii for whitefly control on various plant species, these plant factors may play a role. This was investigated by an experiment studying spore survival on the leaf surface under favourable conditions for the fungus in the laboratory, so that only plant effect would determine the spore survival. The experiment was repeated in the glasshouse using two commonly grown crops. Finally, the susceptibility of whiteflies, reared on various host plants, to V. lecanii was investigated in laboratory and glasshouse environments.

1. Effect on spore survival

(a) Laboratory studies

(i) Materials and Methods. Blastospores of V. lecanii, 1.8×10^7/ml, 84% viable, suspended in standard buffer with wetting agent, pH 7.2, were sprayed on the ventral surface of leaves of potted plants. Five plant species (Table IV - 30) were used, with two potted plants per species. These were kept at 20°C for 11 days in illuminated closed perspex cages resting in a tray of water to maintain approximately 100% relative humidity.

On 1, 4, 6, 8 and 11 days after spraying spore survival on leaf

Table IV - 30

Mortality of blastospores of Verticillium lecanii on five species of plants in the laboratory at 20°C and approximately 100% R.H.

Plant	Plant no.	Dead spores (%)+	Analysis of variance				
			Source of variation	D.f.	Mean square	F*	P
Tobacco *Nicotiana tabacum*	1	14.01	Plant species	4	32.10	0.99	> 0.05
	2	12.78					
Sweet pepper *Capsicum annum*	1	27.35	Error	5	32.47		
	2	9.92					
Tomato *Lycopersicon esculentum*	1	8.26					
	2	9.04					
French bean *Phaseolus vulgaris*	1	11.32					
	2	7.35					
Cucumber *Cucumis sativa*	1	12.29					
	2	10.56					

* At 5% significance level with 4 and 5 d.f. F = 5.2

\+ Mean of 5 observations: number of spores counted ranged between 200 and 600.

surface was estimated. From each potted plant, leaf samples were shaken until wet in two McCartney bottles containing 10 ml of 0.02% Triton X100 in buffer, pH 7.2 and soaked for about 30 min. After vigorous agitation for 1 min. on a whirlimixer, volumes were made up to approximately 25 ml with distilled water, mixed thoroughly and an aliquot studied for spore viability as in section IV, B, 2.

(ii) Results and Discussion. The spores survived well on all five species of plant (Table IV - 30). Variations due to plant species were not significant at the 5% level ($P > 0.05$). Since death of spores by desiccation was avoided while spores were on the plants and the highest mean mortality was 27%, it is unlikely that any of the plant leaf surfaces had substances lethal to blastospores of V. lecanii.

(b) Glasshouse studies

(i) Materials and Methods. Two potted tomato plants and two cucumber plants were sprayed with blastospores as in section IV, E, 1, (a), in two replicate experiments at concentrations of 5.8×10^7 spores/ml, 79% viable in one experiment and 4.7×10^7 spores/ml, 95% viable in the other. After spraying the plants were retained in the glasshouse for 3 weeks. At 5 or 6 intervals spore viablity was estimated as in section IV, E, 1, (a), using two replicate counts from each plant at each interval.

(ii) Results and Discussion. Very large proportions of blastospores died within a few days after spraying and later the proportion dead remained somewhat constant on both tomato and cucumber plants. However, the proportions dead on tomatoes appeared to be higher than on cucumbers. As the proportion of spores dead, except after the first few days, remained more or less constant two mean values for 5 or 6 observations were calculated for the two replicate plants of each plant species (Table IV - 31).

In an analysis of variance with the two experiments with the experimental effect regarded as one main effect and the plant species as another (each with two replicate plants) the interaction was negligible and differences between experiments and between plant species significant whether or not the interaction was included in the error (Table IV - 32).

The differences in survival of spores on the two plant species might be explained by differing desiccation rate due to a different transpiration rate of the leaves, or the morphology of the leaves, compound in tomato and simple in cucumber.

In the glasshouse maintained at an approximate mean air R.H. of 70-80% (min., 54%; max., 90% R.H.), there would have been an opportunity for desiccation of spores even on the leaf surfaces. In the laboratory, however, desiccation was avoided by a humidity near 100% R.H. (section IV, E, 1, (a)) and the spore survival on these plant species did not differ significantly.

The variation in the number of hours of sunshine, and hence of solar radiation, on the first few days after spraying in the two experiments was perhaps responsible for the difference in spore

Table IV - 31

Mortality of blastospores of Verticillium lecanii on tomato and cucumber plants in the glasshouse

Plant	Plant No.	Mean % mortality for 5 or 6 observations after Abbott's correction	
		Experiment 1	Experiment 2
Tomato	1	79.8	94.4
	2	82.3	92.6
Cucumber	1	55.3	68.0
	2	61.3	65.8

Table IV - 32

Analysis of variance for Table IV - 31

Source of variation	D.f.	Mean square	F*	P
Replicate experiments	1	221.13	34.89	< 0.05
Plant species	1	1217.71	192.12	< 0.05
Interaction	1	7.68	1.21	> 0.05
Error	4	6.34	-	

* At 5% significance level with 1 and 4 d.f. F = 7.7

mortality between the two experiments because the temperature and relative humidity were approximately similar in both experiments.

2. Effect on Verticillium lecanii-induced whitefly scale mortality

Three experiments were done to investigate whether whitefly scales reared on five different plant species can be killed with equal ease by V. lecanii. A fourth experiment was done only with tomato plants as it is the most important glasshouse crop among the five plant species tested.

(a) Laboratory studies

(i) Materials and Methods. Whitefly scales established on different potted plants were sprayed with spores and the mortality assessed after 1-2 weeks. The same plant species as in section IV, E, 1, (a), were used in Expts. 1-3 (Table IV - 33).

Scales from eggs laid over an 8-h period (section IV, C, 1) were 19-20 days old (since egg laying) in Expts. 1-3 and 17 days in Expt. 4, at 24°C in glasshouses, by which time they were fully developed pupae. The plants were sprayed thoroughly with blastospore suspension at 3-6 x 10^7 viable spores/ml in standard buffer. The buffer without spores was sprayed on control plants. After spraying, the plants were kept at 20°C for one week in illuminated closed perspex cages, resting in a tray of water to maintain approximately 100% R.H. Then the polythene sheets over the cage doors were replaced with muslin cloth in order to enhance drying of dead scales so that live and dead scales could be easily distinguished. In Expts. 1-3, two weeks and in Expt. 4, one week after spraying, small whole leaves or portions of large leaves were removed from the

plants and examined for live and dead scales.

(ii) Results and Discussion. Most of the scales on the fungus-treated plants were dead and covered with profuse growth of V. lecanii in contrast to the clean plump live scales or pupal skins remaining after adult emergence on the control plants. Control mortality was generally low except on cucumber in Expt. 2 and not due to V. lecanii (Table IV - 33). As there was only one host plant species (tomato) in Expt. 4 the data were not statistically analysed.

A single factor analysis of variance for each experiment showed significant differences between plant species only in Expt. 1 (Table IV - 34) probably because of the very low variation between replicates in that experiment. Single factor analysis across experiments for each plant species revealed no significant differences between experiments so the data were pooled and again showed no significant differences between plant species.

It is concluded that the whitefly scales feeding on the sap of the five host plant species tested do not differ in susceptibility to V. lecanii. However, variation in scale mortality could occur in practice due to incomplete wetting of the ventral leaf surface as the wettability of the leaf surfaces differ among plant species and even among their varieties.

Table IV - 33

Effect of plant species on pathogenicity of blastospores of *Verticillium lecanii* sprayed on whitefly scales in the laboratory at 20°C and about 100% R.H.

Plant	No. of plants & in parenthesis no. of scales examined		Mortality (%)		
	Control	Treated	Control	Treated, after Abbott's correction	Mean, treated
		Experiment 1			
Tomato	1 (55)	1 (49)	22	97	97
French bean	1 (99)	2 (458)	17	82,84	83
Cucumber	1 (202)	2 (98)	19	100,100	100
Tobacco	1 (284)	2 (460)	16	98,98	98
		Experiment 2			
Tomato	2 (350)	2 (826)	38	97,97	97
French bean	1 (252)	3 (1736)	32	85,91,99	92
Cucumber	1 (111)	2 (806)	56	77,100	89
Tobacco	1 (261)	1 (362)	19	100	100
Pepper	1 (80)	3 (200)	20	96,94,89	93
		Experiment 3			
Tomato	1 (862)	2 (1970)	19	84,89	87
French bean	1 (768)	2 (1101)	5	100,95	98
Cucumber	1 (203)	2 (818)	27	100,97	99
Tobacco	2 (534)	2 (827)	5	92,100	96
Pepper	2 (246)	2 (452)	19	80,89	85
		Experiment 4			
Tomato	2 (134)	6 (692)	23	79,97,99,96,100, 96	95

Table IV - 34

Analysis of variance for Table IV - 33

Variable	Expt. no.	d.f.		Mean square		F	F for P=0.05	P
		Variable	Error	Variable	Error			
Plant species	1	3	3	118.29	0.67	177.43	9.28	<0.05
Plant species	2	4	6	31.62	64.86	0.49	4.53	>0.05
Plant species	3	4	5	86.60	20.40	4.3	5.2	>0.05
Replicate expts. (tomatoes)	1-3	2	2	66.15	6.25	10.58	19.00	>0.05
Replicate expts. (cucumbers)	1-3	2	3	78.17	89.67	0.87	9.50	>0.05
Replicate expts. (French beans)	1-3	2	4	106.85	28.29	3.8	6.9	>0.05
Replicate expts. (tobacco)	1-3	2	2	5.60	16.00	0.35	19.00	>0.05
Replicate expts. (pepper)	2-3	1	3	86.70	22.17	3.9	10.1	>0.05
Plant species	1-3	4	22	69.99	46.18	1.50	2.80	>0.05

(b) Glasshouse studies

On cucumber and tomato plants in glasshouses it was found that V. lecanii spores killed whitefly scales (sections IV, D, 2 - 4 and IV, F, 4). In this experiment effect of tobacco plant on whitefly scale mortality, caused by V. lecanii was studied.

(i) Materials and Methods. Whitefly scales of two ages were sprayed on the same day and the scale mortality assessed at intervals.

Two potted tobacco plants bore scales, 15 days old (since egg laying) from eggs laid over 8 h (section IV, C, 1) and two other plants, 8 days old scales (since egg laying), maintained in a glasshouse at 24°C. The spray suspension of conidia (see section IV, C, 1) applied to two plants contained 2.5×10^7 viable spores/ml, spray without spores being used as control on the other two plants. After spraying, the plants were retained in the same glasshouse at approximately 24°C and 80% R.H. On 15, 22 and 28 days after spraying four 32 mm diameter leaf discs/plant were examined under a binocular microscope for scale mortality.

(ii) Results and Discussion. Control mortality was very low and not due to V. lecanii in contrast to fungus-treated mortality (Table IV - 35) in all the three observations of both age groups. Thus it is concluded that whitefly scales on tobacco plants can be killed by high volume spray of conidia of V. lecanii. This is similar to the glasshouse experiments (see sections IV, D, 2-4 and IV, F, 4) on cucumber and tomato plants.

Table IV - 35

Effect of tobacco plant on Verticillium lecanii-induced whitefly scale mortality in a glasshouse

Days after spray	Age of scales from egg laying (days)*	No. of scales		% dead scales	
		Control	Treated	Control	Treated after Abbott's correction
15	8	112	164	0	99
	15	364	291	4	94
22	8	90	343	3	100
	15	209	202	4	92
28	8	83	211	1	99
	15	325	171	22	88

* At 24°C eggs hatch in 6 days (Table IV - 1)

F. SUSCEPTIBILITY OF ENCARSIA FORMOSA AND PHYTOSEIULUS PERSIMILIS TO VERTICILLIUM LECANII

In feasibility studies on the use of V. lecanii as an alternative to chemicals for the control of glasshouse whitefly it is essential to know whether this fungus is harmless to E. formosa and P. persimilis, two natural enemies commonly used in glasshouses. This was investigated in the laboratory and in small glasshouses under high and low relative humidities respectively. In addition the possibility of integrating V. lecanii and E. formosa for whitefly control was studied.

1. Encarsia formosa: laboratory studies

(a) Materials and Methods

Tomato and French bean plants bearing whitefly scales developed at 24°C, two weeks after egg laying over 8 h were obtained by methods described in section IV, C, 1. Then E. formosa adults were allowed to oviposit in the scales. After periods ranging between 8 and 26 days (Table IV - 36) some plants with parasitised scales were thoroughly sprayed with blastospore suspensions in standard buffer and other plants were sprayed with standard buffer as controls. All plants were then kept for one week in an illuminated closed perspex cage, resting in a tray of water to maintain a nominal 100% R.H. Then to allow evaporation to remove condensation the door to the cage was unsealed and covered with muslin cloth. The temperature in the laboratory was 20°C.

After allowing sufficient time for development and emergence of E. formosa adults (i.e. minimum of 30 days after oviposition) emerged and unemerged were scored while holding leaves against the light.

For the production of E. formosa, whitefly scales were developed on plants as in section IV, C, 1. The young flat scales developed to a stage suitable for parasitism by E. formosa in about two weeks, i.e., plump with a convex upper surface. At this stage the plants were transferred to the "E. formosa infesting" glasshouse where 2-3 tobacco leaves bearing approximately 4000 to 5000 black parasitised scales were kept for adult parasite emergence. About 1-4 adult parasites/square inch leaf area were available for parasitism of whitefly scales. After about nine days when the first black scales were formed the plants were transferred to the "whitefly emergence glasshouse" where 17-29 days after original infestation, unparasitised whiteflies emerge. A clean tobacco plant changed fortnightly was kept in this house to catch stray adults. Then E. formosa infested plants were removed at intervals until emergence of adult parasites began, as required for the experiments.

(b) Results and discussion

The V. lecanii spray decreased parasite adult emergence in all age groups, most while the parasites were young, although the 99% unemerged in the 20-day group does not follow this trend. At 26 days, a few adult E. formosa had already emerged before spraying,

so the fungus had little time to act, thus parasite mortality was correspondingly low (26%). Emergence of E. formosa adults in the control varied greatly (Table IV - 36) possibly due to exposure to high humidity. Such exposure while the parasites were either very young or nearly mature (26 days) caused least mortality (unemerged). Fluffy white growth of V. lecanii was seen on many black scales on treated plants but not on control plants. When black scales were crowded the fungal growth coalesced.

It is concluded that, at extreme high relative humidity, V. lecanii kills most parasites inside whitefly scales, unless the fungus is applied shortly before emergence of the adult parasites.

2. Phytoseiulus persimilis: laboratory studies

(a) Materials and Methods

One day after spraying two potted French bean plants with Verticillium lecanii, 4.5×10^7 viable blastospores/ml, several hundred red spider mites, Tetranychus urticae, were released with 50-100 predators, Phytoseiulus persimilis. Subsequently excess spider mites were supplied as food for the predators. Another plant was used as control by omitting V. lecanii from the spray. Immediately after spraying the plants were kept in illuminated closed maximum humidity cages at 20°C (section IV, F, 1) one for the control and the other for the fungus-sprayed plants.

To examine the effect of the fungus on the red spider mite alone three similar plants were set up without the predators in two other cages.

Table IV - 36

Effect of *Verticillium lecanii* infection of parasitised whitefly scales on emergence of *Encarsia formosa* from the scales at high relative humidity in the laboratory at 20°C

Timing of spray: days after *Encarsia* oviposition	Viable spores/ ml x 10^7	Mortality: mean % scales unemerged		No. of plants		No. of insects	
		Control	Treated, after Abbott's correction	Control	Treated	Control	Treated
8	2.0	11	89	1	3	55	31
11	1.5	23	82	2	3	646	421
14	2.0	43	80	1	3	134	885
15	1.5	48	90	1	2	431	1412
17	5.4	40	52	3	8	1075	1205
20	6.8	67	99	1	3	123	163
26	6.8	13	26	1	2	30	310

After one week all the leaves (3-7) from each plant were removed and examined under a binocular microscope for live and dead mites with and without V. lecanii.

(b) Results and discussion

Table IV - 37 shows that mortalities of T. urticae and P. persimilis on V. lecanii-treated plants were higher than in the control. On some dead cadavers of both species the fungus has grown and sporulated. When the total population on the treated plants for both species were considered, there were more live mites than dead mites. On the control plants no T. urticae were dead and only one P. persimilis but without visible V. lecanii.

There are three possibilities for the death of V. lecanii treated mites:-

(1) Drowning in the thick film of viscous blastospore suspension on leaf surface as there was no drying due to high R.H. in the closed cages.

(2) Natural death of the mites and saprophytic growth of V. lecanii on the dead bodies.

(3) Infection by V. lecanii.

As the control mortality was negligible the natural death is ruled out. The most suitable explanation is death due to drowning. It was investigated by dipping 30 predators in blastospore suspension with 1×10^8 viable spores/ml and then releasing on plants infested with red spider mites in a closed cage, at 20°C, under approximately 100% R.H. when, only six died immediately. The

Table IV - 37

Effect of releasing *Tetranychus urticae* and *Phytoseiulus persimilis* on beans sprayed with blastospores of *Verticillium lecanii* and held under very high relative humidity at 20°C in the laboratory

Treatment	Live	Dead	Remarks
T. urticae			
Control	>1000	0	Several eggs and nymphs seen.
Sprayed with *V. lecanii*	>6000	176	>100 dead with profusely grown *V. lecanii*. Also ventral leaf surfaces (sprayed) were free from mites. Upper surfaces had heavy population.
P. persimilis			
Control	55	1	Not due to *V. lecanii*.
Sprayed with *V. lecanii*	176	139	Some dead mites had sporulating *V. lecanii* mycelium.

others multiplied to 78 mites (67 live and 11 dead) fifteen days after dipping without any sign of V. lecanii growing on them. However, on the remains of the prey (T. urticae) left by the predator profuse growth and sporulation of V. lecanii was seen. Thus it is unlikely that V. lecanii infected and killed the mites released on sprayed plants.

3. E. formosa and P. persimilis: glasshouse studies

(a) Materials and methods

Populations of E. formosa and P. persimilis were set up in four small glasshouses two for each species. Four plants on one side of each house were sprayed with V. lecanii blastospore suspensions and four plants on the other side with spray fluid without spores as control. The infestation, spraying and sampling schedules are given in Table IV - 38. The last examination for predators was made on unsprayed young leaves as no predators remained on the old sprayed leaves. The concentrations of spores in the sprays varied between 4.5 and 6.8 x 10^7 viable spores/ml. The physical conditions in the four glasshouses were similar, the temperature controls being set at 20-22°C, the mean relative humidity usually being about 60-70% (Table IV - 41).

(b) Results and discussion

Emergence of Encarsia adults was similar from the scales sprayed with fungal spores and in the controls, without spores (Table IV - 39). As the first spray was applied after black scales appeared, most of the unparasitised whiteflies had emerged

Table IV - 38

Schedule of insect and mite releases, spraying and sampling in the studies on infectivity of *Verticillium lecanii* on the parasite, *Encarsia formosa* and the predator, *Phytoseiulus persimilis* in small glasshouses

Days after first release		*Encarsia formosa*	*Phytoseiulus persimilis*
White-fly	Red Spider Mite	On 6.10.78, released several hundred whitefly adults on tomato plants.	
14		Released a few hundred *E. formosa* scales, weekly until day 47.	
28		Black *E. formosa* scales seen in both houses.	
			On 17.11.78 released several hundred *T. urticae* and about 50 *P. persimilis* adults on each French bean plant.
32 & 42	0	Sprayed in all four glasshouses.	
59	18	Leaf samples collected from all four glasshouses.	
60	20	Sprayed in all four glasshouses.	
70, 89 & 101	28, 47 & 59	Leaf samples collected from all four glasshouses.	

Table IV - 39

Percent dead (unemerged) Encarsia formosa in whitefly scales after spraying blastospores of Verticillium lecanii on tomato plants in glasshouses

Days after spray*			Glasshouse no. 1 (6)[a]		Glasshouse no. 2 (7)[a]	
1st	2nd	3rd	Control	Treated	Control	Treated
28	18		13	30	13	13
38	28	9	11	4	8	8
57	47	28	36	33	21	37
69	59	40	27	35	28	17
		Average	22	25	17	19

* First spray on 7.11.78, second on 17.11.78, third on 6.12.78. No. of insects examined ranged from 226 to 770.

a - Refers to actual glasshouse number and given in Table IV - 41.

before spraying. In the first samples from treated plants a few white and brown scales were seen with sporulating V. lecanii. Some of these could have been parasitised by E. formosa but the proportion is unknown because it is not possible to distinguish parasitised and unparasitised whitefly scales before they turn black.

A higher proportion of P. persimilis were dead on leaves sprayed with V. lecanii than on control leaves (Table IV - 40). However, no growth and sporulation of V. lecanii was seen on the dead predators except once, when 22% dead predators were seen with fungus on a leaf, the whole area of which was covered with a hyphal mat of V. lecanii. New leaves, which grew after spraying, bore no sign of fungal growth on dead predators and the mortality on leaves on the fungus treated plants was slightly lower than that on the control plants.

The higher mortality on fungus-sprayed leaves was probably due to entangling of predators in viscous blastospore suspension soon after spraying. It is unlikely that this would occur as severely on plants in commercial practice because the sprays would be lighter than in the present study.

Table IV - 40

Percent mortality of Phytoseiulus persimilis after spraying blastospores of Verticillium lecanii on French bean plants in glasshouses

Days after spray*			Glasshouse no. 1 (9)[a]		Glasshouse no. 2 (11)[a]	
1st	2nd		Control	Treated	Control	Treated
17			9	16	13	41
28	9		33	94	7	14
47	28		9	35	23	5
			Leaves grown after spraying			
59	40		28	13	31	30
		Average	20	39	18	22

* First sprayed on 17.11.78, second on 6.12.78. No. of mites examined ranged from 31 to 277.

a - Refers to actual glasshouse number and given in Table IV - 41.

Table IV - 41

Temperature and relative humidity in small glasshouses for the experiments on infectivity of Verticillium lecanii on Phytoseiulus persimilis and Encarsia formosa

Spray date	Observation dates	Glass-house no.	°C			% R.H.		
			Max.	Min.	Mean	Max.	Min.	Mean
	1978							
7.11.78	6.11-13.11	6	23	18	20	82	64	72
		7	25	18	20	76	60	70
		9	23	18	20	76	62	70
17.11.78	13.11-4.12	6	24	16	19	76	42	64
		7	24	16	18	78	48	62
		9	23	14	17	76	45	61
	20.11-22.11	11	23	16	18			
6.12.78	4.12-31.12	6	21	16	18	74	50	63
		7	23	16	18	80	48	66
		9	19	13	16	76	48	62
	11.12-18.12	11	22	15	17	76	60	70
	1979							
	2.1-15.1	6	23	13	18	78	54	60
		7	25	15	18	74	50	62
	8.1-15.1	9	19	14	15	68	42	50
	2.1-8.1	11	21	13	14	68	34	50

4. Encarsia formosa: integration with Verticillium lecanii

The efficiency of the hymenopterous parasite, Encarsia formosa, is greatly impaired in short dull days early in the year, presumably because the flight temperature threshold is not reached. E. formosa will fly about the glasshouse to search for prey only above 22°C, but will search actively on leaf surfaces above about 16°C (Scopes and Ledieu, 1979). In addition, the parasite attacks only third instar whitefly scales and if, when it is released, all stages of the pest are present, many will escape attack. When infestations of whitefly are present early in the season before the parasite is established, the pest must be limited by alternative methods. Hence the possibility of using sprays of blastospores of V. lecanii for limitation of the pest before introduction of E. formosa was investigated.

(a) Materials and methods

Whiteflies were established on tomato plants in four compartments of the glasshouse described in section IV, D, 1. V. lecanii was sprayed initially to reduce the pest population before E. formosa was introduced in two compartments maintained at two different temperatures. In another compartment only parasites were released on to the pests to show the effect of poor establishment of parasites. One compartment was used as control to establish that a virile pest population was in use.

The experimental design is given in Fig. IV - 21 and a schedule of insect releases, fungus applications, and sampling in

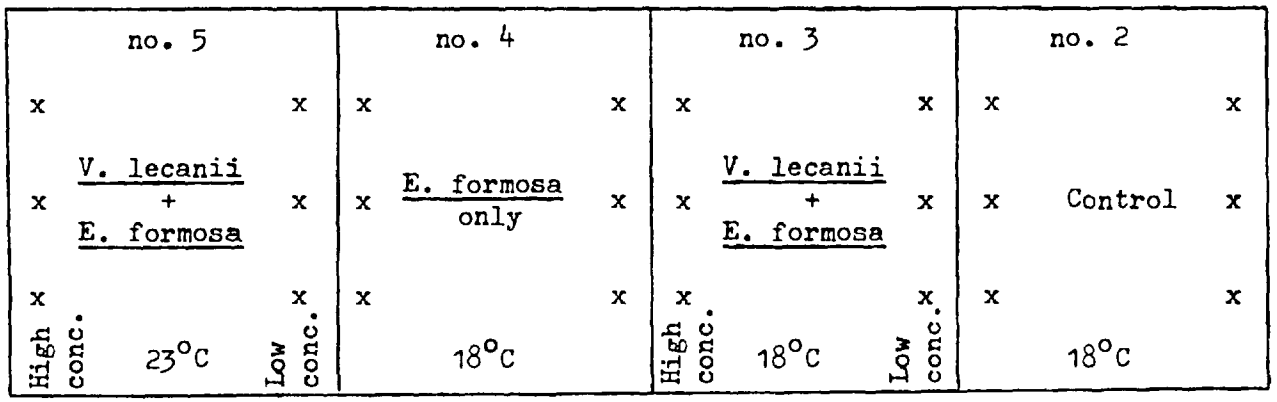


Fig. IV - 21

Integration of Verticillium lecanii and Encarsia formosa for control of whiteflies in glasshouse: experimental layout

x, denotes the plant position

Table IV - 42. To establish the pest population, whitefly adults were released on pest and disease free tomato plants, variety 'Sonato' 40-45 cm in height, grown by normal cultural practices. The whitefly adults were allowed to continue egg-laying until they died naturally so that the population contained pests in all developmental stages.

The details of the blastospore sprays to control the pest in two compartments were as follows:-

	Spray	Concentration, Viable spores/ml	
First:	18 days after first whitefly release	High	5×10^7
		Low	1.5×10^7
Second:	18 days after first spray	High	4.5×10^7
		Low	9×10^6

In the parasite-only compartment, establishment of the pest population was delayed and timed to be at a stage suitable for parasitism when E. formosa was released, which was done at the same time as in the other two treated compartments.

Production of spores, spray suspension, sprayers, application of spray, measurement of temperature and relative humidity were as described in section IV, D, 1. E. formosa was introduced after collection of samples after second spraying (Table IV - 42).

Table IV - 42

Schedule of insect releases, sprays and sampling during integration of *Verticillium lecanii* and *Encarsia formosa* for the control of whiteflies in glasshouses

Dates or weeks after last spray	Control 18°C	*Encarsia* only 18°C	*V. lecanii* + *E. formosa* 18°C	*V. lecanii* + *E. formosa* 23°C
22.1.79 and 26.1.79	Released 10 whiteflies per plant	-	Released 10 whiteflies per plant in both compartments	
6.2.79		Released 20 whiteflies per plant	-	-
9.2.79	Spray, wetting agent in buffer	-	First spray, *V. lecanii*: in both compartments	
12.2.79	Released 10 whiteflies per plant in all 4 compartments			
3rd week after first spray	Sampled two whole leaves per plant in all compartments (From leaf no. 9-14 in 3 cooler compartments*)			(From leaf no. 11-17*)
27.2.79	Spray, wetting agent in buffer	-	2nd spray, *V. lecanii*: in both compartments	
2nd week	Sampled two whole leaves per plant in all compartments (Between leaf nos. 13-22 in 3 cooler compartments*)			(Leaf nos. 19-24*)
3rd week	-	First release, about 200 *E. formosa* scales/plant in all 3 compartments (⌒ 10% only emerged)		
4th and 5th weeks	-	Second release, about 100 *E. formosa* scales/plant in all 3 compartments (most emerged)		
	-	Third release, about 200 *E. formosa* scales/plant in all 3 compartments (most emerged)		

Continued/

Table IV - 42 (Continued)

Dates or weeks after last spray	Control 18°C	Encarsia only 18°C	V. lecanii + E. formosa 18°C	V. lecanii + E. formosa 23°C
9th week	Examined leaf nos. 20 & 21 in all 3 low temperature compartments			Examined leaf nos. 25 & 26
12th week	Examined leaf nos. 24 & 25 in all 3 low temperature compartments			Examined leaf nos. 29 & 30
15th week	Examined scales on one leaflet only from each leaf nos. 50, 51 & 52 for spread of V. lecanii infection in all 4 compartments			
16th week	(a) Examined leaf nos. 40 and 41 in all 4 compartments			
	(b) Examined 5 young leaves below terminal bud for spread of V. lecanii infection on whitefly and E. formosa adults			
	(c) Counted under a binocular microscope the number of whitefly eggs in three young leaflets per plant collected from all four compartments			
	(d) Counted the number of leaves with and without sooty mould on each plant in all four compartments			

* Numbered from base of the plants

Production of E. formosa for release in the glasshouses is described in section IV, F, 1. When emergence of E. formosa adults began, portions of tobacco leaves bearing the required number of scales were cut and one piece left on each tomato plant in the three treated compartments.

On tomatoes whitefly adults and scales, and hence E. formosa are not evenly distributed on the leaf canopies. Eggenkamp-Rotteveel Mansveld et al. (1978) reported that data from random sampling reflected the actual numbers and the distribution of the whitefly and its parasite, E. formosa, very badly. Therefore stratified leaf samples (Southwood, 1978), at various heights along the main stem were taken (Table IV - 42). Scales were counted because they are easy to find and distinguish into parasitized (black) and unparasitized forms (white). As natural pupal mortality is very low, the numbers of white and black pupae are nearly equal to the numbers of adult whiteflies and parasites, respectively, that will eventually emerge from them.

At each sampling (Table IV - 42) two leaves per plant were examined for scale mortality. Also a few leaflets from the treated compartments were incubated at 20°C under high relative humidity to examine for growth of V. lecanii on dead scales. Unparasitised whitefly scales killed by V. lecanii were distinguished from those killed by injury due to feeding by E. formosa. When large numbers of scales were found on some samples collected 16 weeks after the last spray only leaflets, not whole leaves, were examined. Higher leaves were sampled in the warmest

compartment because the plants grew faster.

(b) Results

The two early-season V. lecanii sprays caused 33-67% mortality of scales while mortality in the control infestation was negligible with no signs of fungus spread into the control (Table IV - 43). After introduction of E. formosa, mortality due to the parasite increased and that due to the fungus decreased. E. formosa adults killed many scales by feeding punctures (Table IV - 43). Since the parasite soon spread to the control comparment, killing 55% of the pest scales by 9 weeks after the last fungus spray, the most meaningful measurement of pest control was the condition of the plants at the end of the experiment (16 weeks after last fungus spray), as expressed by the appearance of sooty mould growing on leaves coated with honey dew (Fig. IV - 22 to IV - 24), from pests (Table IV - 46). Plants with V. lecanii + E. formosa had very few leaves with sooty mould ($\sim$ 5%) whereas those with E. formosa alone or neither (control) had many ($>$25%). The proportion of live scales on the former plants was about half that on the latter at the 9 week post-last fungus spray assessment (Table IV - 43). Thus the early-season sprays of fungus greatly improved control of the whitefly.

Over all the observations, the proportion of E. formosa not emerged from scales and believed to be dead, was similar in all compartments with and without fungus (Table IV - 44), except that at 9 weeks after the last spraying 10 to 25% were recorded in the

Fig. IV - 22a

"Sooty mould" on tomato leaves in control glasshouse compartment

Fig. IV - 22b

Stages in "sooty mould" damage to tomato leaflets

Fig. IV - 23

"Sooty mould" on tomato leaves in the "Encarsia formosa only" glasshouse compartment

Fig. IV - 24

Healthy tomato leaves in the Verticillium lecanii + Encarsia formosa glasshouse compartment

Table IV - 43

Integration of Verticillium lecanii and Encarsia formosa to control whitefly on tomatoes in glasshouses: percent** (a) live whitefly scales, (b) dead unparasitised whitefly scales and (c) parasitism of whitefly scales

Week after spray	Leaf number up stem	No fungus, no parasite 18°C			E. formosa only 18°C			Spore concentration ***	V. lecanii + E. formosa 18°C			V. lecanii + E. formosa 23°C		
		a	b	c	a	b	c		a	b	c	a	b	c
3rd after 1st spray	9-17	97	3NF	-	-	-	-	H	63	37F	-	53	47F	-
								L	58	42F	-	64	36F	-
2nd after 2nd spray	13-24	94	6NF	-	-	-	-	H	33	67F		54	46F	
								L	43	57F		67	33F	
9th after 2nd spray	20-26	45	7P	48	64	8P	28	H	31	57Fp	12	29	28Fp	43
								L	36	50Fp	13	24	42Fp	34
12th after 2nd spray	24-30	37	9P	54	15	19P	66	H	6	30Fp	64	7	27Fp	66
								L	10	33Fp	57	15	28Fp	56
16th after 2nd spray	40-41	2	29P	69	7	21P	72	H	1	45Pf	54	2	44Pf	55
								L	1	33Pf	66	1	28Pf	71
15th after 2nd spray	50,51,52	2	81P	17	6	87P	7	H	9	72P	13	5	80P	15
								L	15	82P	9	3	75P	22

* NF - no fungus; F - fungus, P - parasite alone, Fp - more due to fungus than parasite, Pf - more due to parasite than fungus when cause for scale mortalities were considered.

** To obtain percentage values, 1416-2076 scales on 6 plants (12 leaves) in control compartment and 201-1340 scales on 3 plants (6 leaves) per treatment in treated compartments were examined after spraying and 200-9000 scales after E. formosa release.

*** H and L refer to high and low concentration of blastospores.

Table IV - 44

Integration of *Verticillium lecanii* and the parasite *Encarsia formosa* to control whitefly on tomatoes in glasshouses: percent dead (unemerged) *E. formosa* scales, each value based on 100-9000 scales

Weeks after last fungus spray	Treatments each in one glasshouse compartment				
	Control	*E. formosa* only	Spore concentration	*V. lecanii* + *E. formosa*	
	18°C	18°C		18°C	23°C
9	10	16	H*	25	5
			L*	17	6
12	6	5	H	6	6
			L	9	8
16	9	10	H	7	4
			L	5	3

* H and L refer to high and low concentration of blastospores

cooler compartments, probably because all the live parasites had not emerged due to the low temperature. Thus it is unlikely that the fungus had spread to parasitised scales and killed the parasites. Fungus-killed adult E. formosa were rare.

By the end of the experiment the E. formosa had gained good control in all four compartments, including the control, only 2-15% of whitefly scales surviving (Table IV - 43). No fungus spread to compartments not sprayed with fungus. The numbers of whitefly and E. formosa adults on plants without fungus treatment were low and on those with fungus very low (Table IV - 45). Egg numbers average < 2/leaflet in all four compartments but the variation over the plant was too great for this observation to show a difference between treatments (Table IV - 48).

Early in the season at the first two counts (Table IV - 43), there were no consistent differences in the proportions of fungus-killed scales after the first spray (36-47% dead scales) and after the second (33-67% dead scales). Neither were there differences due to spray concentration (high, 37-67%; low, 33-57% dead scales) nor due to temperature (low, 37-67%; high, 33-47%). The factors favouring mortality may have had an additive effect, since the highest mortality, 67% was at a combination of high spore concentration, low temperature and high relative humidity, after the second spray caused by wetting the soil; also 57% died at low spore concentration, low temperature and high relative humidity, a relatively large difference of 10% separating these two values from each other and from the other 6 values, which ranged between

Table IV - 45

Integration of *Verticillium lecanii* and the parasite *Encarsia formosa* to control whitefly on tomatoes in glasshouses: adults on five young leaves below the terminal bud (30 leaves per compartment), 16 weeks after the second spray of spores (27.2.79 to 17.6.79)

Treatment	*Trialeurodes vaporariorum*		*Encarsia formosa*	
	Live	Dead	Live	Dead
Control, 18°C	19	6	36	17
E. formosa only, 18°C	13	6	4	8
V. lecanii + *E. formosa*, 18°C	0	2	3	1
V. lecanii + *E. formosa*, 23°C	4	1	1	1

Table IV - 46

Integration of Verticillium lecanii and the parasite Encarsia formosa to control whitefly on tomatoes in glasshouses: sooty mould on tomato leaves

Treatment	Leaves		
	Total	"Sooty"	"% sooty"
Control, 18°C	420	120	29
E. formosa only, 18°C	400	100	25
E. formosa + V. lecanii, 18°C	420	20	5
E. formosa + V. lecanii, 23°C	420	25	6

Table IV - 47+

Integration of Verticillium lecanii and Encarsia formosa for the control of whiteflies on tomatoes: glasshouse temperature and relative humidity

Spray date	Observation date	Compartment no.	°C			% R.H.		
			Max.	Min.	Mean	Max.	Min.	Mean
9.2.79	5.2-26.2	2	27	18	19	88	53	68
		3	26	17	18	78	46	63
		4	27	18	19	86	60	71
		5	27	19	23	68	42	53
27.2.79	26.2-5.3*	2	25	16	18	90	62	78
	26.2-19.3	3	27	17	19	80	52	66
		4	29	17	19	88	66	75
		5	31	19	23	78	48	61

* Thermohygrograph was not maintained between 5.3.79 and 29.5.79 in the control compartment

\+ More details in Appendix IV - 22

Table IV - 48

Integration of Verticillium lecanii and Encarsia formosa to control whitefly on tomatoes in glasshouses: number of whitefly eggs on three young leaflets/tomato plant, 16 weeks after second spraying

Plant number	Treatments, each in one glasshouse compartment			
	Control	E. formosa only	V. lecanii + E. formosa	
	18°C	18°C	18°C	23°C
1	0	0	0	0
	0	0	0	0
	4	1	0	1
2	1	0	0	0
	0	0	0	0
	0	0	0	0
3	0	0	0	0
	0	1	0	0
	1	0	0	0
4	0	0	22	0
	1	0	0	1
	1	0	0	0
5	1	0	0	0
	20	2	0	0
	1	3	0	0
6	0	1	0	4
	0	2	0	3
	0	21	0	0
Total	30	31	22	9
Average eggs/ leaflet	1.6	1.7	1.2	0.5

33% to 47% (Table IV - 43).

(c) Discussion

Okada and Santa (1978) recognised three steps concerning the parasite-host (P/H) ratio of adults until successful control was obtained. The first step is in the period after releasing the parasite until P/H reaches 0.1, when the whitefly population grows normally as an untreated one. During the second step the P/H ratio reaches about 10 when the whitefly population was maintained stationarily. During the third step the whitefly population decreased rapidly. They expected the control of whitefly by E. formosa to be about 90% at the second step and more than 99% at the third step. In the present study the control of whitefly scales had almost reached the third step at termination of the experiment.

Scopes and Ledieu (1979) consider that, as a general rule, it is unwise to introduce E. formosa if more than one adult whitefly is found on ten plants. V. lecanii did not reduce heavy experimental whitefly populations to this standard, although reduction may be greater in larger glasshouses where relative humidity may be higher than in small ones, and more spore sprays could be used to give greaterreduction. Despite the moderate population reduction achieved by the fungus, the effect was sufficient to allow E. formosa to control the pest (Fig. IV - 25) with only slight crop damage in a period of 14 weeks, which compares favourably with the 13 weeks expected by Scopes and Ledieu (1979). There was no evidence that the fungus killed more than an insignificant

Fig. IV - 25

From left to right : whitefly scale population (parasitised and unparasitised) on the ventral surface of tomato leaflets from "Encarsia formosa only","Verticillium lecanii + E. formosa"and the control glasshouse compartments

proportion of parasites.

Thus V. lecanii can be recommended for early season whitefly control prior to use of E. formosa. It has the advantages of not harming the predatory mite P. persimilis (section IV, F, 3) used to control red spider mites, nor the plants. No phytotoxicity, hardening or infection of the plants were observed and there is no record of V. lecanii attacking plants. In contrast, early in the season plants are delicate and particularly susceptible to chemical phytotoxicity.

The alternative to V. lecanii is to choose chemicals which will be least harmful to natural enemies. P. persimilis is highly susceptible to many pesticides (ADAS, MAFF, 1978). Most insecticides are effective only against adult whiteflies so that repeated treatments at 3-5 day intervals are essential for several weeks before control can be achieved. In comparison fortnightly or monthly sprays of V. lecanii are adequate to kill a large population of scales (section IV, D, 4) and keep the pest under control.

Blastospores of V. lecanii in aqueous suspension when sprayed on black parasitised whitefly scales infected and killed a large proportion of E. formosa inside under very high relative humidity (R.H.) in the laboratory (section IV, F, 1) but no harmful effect was observed under lower R.H. in the glasshouses (section IV, F, 3).

G. EXPOSURE OF OPERATOR TO VERTICILLIUM LECANII

Cultures of V. lecanii grown on agar and in liquid media in the laboratory were handled with bare hands for about 2.5 years. Only a white coat was worn as protective clothing. In the glasshouses crops were sprayed with viable conidia and blastospores suspended in standard buffer solution on thirteen occasions using pneumatic hand sprayers. During spraying spray drifts fell on exposed hands and face. Disposable face masks were used only during the first two sprayings. Sometimes spray droplets fell in the eyes but were not washed until spraying was over. At the end of the sprayings some of which lasted up to 2 h, hands and face were washed with soap and water. Concentrations of spore suspensions sprayed ranged from 3.5×10^6 to 1.0×10^8 viable spores/ml. No harmful effect of the pathogen was noticed even one year after the last spraying. This together with confirmation that strain C-3 will not grow at temperatures above 31°C (section IV, B, 1) is strong evidence in support of the safety of V. lecanii.

H. DISCUSSION

The feasibility of integrating a pathogen into the programme for the control of glasshouse whitefly depends on the following attributes of a microbial control agent:

1. Control of pest to sub-economic level
2. Virulence
3. Predictable control
4. Easy application
5. Rapid spread
6. Low cost
7. Effect on beneficial parasites and predators
8. Persistence in the pest environment
9. Easy production
10. Good storage
11. Safety

Viable conidia and blastospore suspensions of Verticillium lecanii sprayed in the evening at high volume to wet the developing scales caused very high scale mortality on cucumbers in glasshouses to a sub-economic level at all seasons when the conditions were favourable (section IV, D, 2-4).

In such conditions of temperature and humidity the fungus was virulent, killing scales in about two weeks and control of one generation of scales was predictable.

These conditions have been defined. Ambient temperature in the

glasshouses were largely within a broad range (18-27°C) which was shown to support growth of V. lecanii on an artificial medium in the laboratory (section IV, B, 1). This agrees with the conclusions of Roberts (1970) for other fungal insect pathogens. He stated that temperatures which support moderate growth of the fungus are adequate for disease initiation and development. Temperatures above this range caused unfavourably low relative humidity which could not be corrected by artificial humidification, under which scale mortality was lower in comparison with optimum temperature-compartments. The relative humidity in the glasshouses with cucumbers was generally high enough for adequate scale mortality, but with tomatoes the relative humidity was lower as the surface soil was dry. Hence the scale mortality was moderate.

Control of scales was best at or slightly below 23°C combined with wetting the soil before spraying spore suspensions. At higher temperatures timing the spore spray was critical because whitefly pupae develop fast and may emerge as adults before V. lecanii can infect and kill them.

The eggs were not susceptible to V. lecanii (section IV, C, 2). This confirms the findings of Hussey (1958). The best stage of the pest at which to aim control is the scale a few days after settling of the crawler, i.e. 10-12 days after egg laying (Table IV - 1), so that the fungus has adequate time to infect and kill. Spraying in the evening is recommended because the spores can escape from the harmful ultra-violet rays of sunlight.

Spore sprays were easy to apply but effort was needed to ensure

good coverage underneath young scale-bearing leaves. In the glasshouse, mortality usually increased with high spore concentration except when humidity was low (section IV, E, (b)). This agrees with the laboratory data on bioassay (section IV, C, 1) in which LC_{50}s between humid and dry conditions varied by X10 and between dry and wet conditions by X100. This is not surprising, as expressed by Roberts and Yendol (1971) because the fungal spores must germinate outside their hosts. The mere presence of the spores is not adequate to ensure death of exposed insects because environment is a factor of paramount importance in disease induction. As microbial insecticides, except in very favourable conditions, fungi tend to be required in uneconomically high concentrations (Burges and Hussey, 1971). Therefore insect control using V. lecanii must be undertaken with either the environment or the crop requirements as the overriding consideration.

In the glasshouse (section IV, D and F) V. lecanii did not spread from dead scales bearing sporulating fungus to healthy newly developed scales on new leaves. Even though dead fungus-covered adults were seen, the healthy whitefly population built up continuously from survivors. The fungus did not spread even to unsprayed leaves left accidentally on sprayed plants (section IV, D, 4). This is an important finding in assessing the potential of the fungus as a microbial control agent. It is concluded that V. lecanii must be sprayed repeatedly and thorough spray coverage is essential on every new generation of scales.

Such frequent sprays would be uneconomic. This contrasts with the findings of Hall and Burges (1979) with aphids. One spray of spore suspension was adequate to control the aphid, Myzus persicae, on chrysanthemums throughout the crop season. This is possibly for the following three reasons:-

(1) High relative humidity caused by the polythene blackout, used to induce flowering of chrysanthemums.

(2) When the chrysanthemum plants are watered from above, the spores in slime heads on cadavers are dispersed to healthy aphids. The water film on the leaves remains long in the crowded canopy of chrysanthemum plants in the beds.

(3) M. persicae adults and nymphs are partially gregarious and move actively on the plants, thus contacting sporulating fungus on cadavers of aphids.

In contrast the whitefly scales are sedentary. Adults emerge from healthy scales missed by the fungus spray and fly away from the fungus to the upper part of the plant, to the young leaves. These adults do not contact spores unless soon after emergence they crawl over dead scales. The cucumber plants are watered at the bottom and the leaves are spaced far apart on the stem, many high above soil level. The new young scales on upper leaves of cucumber plants are not likely to come in contact with spores from dead old scales, which are on the old leaves lower down the plant. With cucumbers, a small-scale experiment to control Aphis gossypii showed that several sprays of V. lecanii were needed during a single crop (Hall, 1978).

Whitefly control by the parasite E. formosa fails early in the season because of low temperature and perhaps dull weather. Early season conditions suit V. lecanii and it proved possible (section IV, F, 4) to use the fungus then and the insect parasite later, since the fungus did not significantly attack E. formosa or the predatory mite P. persimilis (section IV, F). Used in this way, one or two early season sprays would form a commercially economical contribution to integrated control of the whitefly.

As cucumbers are grown in glasshouses with a minimum temperature of day, 21°C; night, 19°C and under humid and moist conditions, the environment was very suitable for V. lecanii. For an early crop of tomatoes the recommended minimum night temperature is 15°C and the day temperature is 18°C. But in the day time on bright sunny days the temperature inside the glasshouses rises, sometimes up to 24°C (ADAS, MAFF, 1977ℓ) The advantage with V. lecanii is that it can grow considerably even at 15°C and kill whitefly scales which too grow very slowly at 15°C (Table IV - 1). But this temperature is too low for E. formosa to parasitise efficiently (Table IV - 2).

In the glasshouses the spore viability on the leaf surfaces was lost faster than in the laboratory where plants were kept in cages with near saturated relative humidity. Also spore survival on cucumber leaves was higher than on tomato leaves in the glasshouses, which may be due to the differences in evapotranspiration rates from the two different types of leaves. The species of plant per se had no effect on V. lecanii (section IV, E).

The fungus is easy to produce on both liquid and solid media (section II). Some progress has been made with the problem of storing blastospores. Suspensions of blastospores survived well for long periods (LT_{50} = 970 days; extrapolated value) when stored at -20°C after thoroughly washing to remove culture medium and then suspended in 10% skimmed milk. Also spore survival was good in three other media containing sugars and gelatin or serum. Freeze-drying in some sugar solutions and glucose with serum too gave moderately high survival (50%). These results suggest that blastospores could be stored deep frozen in the less expensive media. Storage at -20°C is costly for commercial use and makes transport of spores difficult. Freeze-drying of spores too is expensive but transport is easy and is probably commercially more practicable. For experiments blastospores can be held at 2-4°C for short periods (a few days), after harvesting until use. In the U.S.A. filtered mycelial fragments of Hirsutella thompsonii from cold storage was formulated on the day of field application (McCoy et al., 1972).

In the glasshouse experiments, conidia and blastospores were equally effective against whitefly (section IV, D, 3). Thus mass production of conidia may be the best commercial approach. In Brazil conidia of M. anisopliae grown on boiled rice, dried and ground into a powder named Metaquino was stored at 7°C (Aquino et al., 1975; Guagliumi et al., 1974; Moura Costa& Magalhaes, 1974; Moura Costa et al., 1974).

Even though prolonged storage methods to maintain high viability for commercial purposes are yet unknown, V. lecanii could be cultured fresh for immediate application as with macrobial parasites and predators such as E. formosa and P. persimilis.

The virulence of V. lecanii (C-3, GCRI) has not apparently been affected by repeated subculturing on artificial media (Hall, 1977). Ramaseshiah and Dharmadhikari (1968) too found in India that their strain of V. lecanii has not lost its virulence by repeated subculturing. Some other workers, Nagaich (1973), Hussey (1958) and Barson (1976) reported loss of virulence on repeated subculturing on artificial media. In the present study possible problems were averted by storage of many seed cultures from a very early subculture of C-3 and using these for all experiments.

V. lecanii was regarded as safe to man for a number of reasons. It does not grow above 30°C. It has not been reported as a harmful agent to human beings so far though in many temperate and tropical countries it has been used by many research workers. Hall (1977) has reported some results of safety tests with the strain C-3, GCRI. Absence of records in man and other vertebrates in medical and veterinary history is impressive evidence. My own experience adds further evidence in that I had no abnormal effects due to V. lecanii though I have used it in experiments for 2.5 years purposefully without adequate protective clothing. Tate and Lyle Ltd. are currently commissioning full safety testing on their industrial product of conidia.

No studies were made on the effect of insecticides and fungicides on V. lecanii. However, Olmert and Kenneth (1974), Wilding (1972b)and Easwaramoorthy and Jayaraj (1977b) found that some commonly used chemical pesticides were harmful to V. lecanii in their in vitro studies. Wilding has also found that dimethirimol was harmless to V. lecanii. This is confirmed by routine use of this fungicide in glasshouse experiments (section IV, D, 2-4). If chemical pesticides are carefully chosen and applied in the same way as for protecting P. persimilis and E. formosa (Scopes and Ledieu, 1979) it is possible to reduce or avoid harmful effects on V. lecanii.

Based on the above facts, it appears that V. lecanii will not replace E. formosa as the major component for whitefly control in the integrated pest control programme for tomatoes and cucumbers in glasshouses but could play a useful role in early crops of many plants at the beginning of the year when E. formosa is inefficient due to the cold and perhaps dull weather, in addition to its prospects as the major control agent for aphids on year-round chrysanthemums.

V. BACILLUS THURINGIENSIS

A. INTRODUCTION

The potency of Bacillus thuringiensis against many lepidopterous larvae has led to its development as a "biological insecticide". Preparations based on this bacterium contain two active ingredients, the spore and the crystal of toxic protein, and sometimes a third, the exotoxin. The crystal is the most important ingredient against many lepidopterous insects. It contains the δ-endotoxins which have been shown to be safe to man and effective in the practical control of many phytophagous lepidopterous insects. In contrast to chemical insecticides, δ-endotoxins do not attack beneficial insects and offer the hope of controlling pest insects with little or no effect on the rest of the ecosystem. Consequently, the commercial production of formulations of microbial insecticides based on B. thuringiensis has grown rapidly since the first product was registered for commercial use in 1960. Already the commercial products Bactospeine (two formulations, one with serotype 1 and the other with serotype 3a3b), Dipel and Thuricide (both with serotype 3a3b) are being sold on a considerable scale internationally. None of these have the exotoxin of this bacterium, because this toxin has not been submitted for safety clearance (Burges, 1977).

Bonnefoi and de Barjac (1963) discovered that variants within the species B. thuringiensis could be differentiated serologically by comparing antibodies to their flagellar proteins ("H-antigens"), which were given numbers. This serotyping proved to be a very reproducible and reliable technique, and is now the principle tool

used in the classification of varieties, which have been given names (Dulmage, 1979). Classification has since been refined by the procedures of Norris (1964) and Norris and Burges (1965), who discovered that electrophoretic patterns of the esterases produced in vegetative cells of B. thuringiensis could also be used to distinguish varieties of this organism. Significantly there was a close relationship between the classifications made by H-antigen serotyping and those made by esterase analysis. Esterase patterns are now used as a supplement to the serotyping procedures developed by Bonnefoi and de Barjac.

The δ-endotoxin is made up of one or more proteins contained in the bipyramidal crystalline body. The pathogenicity of B. thuringiensis depends on either mainly toxicosis, or on both infectivity of the spores and toxicosis, depending on the host species and bacterial variety involved (Burges et al., 1976). Numerous investigations have shown that the crystals produced by different serotypes, and even different isolates of the same serotype of B. thuringiensis, can have different spectra of activity against various species of Lepidoptera (Burgerjon and Dulmage, 1977). Early attempts to measure the potency of different preparations were based on viable spore counts. An important advance in the problem of standardization was the realisation that the insecticidal activities of different preparations cannot be consistently measured by counting the spores, because of the different potencies of crystals from different strains and because the spore:crystal ratios vary enormously (Burges, 1967). Bioassay with insects has taken the place of the spore count. These assays are difficult,

demanding of time and manpower, and require large numbers of insects. The technology for standardization of the potency of commercial B. thuringiensis products is complex and well developed. It has reached a stage well in advance of that of other types of microbial insecticides, e.g.the viruses.

B. BIOASSAY OF BACILLUS THURINGIENSIS ISOLATES AGAINST GALLERIA MELLONELLA

1. The standardization and measurement of the potency of Bacillus thuringiensis products

In the development of bioassay methods, a major concern is to maximise precision. Variation due to differences in successive batches of assay larvae can largely be eliminated by comparison of the results of a test preparation with those of a reference standard bacterial powder included in each batch, to form potency ratios. Comparison with the same reference standard also helps to eliminate variation between different laboratories conducting assays and between different assay techniques.

The adoption of one batch of stable bacterial preparation, E61 (H-serotype 1), as the international standard has provided a recognised material with which other materials can be compared. Such measurement of the potency of materials should work well with products containing the same strain of bacterium as the standard, that is when the standard is homologous (Burges, 1967). All insect species should give similar potency ratios when test and standard materials are strictly homologous. The standard can be regarded as only partially

homologous with products containing other strains of the bacterium. With these strains, different species of assay insect give very different comparative toxicity values between bacterial products as the susceptibilities of the different insect species to the various bacterial strains are unlike (Burges, 1967). Thus, when the standard is incompletely homologous, it is impossible to rely on units based on only one species of assay insect in measuring the insecticidal activity of different products.

The standard material is used as a basis for establishing potency units. It is assigned a specific potency expressed in units of activity, the unit being the "International Unit" or IU. The first standard, prepared in France and called E-61, was recommended as an international standard in 1966 (Burges et al., 1967) and assigned a potency of 1,000 IU/mg.

Later other more active isolates, many from different H-serotypes, were used in industrial products, and test insects were employed in which E61 was only moderately active. This created the dual problems of having to use relatively high concentrations of E61 in the standard assay and of having a non-homologous standard.

When an isolate in serotype 3a3b, code named HD-1, was selected for most commercial products of B. thuringiensis, these problems were overcome by selecting a particular batch of HD-1 powder, labelled 'HD-1-S-1971', for use as a primary reference standard for products in the USA. This was assigned a potency of 18,000 IU/mg on the basis of comparative assays against E-61 with Trichoplusia ni as the test insect.

G. mellonella is more susceptible to H-serotype 5a5b than to some other strains (Burges and Bailey, 1968; Vankova, 1964: Burgerjon and Biache, 1967) and only slightly susceptible to many of the commoner strains, including H-serotype 1 (E61) and 3a3b (HD-1-S-1971). An extensive programme of research was conducted with commercial Thuricide at a time when it contained H-serotype 5a5b (Burges and Bailey, 1968; Burges et al., 1976; Burges, 1976b, 1977). One of the best batches of "Thuricide" was a wettable powder, some of which has been carefully stored as the standard "G" for bioassays against G. mellonella in their work.

2. The international co-operative screening programme

By 1975, the problem of studying known varieties of B. thuringiensis and of finding improved strains for industrial use had grown so large that it was beyond the scope of any single organisation if significant progress was to be made. So Dulmage (1979) enlisted the help of a group of scientists from many laboratories around the world in a programme to explore the δ-endotoxins of B. thuringiensis and their spectra of activity. Each of more than 300 isolates of B. thuringiensis in Dulmage's culture collection were grown in the same medium and under the same conditions in shake flasks, using sufficient broth in each fermentation to yield about 20 g of formulation by the acetone-coprecipitation harvest process of Dulmage et al. (1970). Each product was distributed to the participating scientists, who tested it against the particular insect species used in their laboratories.

Thus each scientist tested the same material from each isolate, and this eliminated any influence that might have arisen from the fermentation method and chance variations in the fermentation. In this way a spectrum of activity for each of the formulations was assembled.

The susceptibility spectrum of G. mellonella is rather different from those of most other well studied hosts of B. thuringiensis (Burges et al., 1978). It was therefore regarded as a very useful host to include in the Dulmage-programme by H. D. Burges and P. Jarrett. As a limited part of their programme I have studied 59 isolates by 2-concentration, range-finding, assays and 18 isolates by 5-concentration definitive assays.

3. Materials and methods

An artificial food was used for the bioassays with G. mellonella. The insect stock has been bred in the laboratory since 1963 and was already adapted to an artificial food when obtained from Dr Y. Tanada, Berkeley, California.

Details of breeding and bioassay are given by Burges and Bailey (1968) and Burges (1976a). The artifical food contains baby cereal, clear honey, glycerine, debittered yeast powder and water in the ratio of 33:23:21:15:8. For bioassay each bacterial powder, coded by "HD" followed by a number, supplied by Dulmage was homogenised in phosphate buffer solution (0.0425 g KH_2PO_4/litre of distilled water), pH 7.2 in a glass homogeniser (Griffith's tube), serially diluted and the diluted suspensions were used in place of

the water in preparing portions of the artificial food. Small quantities of the food were placed in small gelatin capsules each with one larva (7 days old at 30°C, average weight about 0.2 mg). After 7 days on the treated food mortality was assessed. At each bacterial concentration, 25 larvae were used. Initially 2-concentration assays were done to find the activity range. For active isolates (HD powders) and many of the less active ones, these were followed by more accurate 5-concentration assays. These assays were set up in groups of three to five isolates and one standard powder ('G' powder, serotype 5a5b). For each group of assays evenly sized larvae were selected visually from a single breeding batch derived from eggs laid over 24 h. Prior to use all bacterial powders were stored at 2°C.

4. Results and discussion

(a) Objectives of the present work

At the time the present work was started, it was obvious that the activities of the isolates against G. mellonella fell into three groups, inactive, moderately active and active, as illustrated by Jarrett et al. (1979). Since the most important isolates were the active ones, most work to obtain accurate results was devoted to them and a policy had been adopted of following the 2-concentration range-finding assays by accurate 5-concentration assays.

The objective of my present 5-concentration assays with six active isolates was to obtain accurate values for them because they had not yet been assayed. For the same reason the activity of less active

isolates was obtained less accurately with replicated 2-concentration assays, fitting a standard slope to each assay to estimate the LC_{50}. There was some evidence that slopes for less active isolates were lower than those for very active isolates, so the present 5-concentration assays on selected less active isolates were undertaken to decide the best slope to use.

(b) Precision of the assays

Examination of 14 consecutive assays of the G-standard, involving 14 consecutive batches of insects in the present study, showed that the assay was highly stable, reproducible and precise (Table V - 1). The mean LC_{50} value of 0.00093% bacteria in the food for these 14 assays agrees well with the mean value of 0.0010% for 32 consecutive assays by Burges et al. (1978) and with a mean of 0.0012% obtained with three assays conducted 13 years earlier in 1965 (Burges et al., 1976). There is also good agreement with an even earlier 1963 value of 0.0031 (Burges and Bailey, 1968) which is expected to be a little higher than present values because the exposure period was only 6 days instead of 7. The mean (arithmetic) slope of the probit lines for the 14 assays in the present study was 3.6 compared with 4.0 obtained by Burges et al. (1978).

Only one of the 14 plus 32 assays showed significant heterogeneity of the points about a probit line. This would be expected by chance.

The precision of 5-concentration assays of test isolates that were highly active in G. mellonella, i.e. with an LC_{50} of 0.04%

Table V - 1

Bioassay of Bacillus thuringiensis against Galleria mellonella:

LC_{50}s and slopes for "Standard G powder" (serotype 5a5b)

LC_{50} with 95% fiducial limits: % bacteria in food	Slope ± S.E.	Heterogeneity of points about the probit line		
		χ^2	df	P
0.00091 (0.0006, 0.0012)	3.0 ± 0.5	4.86	3	0.20
0.00092 (0.0007, 0.0011)	5.1 ± 1.0	1.02	3	0.80
0.00070 (0.0005, 0.0009)	2.5 ± 0.4	3.04	3	0.30
0.00103 (0.0008, 0.0014)	4.7 ± 0.8	6.13	3	0.10
0.00077 (0.0006, 0.0009)	3.3 ± 0.5	0.57	3	0.90
0.00079 (0.0006, 0.0010)	2.5 ± 0.4	2.59	3	0.50
0.00107 (0.0004, 0.0014)	4.6 ± 1.7	0.05	1	0.80
0.00120 (0.0009, 0.0016)	4.0 ± 0.7	5.32	3	0.20
0.00079 (0.0006, 0.0010)	3.6 ± 0.7	0.77	3	0.90
0.00166 (0.0012, 0.0020)	5.4 ± 1.4	0.98	3	0.80
0.00082 (0.0004, 0.0014)	2.1 ± 0.4	9.58	3	0.02
0.00071 (0.0005, 0.0009)	3.0 ± 0.5	0.97	3	0.80
0.00084 (0.0007, 0.0010)	3.6 ± 0.6	0.36	3	0.95
0.00076 (0.0004, 0.0011)	3.1 ± 0.6	5.90	3	0.10
Mean* = 0.00093	3.6*			

* Arithmetic mean

or less (Table V - 3), was as good as that of the standard (Table V - 1). This agrees with the results of Burges et al. (1978).

With 5-concentration assays, a small improvement in the precision of the LC_{50} of the test isolates was obtained by Burges et al. (1978) by correcting for slight differences in the susceptibilities of consecutive batches of larvae by the following equation:

$$\text{Corrected } LC_{50}n = \frac{\text{Test } LC_{50}n \times \text{Mean Standard } LC_{50}\ (0.001)}{\text{Standard } LC_{50}n}$$

where "Test $LC_{50}n$" and "Standard $LC_{50}n$" are values for the LC_{50} of the test powder and the LC_{50} for the standard with the same batch of insects respectively. Mean standard LC_{50} for 32 consecutive assays was 0.0010% (Burges et al., 1978). The same calculation was used in the present study.

(c) Slopes of the assay

Twenty four 5-concentration assays with the 12 relatively inactive isolates (all except serotypes 5 and 7) in my study had probit lines with a mean slope of 3.06 ± 0.40 (Appendix V) while the twelve 5-concentration assays with the six active isolates (serotypes 5 and 7) had a mean slope of 3.3 ± 0.20. Thus in the present study the slopes of probit lines of active and inactive isolates were not significantly different. In contrast Burges et al. (1978) reported a mean slope of 4.00 ± 0.22 for probit lines of a larger number of assays, 27, with active ($LC_{50} < 0.01\%$) isolates.

Table V - 3

Bioassay of *Bacillus thuringiensis* isolates with *Galleria mellonella*: 5-concentration assays

Code of bacterial formulation		Serotype		No. of assays	LC_{50} with 95% fiducial limits: % bacteria in food
Isolate	Fermentation no.	H	Crystal+		
HD352	R758C	3*	Not known	2	11.0 (6.49, 18.6)
HD71	R636A	3a	Al.	2	2.42 (1.95, 3.00)
HD343	R745A	3	Not known	2	2.04 (0.970, 4.29)
HD147	R754A	10*	Not known	2	1.94 (1.55, 2.42)
HD16	R615A	3a	Not known	2	1.72 (1.35, 2.19)
HD28	R622C	1*	Th.	2	1.42 (1.16, 1.73)
HD27	R619C & R725B	1	Th.	3	1.23 (0.419, 3.58)
HD247	R770C	3a3b	K-1	3	0.977 (0.858, 1.11)
HD27	R619C	1	Th.	2	0.756 (0.409, 1.40)
HD40	R603A	3a	Not known	2	0.699 (0.584, 0.836)
HD97	R645B	1	Th.	2	0.479 (0.411, 0.559)
HD 1S 1971		3a3b		3	0.0654 (0.0530, 0.0803)
HD322	R734A	5a5b	Not known	2	0.0026 (0.0021, 0.0034)
HD184	R755A	5a5b	Gal.	2	0.0026 (0.0022, 0.0030)
HD175	R754C	5a5b	Gal.	2	0.0019 (0.0016, 0.0023)
HD361	R752B	5*	Not known	2	0.0014 (0.0012, 0.0016)
HD359	R751C	5	Not known	2	0.0013 (0.0011, 0.0016)
HD283	R750B	7	Aiz.	2	0.0013 (0.0011, 0.0015)

* Serotype 3, 10, 1, 5 means that the study of sub-antigenic factors 'a' and 'b' have not been made yet.

+ Gal. = var. *galleriae*; Th. = var. *thuringiensis*;
Aiz. = var. *aizawai*; Al. = var. *alesti*;
K-1 = var. *kurstaki*, type K-1

A slope of 3.0 was, therefore, adopted for use in 2-concentration assays of moderate and low activity isolates and a slope of 4 for the active isolates in the whole Galleria-programme, including my work (Tables V - 2 and V - 3).

(d) Relationships of the potencies of different bacterial isolates

Jarrett et al. (1979) reported that the activities of the isolates fell into three distinct arbitrary categories, active ($LC_{50} < 0.04\%$ bacterial powder in the insects' food), inactive ($> 4\%$) and moderately active between these values. My results (Tables V - 2 and V - 3) agree with their findings.

In their studies all the active isolates were in three H-serotypes, 5a5b, 7 and a few in 6. Among these, all isolates of one crystal-type ("galleriae"), most of those of another "aizawi" and a few "entomocidus" were in the active group. They also reported that this distribution of activity against G. mellonella is much simpler than the distribution against other susceptible Lepidoptera. They found that no isolates were significantly better for the practical control of G. mellonella than the serotype 5a5b used in commercial "Thuricide" in the 1960s but now discontinued.

In the present study too, the active isolates were in the serotypes 5 and 7. No serotype 6 was tested. No isolate was more active than serotype 5a5b used as standard "G" against G. mellonella. Thus the results in both studies agree well.

One isolate, HD 325, H-serotype 5a5b had an LC_{50} of 0.38% (Table V - 2). Thus it is a moderately active isolate. The

Table V - 2

Bioassay of *Bacillus thuringiensis* isolates with *Galleria mellonella*: LC_{50}s determined by fitting lines with slopes of 3 or 4 to the results of 2-concentration assays

Code of bacterial formulation		Serotype		No. of assays	LC_{50} (mean): % bacteria in food
Isolate	Fermentation no.	H	Crystal*		
HD336	R751A	3a3b	Not known	2	0.90
HD270	R749B	3a3b	K-1	2	0.30
HD332	R740A	3a3b	Not known	2	0.40
HD269	R749A	3a3b	K-73	2	0.26
HD252	R747A	3a3b	K-1	2	2.04
HD327	R736B	3a3b	Not known	2	2.2
HD323	R734B	3a3b	Not known	3	1.1
HD349	R758B	3a3b	Not known	2	0.73
HD203	R755C	3a3b	K-1	2	0.10
HD250	R758A	3a3b	K-1	3	1.8
HD247	R770C	3a3b	K-1	2	0.56
HD350	R737A	1+3a3b (mixed)	Not known	2	0.14
HD343	R745A	3	Not known	2	1.2
HD364	R753C	3	Not known	2	1.9
HD342	R744B	3	Not known	2	0.38
HD344	R745B	3	Not known	1	2.2
HD363	R753B	3	Not known	2	0.32

Continued/

Table V - 2 (Continued)

Code of bacterial formulation		Serotype		No. of assays	LC_{50} (mean): % bacteria in food
Isolate	Fermentation no.	H	Crystal*		
HD339	R743A	3	Not known	1	0.35
HD338	R742B	3	Not known	2	0.62
HD340	R743B	3	Not known	3	0.37
HD348	R748B	3	Not known	2	2.1
HD345	R747B	3	Not known	2	1.1
HD346	R746B	3	Not known	2	2.1
HD341	R744	3	Not known	3	0.19
HD347	R748A	3	Not known	3	1.8
HD352	R758C	3	Not known	1	1.8
HD337	R742A	3	Not known	2	0.15
HD328	R738A	4a4c	Not known	2	0.40
HD326	R736A	1	Not known	1	0.82
HD317	R757A	1	K-1 + Th	2	0.20
HD138	R728A	1	Th	2	0.59
HD39	R785A	1	K-1	1	4.9
HD26	R783A	1	Th	1	0.25
HD110	R759A	6	Ent.	2	0.15
HD301	R756B	9	Tol.	2	0.26

Continued/

Table V - 2 (Continued)

Code of bacterial formulation		Serotype		No. of assays	LC_{50} (mean): % bacteria in food
Isolate	Fermentation no.	H	Crystal*		
HD124	R760B	9	Tol.	2	0.45
HD324	R735A	8	Not known	1	1.8
HD147	R754A	10	No serum available	1	0.74
HD334	R741A		No crystals	1	0.37
HD351	R737B		No crystals	2	1.48
HD362	R753A		No crystals	3	0.70
HD333	R757C		No crystals	2	1.8
HD357	R751B		Self agglutinating	2	0.61
HD330	R739A		Self agglutinating	1	0.96
HD329	R738B		Self agglutinating	1	1.3
HD335	R741B		Not known	1	0.34
HD331	R757B		Not known	2	1.5
HD395	R780A		Not known	1	2.2
HD462	R781A		Not known	1	1.6
HD175	R754C	5a5b	Gal.	1	0.0016
HD322	R734A	5a5b	Not known	1	0.0017
HD149	R754B	5a5b	Gal.	1	0.0105
HD184	R755A	5a5b	Gal.	1	0.0032

Continued/

Table V - 2 (Continued)

Code of bacterial formulation		Serotype		No. of assays	LC_{50} (mean): % bacteria in food
Isolate	Fermentation no.	H	Crystal*		
HD224	R756A	5a5b	No serum available	1	0.018
HD359	R751C	5	Not known	1	0.00094
HD361	R752B	5	Not known	1	0.0016
HD360	R752A	5	Not known	1	0.0069
HD283	R750B	7	Aiz.	1	0.0014
HD325	R735B	5a5b	Not known	1	0.38

* Aiz. = var. aizawai; Gal. = var. galleriae;

K-1 = var. kurstaki, type K-1; K-73 = var. kurstaki, type K-73;

Ent. = var. entomocidus; Th. = var. thuringiensis;

Tol. = var. tolworthi

crystal serotype is not known yet. Burges et al. (1978) too reported that some isolates of H-serotype 5 are moderately active or inactive, but none of these have crystal antigen galleriae. In their studies, crystal antigen galleriae consistently conferred high activity against G. mellonella. Thus it is probable that HD 325 does not have the crystal antigen galleriae.

(e) Role of the spore and crystal in potency

Spores, as well as crystals, play an important role in the pathogenicity of H-serotype 5 in G. mellonella (Burges et al., 1976). Pure spores were X1000 more potent than pure crystals, suggesting that spores are more important than crystals. However, a 1:1 mixture of spores and crystals was X10 more potent than pure spores, proving that both spores and crystals play important roles in the normal pathogenicity of B. thuringiensis in G. mellonella. Mixing spores of serotype 1 instead of spores of serotype 5 with crystals of serotype 5 reduced potency of the mixture by X100 and pure serotype 1 spores were almost inactive in G. mellonella (Burges et al., 1976). Thus the serotype of the spore is important and spores as well as crystals carry important pathogenicity factors.

Similar conclusions can be reached by a consideration of assay slopes in the light of theoretical reasoning described by Meynell and Meynell (1965). There are two general hypotheses that describe how a host might respond to a harmful entity when more than one unit of this entity is required on the average at each response site (the host insect).

These are:

(1) the hypothesis of independent action, which postulates that the units act independently at each site, so that a response might be caused by only one of several units present; and

(2) the hypothesis of co-operative action, which postulates that units are not independent within the site and must always act together to produce a response (Meynell and Meynell, 1965).

The hypothesis of co-operative action predicts that the maximum slope of the dose-response curve is ∞, whereas independent action predicts it will be far smaller ($b \simeq 2$ on a probit mortality-log dose plot). It is noteworthy that the slopes observed in toxicity titrations with chemical poisons are usually far greater ($b \simeq 20$) than those from infectivity titrations with pathogens that do not involve toxins ($b \leq 2$) (Meynell and Meynell, 1965), suggesting that insecticide molecules co-operate *in vivo*, whereas incoulated living micro-organisms are independent (Meynell, 1957).

In bioassays of *B. thuringiensis* isolates against *G. mellonella*, average slopes of 3 to 4 were obtained (present work and Burges *et al.*, 1978). This indicates that both infectivity due to spores and a toxic effect due to crystals were responsible for the larval mortality.

The average slope of 4 for very active isolates and 3 for the less active isolates might indicate a relatively greater importance of crystals in the most active isolates. However, because variability in the assays also has the effect of lowering the slope, and the less active isolates involve relatively large amounts of

foreign bacterial and spent fermentation material being added to the insects' food, which may increase variation in the insects' response, the above suggestion is too speculative to be meaningful, particularly since in the work of Burges et al. (1976) pure spores were X1000 more active than pure crystals.

δ-endotoxin injected into the haemocoel of an insect will not harm it. However, if a susceptible insect eats δ-endotoxin, its gut will become paralysed, there will be considerable damage to the intestinal wall and a toxaemia and/or septicaemia will later develop. In some insects, toxaemia due to the crystal plays the dominant role, in others such as G. mellonella, the presence of the spore supplements these effects (Angus, 1954; Heimpel and Angus, 1959; Burges et al., 1976).

(f) Spectra of activity of different strains of B. thuringiensis in different insects

Toxins from different strains of B. thuringiensis have different spectra of insecticidal activity (Dulmage, 1979). For example, the δ-endotoxins produced by many B. thuringiensis var. kurstaki powders with crystal serotype K-73 are very toxic to Trichoplusia ni and Heliothis virescens, but have little or not toxicity toward Bombyx mori. In contrast, the δ-endotoxins produced by most isolates of var. alesti have very little toxicity toward T. ni and H. virescens but are very toxic to B. mori. This indicates that both bacteria-related factors and insect-related factors play a role in host susceptibility.

Dulmage divided powders in particular H-serotype-crystal serotype groupings into further groups based on activities in H. virescens and T. ni. Activity in G. mellonella is sometimes correlated with activity in H. virescens or with that in T. ni or with both and sometimes not. It is particularly noteworthy that some groups of isolates with little activity against both H. virescens and T. ni included isolates all inactive against G. mellonella, while other such groups contained only isolates very active in G. mellonella.

Lecadet (1970) suggested that the varying spectra of activity of the different δ-endotoxins reflect differences in the rate of their digestibility by the various insect species. Thus the toxic entities may be the same in all these endotoxins, but that relatively minor differences in the structure of the proteins that make up the crystal affect the ability of the gut juices to break down the proteins and free the toxic entity. The fact that two different insect species often varied greatly in their susceptibilities to the same bacterial powder suggests that factors such as gut pH (the crystals dissolve most readily at high pH) and the exact nature of the gut enzymes are also important.

In the International Co-operative Screening Programme it was almost always possible to find a δ-endotoxin more active than HD-1 against a particular insect species (Dulmage, 1979). Herein lies the present practical value of screening isolates of B. thuringiensis and the importance of the differences in spectra that are being found. However, in no case was it possible to

predict activity against all other insects from the assays against any single insect species, which shows that we have much more to learn about the interactions of the different bacterial powders and the various host insect species. Also we need to know more about the value of strain selection or induced mutations in producing higher yields of δ-endotoxins in fermentations.

VI. GENERAL DISCUSSION

The potential of utilizing micro-organisms for the control of arthropod pests has been proposed for many years. Many attempts have been made to introduce pathogens as self-perpetuating biological control agents. The production of pathogens attracted the attention of industry. The milky disease bacteria, Bacillus popilliae and Bacillus lentimorbus in the 1950s, and Bacillus thuringiensis in the 1960s were the first insect pathogens to be successfuly developed by industry as microbial insecticides. A major effort to develop the nuclear polyhedrosis baculovirus (NPV) of Heliothis spp. was initiated in the 1960s. This NPV was registered for commercial use in 1974, followed by the registration of NPVs of two forest pests, the gypsy moth and tussock moth, in 1977 and 1978, respectively (Allen et al., 1978). The development of more bacteria and viruses continues and a greater range of pathogens are under trial. Experimental use permits have been issued for Nosema locustae for locust control in western USA, Hirsutella thompsonii against the mite on citrus in Florida and Verticillium lecanii for the control of aphids on chrysanthemums in commercial glasshouses in the United Kingdom. The vast array of pest species and the great number of potential pathogens suggest that many valuable inter-relationships are waiting to be exploited.

The methods of using pathogens fall conveniently into three categories: (1) Inoculative release with permanent establishment.

(2) Inundative release with permanent establishment.

(3) Inundative release without permanent establishment.

In the first category, introduction in small quantities or over limited areas results in establishment and spread of the pathogen either vertically, horizontally, or both through the host population with transmission to subsequent generations. Many reviews have summarised introductions of pathogens. For instance, Burges and Hussey (1971) listed 21 examples and Burges (in press) listed 17 more since 1970. In both lists some of the introductions were made purposefully by man, others were accidental or natural. Thus polyhedrosis virus introduced against Malacosoma disstria, the forest tent caterpillar in Canada spread and persisted causing heavy mortality (Stairs, 1965). Granulosis virus introduced against Hyphantria cunea, the fall webworm limited a major introduced pest to a minor level in Yugoslavia (Vasiljevic', 1964). Bacillus popilliae and B. lentimorbus reduced Popillia japonica, the Japanese beetle from major to minor pest status within 3 years of introduction of the bacteria in the USA (Hall, 1964). In Australia, Dedalenus siricidicola, a nematode introduced against the wood wasp, Sirex spp. was spread by the wasps causing 75% infection in most areas (Burges, in press).

In the second category, high levles of inoculum of a pathogen are applied in order to rapidly reduce heavy pest infestations and prevent immediate damage, while still achieving regulation of future infestations through establishment and transmission to subsequent generations. For example, Hall and Burges (1979) reported that one spray of spore suspension of V. lecanii gave excellent control of aphids on chrysanthemums which was maintained

throughout the crop-season in commercial glasshouses in England. Petersen (1976) reported that mass release of the nematode, Romanomermis culicivorax against 16 potential mosquito hosts in Louisiana, USA, gave significant parasitism, often 90%, continuing at many sites in the second and third year. He has also reported that release of another nematode, Diximermis peterseni at one site in Louisiana produced 80% infestation even in the fifth year.

In the third category, high levels of inoculum are introduced, resulting in a rapid but temporary suppression of the pest population. Permanent establishment does not occur because of environmental, physiological or epidemiological factors. Bacillus thuringiensis is always applied inundatively for control of pests. Despite continued use of the bacterium in a given area, it never establishes itself as a permanent regulatory agent, nor does it show any significant capability for being either horizontally or vertically transmitted. The bacterium replicates in infected insects and then disappears from the insects' environment.

From the above examples it can be seen that pest control by pathogens is not limited to any one group of host insects nor to any one group of entomopathogens. Individual pest problems have and will continue to dictate where and when and what types of micro-organisms are sought for these purposes. Since entomopathogens are at least to some degree host specific, they are likely to be most useful when integrated with other methods of control, because most crops bear many different pests.

For introduction, pathogens may be obtained through exploration

or from existing collections. Compared with insects, mites are a smaller, less studied group of pests and naturally fewer pathogens have been recorded. Little is known about the role of pathogens in naturally occurring mite limitation. In the present work, information about the pathogens of blackcurrant mites was sparse, so it was expected that a survey for naturally occurring pathogens would yield new pathogens. The discovery of only one type of pathogen, a fungus, was reasonable for two reasons: (1) Mainly fungi have been recorded from mites (Lipa, 1971). (2) The blackcurrant mite feeds most of its life inside the bud, which is not likely to harbour pathogens that it can acquire by the most common route – *per os* – so fungi, which may attack through the cuticle in the inter-bud phase of the mite life cycle, are the most likely pathogens. In the bud, the mites are protected from airborne spores. The mites are most likely to carry spores of *V. lecanii* from old bud to new bud, particularly since this fungus grows saprophytically on decaying parts of opened big buds at the time of mite dispersal. Mites may also acquire spores during the wandering period before entering a new bud. In the laboratory tests only *V. lecanii* proved virulent. The results suggested that *V. lecanii* might be worth further study of inundative release with a view to permanent establishment.

The study of *V. lecanii* was continued on whitefly in glasshouses, where there was opportunity for establishment for the duration of a crop on which biological control of pests was already important. However, many entomopathogens cannot be transmitted

effectively within susceptible host populations or persist in the host habitat. Even so, inundative introductions of non-colonizing pathogens offer opportunities for pest management programmes because the pathogens do not directly harm beneficial organisms in the environment. Pests can be controlled through the repeated application of these pathogens. While requiring more effort and expense than successful colonization, this procedure is often more satisfactory than the more disruptive control methods using chemical insecticides.

V. lecanii was already known as a virulent pathogen of glasshouse whitefly. In the present study, heavy populations of this pest were curbed but not eliminated by repeated spore sprays. In glasshouses five sprays at 2-week intervals or three sprays at 4-week intervals reduced the adult population by 90 to 99%. The fungus did not spread to untreated leaves in the treated glasshouses. It did not affect the natural enemies used on various glasshouse crops. Thus it is concluded that V. lecanii is a pathogen suitable for inundative release without permanent establishment among whitefly populations on glasshouse cucumbers and tomatoes.

Regular sprays of V. lecanii on whiteflies for the whole duration of the crop may be uneconomic. However, it was shown that the fungus could be integrated into control programmes that relied on the parasitic insect, Encarsia formosa, to control whitefly. This parasite failed in early season dull light and low temperature, conditions in which V. lecanii thrived. Thus V. lecanii in the early stage of a crop was successfully followed by E. formosa.

Many crops attacked by whitefly, e.g. cucumber and tomato, are replaced annually and the glasshouses treated with insecticide and steam in between crops, precluding perennial persistence of pathogens. On the other hand, a commercial glasshouse containing chrysanthemum flower beds of all ages is never empty. Hall and Burges (1979) showed that the fungus applied to a bed to control aphids persisted to give crop-long control. However, its spread about a glasshouse was not enough to achieve predictable pest control by natural spread from bed to bed, so it had to be sprayed once on to every bed shortly after planting the chrysanthemum cuttings. Thus this fungus can be used in glasshouses individually, with or without persistence.

Fungi and nematodes are more dependent on the micro-environment than spore-forming bacteria and occluded viruses, so attention was paid to the effect of temperature and relative humidity on the pathogenicity of V. lecanii to whitefly. Equally important was a sound study of the pathogen, especially, for instance, temperature requirements and spore viability during storage. In the commercial glasshouses in temperate countries, which are normally large, V. lecanii can be used for whitefly control because the humidity is usually high and the temperature is favourable for the fungus during the crop-season. However, the shelf-life of spores proved to be very short at room temperature, which is a limiting factor for economic commercial sale of spores by industry. On the other hand, spores can be cultured on inexpensive media for immediate use for pest control, or they can be maintained in cold storage.

An important aspect of the development of a microbial control agent is the selection of the most suitable strains or wild biotypes. Of the several microbial insecticides that have been registered, only B. thuringiensis is advanced enough to warrant strain selection. B. thuringiensis is registered and used commercially against some lepidopterous pests. The range of Lepidoptera known to be susceptible is wide and growing, whereas the polyhedrosis and granulosis viruses are numerous, but much more specific. Production and formulation methods for B. thuringiensis are well developed, as is the technology for standardization of the potency of isolates. In fact, the technology has reached a stage well in advance of that of other types of microbial insecticides, e.g. the viruses.

Over 360 bacterial isolates are classified by 15 H-serotypes with sub groups recognised by crystal toxin serotypes (Jarrett et al., 1979). The performance of isolates is measured by bioassay to indicate virulence. These isolates were screened in an international programme against many susceptible hosts, including larvae of Galleria mellonella.

I contributed to the bioassay programme using G. mellonella by establishing that a slope of 3 should be used in estimations of LC_{50}s from 2-dose assays of inactive and moderately active strains. I also contributed by testing some active isolates by 5-concentration assays in an attempt to find a better strain than the commercially used strain. No isolates were better than the commercially used serotype 5a5b in Thuricide in 1963, but discontinued now. However, in the international bioassay programme a total of 10 strains superior to the present commercially used strain (HD-1,

serotype 3a3b) were found against 10 other species of insects (Dulmage, in press). Of these six strains were given to the industry for development.

The future and ultimate direction of the development of these microbial insecticides should be towards finding: (a) One super strain of B. thuringiensis for all hosts or (b) Several special products for particular host groups. For instance, the new israelensis variety of B. thuringiensis is particularly active against mosquitoes. It requires concerted effort both to improve production and to develop formulations suitable for application to water, a very different substrate to the foliage for which the formulations used in agriculture are designed. Co-operative world-wide testing of products against different disease vectors is also necessary in different aquatic habitats.

Sometimes a suitable strain may not exist in the wild or there may be room for improvement. This may indicate long term work, such as genetic manipulation to create a suitable organism. Such programmes are likely to be beyond the scope of individual research centres and co-operative programmes will be imperative. If a pest develops resistance to pathogens, the resistance can be overcome by changing strains. This could be regarded as the ultimate goal of strain manipulations.

SUMMARY

Three projects are presented, each illustrating consecutive stages in the development of a microbial control programme.

1. The initial project was a survey for fungal pathogens of the blackcurrant mite, Cecidophyopsis ribis. Samples of big buds of blackcurrants were received from various parts of England and examined in the laboratory for diseased mites. All the mites from a few buds from four of 39 localities in 1977 and one of 15 localities in 1978 were found dead and associated with Verticillium lecanii. No other fungal pathogens were found. In all the other big buds there were healthy active mites.

2. In the laboratory, infectivity of five commonly used fungal pathogens of insects and mites was studied on C. ribis. V. lecanii killed all the mites within 2-4 days. It grew and sporulated on individual dead mites though no live mites could be seen with fungal growth. The other fungal pathogens were much less infective than V. lecanii.

3. In the second project, the potential of V. lecanii was studied on a more amenable host, the glasshouse whitefly, Trialeurodes vaporariorum, in a more accessible habitat, the foliage of glasshouse crops. Most emphasis was placed on this project.

4. In ten paired bioassays, whitefly pupae were treated with spores on tobacco leaf discs and incubated at varying degrees of moisture availability, graded as wet, humid and dry. The

pathogenicity of both conidia and blastospores was drastically reduced by low ambient humidity. The Log LC_{50}s were about 6 in dry, 5 in humid and 4 in wet conditions. The slopes of probit lines of the ten paired assays did not differ significantly. Thus it was concluded that both spore types responded similarly to the different experimental conditions in each assay. The slopes of probit lines were below two, except in one assay, suggesting that the scale mortality was due more to infectivity than to toxins produced by V. lecanii. In the laboratory, the main susceptible stages were scales and pupae. Whitefly adults were killed by V. lecanii, but fresh as well as old eggs were not susceptible.

5. In three glasshouse experiments on cucumbers, a single spray of either conidia or blastospores gave excellent control of one generation of young whitefly scales at temperatures favourable to the fungus. Spore concentrations exceeding 10^7 viable spores/ml of spray would be unrewarding. A few insects survived due to imperfect spray coverage, because the fungus did not spread from scale to scale. Spraying at two or four-weekly intervals was necessary for continuous pest control, which would probably be uneconomical. In the fungus-treated glasshouses some adults were dead and covered with profuse growth of V. lecanii. The control plants became sticky with "honey dew" and covered by sooty mould. Later some leaves wilted and died prematurely. In contrast, the plants protected by V. lecanii were healthy with only a few leaves bearing sooty mould. The

systemic fungicide, dimethirimol, was used to control cucumber mildew without harming V. lecanii.

6. Survival of V. lecanii was unaffected by the five species of crop plants tested. The blastospores survived well on leaves of all five species when death of spores by desiccation was avoided by maximum humidity in the laboratory. In the glasshouse, most blastospores died on the ventral leaf surface within a few days after spraying, more on tomato leaves than on cucumber leaves, perhaps due to the differences in evaporation and transpiration rates depending on the morphology of the leaf.

7. Whitefly scales reared on different plant species were killed easily in both laboratory and glasshouse by spraying a spore suspension of V. lecanii.

8. In the laboratory at maximal relative humidity V. lecanii sprays killed whitefly scales of all ages so that most E. formosa inside them did not survive, particularly from young scales. In the glasshouse under lower humidity, significant numbers of E. formosa were not killed by the fungus.

9. In the laboratory, under extreme high humidity, some Tetranychus urticae and Phytoseiulus persimilis released on plants already treated with V. lecanii were found dead with fungal growth on cadavers, probably after drowning in the thick film of viscous blastospore suspension. Many P. persimilis adults survived and multiplied after dipping in a suspension of viable blastospores and subsequent incubation under high humidity on an untreated plant, bearing T. urticae as food.

In the glasshouse, sprays of V. lecanii killed a high proportion of P. persimilis which were already established on plants before spraying. On control plants less mortality was observed. However, the mortality of predators on new leaves, which grew on fungus-treated plants after spraying, was more or less similar to that on the control plants. Entangling of predators in the viscous blastospore suspension soon after spraying may be the cause of higher mortality on sprayed leaves.

10. In the glasshouse two early-season sprays of V. lecanii were integrated with later release of E. formosa for whitefly control on a tomato crop. The early sprays of the fungus, at a time when E. formosa is normally ineffective, greatly improved control of the whitefly. The proportion of E. formosa not emerged from scales and believed to be dead was very low and similar in all compartments with and without fungus. Fungus-killed adult E. formosa were rare. By the end of the experiment E. formosa had gained good control in all the glasshouse compartments including the control, only 2-15% whitefly scales surviving. V. lecanii did not spread to control compartments. However, plants treated with V. lecanii + E. formosa had very few leaves with sooty mould (about 5%), whereas those with E. formosa alone, or neither (control), had many ($>$ 25%). The proportion of live whitefly scales on the former plants was about half that on the latter two groups at the assessment 9 weeks after the last fungus spray. Thus V. lecanii

is recommended for practical early season whitefly control prior to the use of E. formosa.

11. Growth of V. lecanii on Sabouraud dextrose agar was negligible at 2°C. It increased with temperature up to an optimum near 25°C, then decreased rapidly. There were very small irregular discoloured colonies at 30°C and no growth at 31.5°C.

12. In attempts to improve the shelf life of the normally short-lived blastospores, using spores cultured for 2-11 days, survival at 2°C increased with spore age in distilled water and fresh Sabouraud Liquid Medium. Survival in the latter was better.

13. The effects of 18 suspending media on survival of blastospores were studied. At 2°C, irrespective of the type of suspending fluids, blastospores lost viability within approximately two months. At -20°C, blastospores in distilled water were not injured by freezing and thawing, and survived well (LT_{50} = 382 days); the suspending media were mostly deleterious except four media in which blastospores had an LT_{50} exceeding one year;

10% skimmed milk prolonged the survival (extrapolated LT_{50} = 970 days) in comparison to distilled water. In eleven media spores survived longer at -20°C than at 2°C and in the other seven media the temperature effects were not significantly different. Freeze-drying killed most blastospores, except in a few media in which 43-55% on average survived, and the best medium was 7.5% glucose serum.

14. Exposure of the operator intermittantly to V. lecanii for about 2.5 years without protection to hands and face had no harmful effect.

15. In the third project, isolates of Bacillus thuringiensis were screened against the wax moth, Galleria mellonella, as a part of an international programme to find better bacterial strains. The predetermined bioassay technique was precise and reproducible. My contribution to the studies with G. mellonella was to establish that a slope of 3.0 should be used to estimate LC_{50}s from 2-concentration assays of inactive and moderately active isolates in contrast to the slope of 4.0 for active isolates. I also made definitive 5-concentration assays on some active isolates. My results fitted the general conclusion for the G. mellonella programme, viz. the activities of the isolates fell into three distinct arbitrary categories, active ($LC_{50} < 0.04\%$ bacterial powder in the insects' food), inactive ($> 4\%$) and moderately active between these two values. Most active isolates were in H-serotypes 5a5b and 7. No isolates were significantly better for practical pest control than the best commercial product.

16. The perspectives of the three projects viz. a survey for pathogens, the potential of using a pathogen for pest control and the selection of virulent strains of a pathogen, in integrated pest control are discussed.

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Appendix IV - 1[+]

Survival of blastospores of Verticillium lecanii of different ages suspended in fresh Sabouraud Liquid Medium (SLM) and in distilled water on 10.7.78 and stored at 2°C

(Expt. 1)

Age of spores (days)	Medium		Period of storage (days)					
			0	5	8	16	19	33
2	Fresh SLM	D*	6	355	354	442	453	481
		T	238	833	390	449	455	482
	Distilled water	D	6	309	292	312	413	755
		T	238	407	305	331	422	757
3	Fresh SLM	D	23	177	227	380	210	602
		T	312	509	276	403	213	609
	Distilled water	D	23	138	207	278	244	616
		T	312	309	266	314	280	636
7	Fresh SLM	D	2	7	15	178	227	368
		T	137	440	222	669	348	378
	Distilled water	D	8	56	41	175	183	391
		T	214	481	247	332	246	406

* D = Dead spores; T = Total number of spores examined

[+] Corresponds to Table IV - 4

Appendix IV - 2[+]

Survival of blastospores of *Verticillium lecanii* of different ages suspended in fresh Sabouraud Liquid Medium (SLM) and in distilled water on 18.8.78 and stored at 2°C (Expt. 2)

Age of spores (days)	Medium	P, Period of storage (days); D, No. of dead spores; T, No. of total spores examined								
3	Fresh SLM	P	0	1	3	8	15	16	31	64
		D	1	12	7	69	408	258	201	264
		T	209	520	274	554	558	483	221	266
	Distilled water	D	1	4	47	117	109	404	348	262
		T	209	433	401	417	120	560	367	262
5	Fresh SLM	P		0	6	13	19	33	68	71
		D		8	61	123	218	108	303	403
		T		386	744	267	380	124	303	405
	Distilled water	D		6	58	309	248	246	541	480
		T		373	345	373	312	276	544	485
7	Fresh SLM	P	0	4	11	17	32	66	69	
		D	6	9	19	30	221	252	127	
		T	448	557	334	314	318	254	129	
	Distilled water	D	26	8	49	171	309	254	-	
		T	432	413	197	364	323	255	-	
9	Fresh SLM	P		0	2	9	16	46	64	
		D		7	4	4	38	364	253	
		T		489	351	310	218	370	253	
	Distilled water	D		1	7	51	112	262	256	
		T		165	419	268	166	266	257	
11	Fresh SLM	P	0	1	7	8	16	44	65	
		D	1	5	5	7	35	504	418	
		T	364	300	350	334	209	542	427	
	Distilled water	D	6	3	11	13	153	306	438	
		T	412	364	509	456	335	312	447	

[+] Corresponds to Table IV - 4

- not examined

Appendix IV - 3

Survival of blastospores of Verticillium lecanii suspended in liquid media on 2.8.78 and stored at 2°C and at -20°C (Expt. 1)

Medium	Storage temperature (°C)		Period of storage (days)							
			0	1	2	5	15	33	64	92
Spent SLM+	2	D*	29	15	9	72	221	279	113	-
		T	228	410	645	668	269	297	122	-
	-20	D	11	23	123	182	164	253	460	-
		T	328	225	503	390	283	332	586	-
Fresh SLM	2	D	29	5	77	27	338	191	217	258
		T	228	46	366	104	391	192	220	258
	-20	D	44	70	86	67	104	178	447	-
		T	299	271	500	213	216	316	584	-
5% inositol	2	D	29	61	37	47	258	165	228	171
		T	228	206	319	231	360	190	233	175
10% skimmed milk	-20	D	29	34	35	54	194	194	426	-
		T	228	279	323	337	258	206	442	-

* D = Dead spores; T = Total number of spores examined

+ SLM - Sabouraud Liquid Medium

- not examined

Appendix IV - 4

Survival of blastospores of Verticillium lecanii suspended in liquid media on 8.8.78 and stored at 2°C and at -20°C

(Expt. 2)

Medium	Storage temperature (°C)		Period of storage (days)								
			0	1	2	5	8	16	34	65	86
Potassium dihydrogen phosphate 0.0425 g/ litre of distilled water	2	D*	7	16	13	98	125	317	237	309	-
		T	306	345	327	233	274	362	249	312	-
	-20	D	11	38	21	37	52	43	183	176	114
		T	281	512	353	369	706	431	448	409	415
10% glycerol	2	D	7	121	80	157	179	216	217	204	-
		T	306	545	410	529	553	345	228	204	-
	-20	D	31	169	87	205	238	263	424	224	-
		T	238	412	301	444	524	375	485	236	-
Hank's solution	2	D	7	18	38	210	266	440	649	521	-
		T	306	554	1034	877	698	610	667	526	-
	-20	D	5	311	278	388	294	256	398	227	-
		T	231	421	334	430	308	288	411	229	-
Distilled water	2	D	7	14	18	256	209	213	173	477	-
		T	306	510	491	521	448	350	186	485	-

* D = Dead spores; T = Total number of spores examined

- not examined

Appendix IV - 5

Survival of blastospores of Verticillium lecanii suspended in liquid media on 20.9.78 and stored at 2°C and at -20°C (Expt. 3)

Medium	Concentration (spores/ml)	Storage temperature (°C)		Period of storage (days)								
				0	5	15	33	64	99	131	257	300
5% sodium glutamate	6.8×10^7	2	D*	7	18	199	347	196	-	-	-	-
			T	310	326	783	505	301	-	-	-	-
		-20	D	13	67	71	89	32	162	210	217	488
			T	282	189	445	299	132	311	254	225	506
7% peptone + sucrose	5.5×10^7	2	D	12	21	107	314	171	-	-	-	-
			T	276	237	385	418	224	-	-	-	-
		-20	D	31	73	126	301	101	231	272	294	418
			T	497	238	555	529	207	309	307	422	428
7% peptone + dextrose	7.8×10^7	-20	D	11	60	153	221	135	262	265	359	422
			T	302	253	441	378	216	353	316	403	456
3% gelatin + dextrose	7.7×10^7	2	D	18	24	180	207	128	-	-	-	-
			T	382	223	505	416	238	-	-	-	-
Distilled water	1.1×10^8	2	D	19	141	247	587	199	-	-	-	-
			T	474	380	321	628	204	-	-	-	-
Horse serum	5×10^7	-20	D	16	53	105	130	70	323	144	329	377
			T	259	221	549	362	235	560	272	401	436

* D = Dead spores; T = Total number of spores examined

- not examined

Appendix IV - 6

Survival of blastospores of Verticillium lecanii suspended in liquid media on 26.10.78 and stored at 2°C and at -20°C (Expt. 4)

Medium	Concentration (spores/ml)	Storage temperature (°C)		Period of storage (days)										
				0	4	8	14	25	35	63	70	125	256	264
10% glycerol	3.5×10^7	2	D*	148	164	-	228	-	312	-	249	121	-	-
			T	237	274	-	301	-	328	-	250	121	-	-
		-20	D	121	179	198	106	-	63	86	-	285	224	-
			T	240	304	284	149	-	70	91	-	286	224	-
Horse serum	2.0×10^8	2	D	36	215	-	175	-	164	-	125	192	-	-
			T	412	450	-	249	-	267	-	154	252	-	-
		-20	D	65	117	222	160	91	196	196	-	270	369	373
			T	384	436	584	469	301	479	361	-	426	420	404
5% sodium glutamate	3.5×10^7	2	D	50	64	-	70	-	147	-	167	306	-	-
			T	356	285	-	195	-	329	-	212	309	-	-
Distilled water	1.8×10^7	2	D	43	47	-	297	-	152	-	226	306	-	-
			T	405	229	-	356	-	156	-	227	306	-	-
5% inositol	2.0×10^7	2	D	40	92	-	148	-	180	-	228	313	-	-
			T	296	389	-	201	-	200	-	228	341	-	-

Continued/

Appendix IV - 6 (Continued)

Medium	Concentration (spores/ml)	Storage temperature (^{o}C)		Period of storage (days)					
				0	4	14	35	70	125
10% skimmed milk	2.8×10^{7}	2	D	38	72	116	305	296	204
			T	413	349	238	338	301	204

* D - Dead spores; T = Total number of spores examined

- not examined

Appendix IV - 7

Survival of blastospores of Verticillium lecanii suspended in liquid media on 3.11.78 and stored at 2°C and at -20°C (Expt. 5)

Medium	Concentration (spores/ml)	Storage temperature (°C)		Period of storage (days) 0	6	17	42	62	129	256	262
7% peptone + dextrose	1.9×10^7	2	D*	19	38	413	329	173	-	-	-
			T	263	216	470	364	176	-	-	-
		-20	D	12	77	126	137	252	342	102	377
			T	204	332	437	206	367	418	111	407
7% peptone + sucrose	1.2×10^7	2	D	45	108	120	147	289	-	-	-
			T	309	511	212	156	301	-	-	-
		-20	D	32	100	179	42	314	218	117	53
			T	259	280	520	65	483	240	119	56
3% gelatin + sucrose	1.5×10^7	2	D	28	274	201	177	41	-	-	-
			T	311	505	235	191	316	-	-	-
		-20	D	31	67	40	29	41	91	170	64
			T	313	578	453	216	316	494	428	167
3% gelatin + dextrose	1.5×10^7	2	D	32	106	324	96	116	-	-	-
			T	341	177	404	115	125	-	-	-
		-20	D	21	23	110	80	153	319	174	276
			T	229	330	709	207	340	624	305	455
10% glycerol	1.7×10^7	2	D	92	103	161	124	305	133	-	-
			T	388	356	411	180	397	136	-	-
		-20	D	32	191	262	120	219	255	130	323
			T	349	369	355	137	236	256	130	323
10% honey	1.5×10^7	2	D	34	67	128	200	268	257	-	-
			T	352	321	211	255	327	258	-	-
		-20	D	12	49	186	54	145	333	166	408
			T	169	275	432	113	254	340	182	421

* D = Dead spores; T = Total number of spores examined

- not examined

Appendix IV - 8

Survival of blastospores of Verticillium lecanii suspended in liquid media on 9.11.78 and stored at 2°C and at -20°C (Expt. 6)

Medium	Concentration (spores/ml)	Storage temperature (°C)		Period of storage (days)										
				0	3	6	14	40	63	130	132	151	207	256
Potassium dihydrogen phosphate, 0.0425 g/litre distilled water	1.1×10^7	2	D*	10	46	50	39	177	143	-	-	-	-	-
			T	173	334	292	121	201	156	-	-	-	-	-
		-20	D	10	52	80	155	45	201	123	184	256	116	102
			T	173	286	382	451	153	538	173	229	352	145	121
7.5% glucose broth	1.3×10^7	2	D	10	35	31	13	131	31	-	-	-	-	-
			T	173	352	248	149	171	32	-	-	-	-	-
		-20	D	10	89	125	155	100	218	165	266	290	271	118
			T	173	522	484	514	150	307	167	266	300	280	118
5% lactose in 10% glycerol	2.4×10^7	2	D	10	85	46	56	166	139	-	-	-	-	-
			T	173	346	253	197	184	151	-	-	-	-	-
		-20	D	10	83	98	159	81	468	260	106	283	316	130
			T	173	405	409	411	201	745	285	116	304	338	143

Continued/

Appendix IV - 8 (Continued)

Medium	Concentration (spores/ml)	Storage temperature (°C)		Period of storage (days)										
				0	3	6	14	40	63	130	132	151	207	256
Fresh Sabouraud Liquid Medium	$1.5x10^7$	2	D	10	55	44	75	80	71	-	-	-	-	-
			T	173	325	306	221	102	72	-	-	-	-	-
		-20	D	10	76	89	166	135	245	222	149	327	373	158
			T	173	405	205	345	206	310	223	150	339	383	160
Spent Sabouraud Liquid Medium	$1.7x10^7$	2	D	10	100	75	160	98	224	-	-	-	-	-
			T	173	506	209	242	104	231	-	-	-	-	-
		-20	D	10	204	322	162	98	124	132	189	231	250	135
			T	173	434	459	186	104	126	132	189	231	251	135
Hank's solution	$1.2x10^7$	2	D	10	73	42	36	155	109	-	-	-	-	-
			T	173	550	235	155	171	118	-	-	-	-	-
		-20	D	10	87	180	160	134	133	88	219	139	52	251
			T	173	200	285	251	162	134	88	219	140	52	251

* D = Dead spores; T = Total number of spores examined

- not examined

Appendix IV - 9

Survival of blastospores of *Verticillium lecanii* suspended in liquid media on 30.11.78 and stored at -20°C (Expt. 7)

Medium	Concentration (spores/ml)		Period of storage (days)					
			0	35	77	130	256	263
Horse serum	1.0×10^8	D*	42	47	101	320	385	362
		T	519	308	425	502	470	425
Distilled water	7.8×10^7	D	42	28	59	85	227	253
		T	519	371	435	511	489	536
10% skimmed milk	1.0×10^8	D	42	27	25	122	124	220
		T	519	255	479	417	472	649
5% sodium glutamate	1.0×10^8	D	42	55	312	422	250	362
		T	519	294	588	503	286	416
7.5% glucose serum	1.0×10^8	D	42	36	50	139	133	265
		T	519	254	260	416	311	626
5% inositol	5.0×10^7	D	42	239	472	216	343	277
		T	519	514	632	261	408	304
7.5% glucose broth	7.2×10^7	D	42	114	276	351	266	234
		T	519	311	416	408	300	268

* D = Dead spores; T = Total number of spores examined

Appendix IV - 10

Survival of blastospores of *Verticillium lecanii* suspended in liquid media on 7.12.78 and stored at 2°C and at -20°C (Expt. 8)

Medium	Concentration (spores/ml)	Storage temperature (°C)		Period of storage (days)									
				0	4	11	21	34	47	63	131	137	256
7% peptone + dextrose	4.7×10^6	2	D*	8	3	23	-	-	56	167	65	69	-
			T	267	97	77	-	-	63	185	65	77	-
		-20	D	7	17	48	55	95	25	165	53	105	90
			T	258	138	152	148	136	41	205	57	118	94
7% peptone + sucrose	7.0×10^6	2	D	8	6	21	-	-	70	122	148	250	-
			T	267	128	152	-	-	78	148	148	255	-
		-20	D	4	17	15	66	176	72	177	162	179	164
			T	324	150	83	144	233	101	218	174	184	172
3% gelatin + sucrose	9.7×10^6	2	D	8	7	18	-	-	191	99	102	102	-
			T	267	103	73	-	-	267	117	104	112	-
		-20	D	2	12	7	17	11	4	12	13	55	51
			T	228	163	266	321	184	93	339	121	581	327
3% gelatin + dextrose	9.8×10^6	2	D	8	9	11	-	-	69	164	144	118	-
			T	267	164	125	-	-	122	184	159	161	-
		-20	D	5	6	8	24	21	23	123	83	77	348
			T	188	326	95	200	226	166	535	298	263	626

Continued/

Appendix IV - 10 (Continued)

Medium	Concentration (spores/ml)	Storage temperature (°C)		Period of storage (days)									
				0	4	11	21	34	47	63	131	137	256
10% honey	3.3×10^6	2	D	8	5	20	-	-	40	96	58	67	-
			T	267	86	108	-	-	105	159	59	73	-
		-20	D	9	19	20	143	124	69	278	64	167	63
			T	227	120	50	151	139	69	278	64	167	63
5% lactose in 10% glycerol	7.5×10^6	2	D	8	23	16	-	-	51	164	108	117	-
			T	267	247	113	-	-	62	184	108	117	-
		-20	D	10	11	27	55	106	142	123	47	175	122
			T	255	161	121	185	238	225	535	51	230	128
Fresh Sabouraud Liquid Medium	6.8×10^7	-20	D	4	-	100	174	197	-	-	319	324	343
			T	269	-	338	433	387	-	-	417	375	408
Distilled water	1.2×10^8	-20	D	8	19	18	-	-	37	25	134	109	268
			T	267	414	356	-	-	410	346	503	444	839

* D = Dead spores; T = Total number of spores examined

- not examined

Appendix IV - 11

Survival of blastospores of *Verticillium lecanii* suspended in liquid media on 14.12.78 and stored at -20°C (Expt. 9)

Medium	Concentration (spores/ml)		Period of storage (days)							
			0	4	14	21	33	63	130	257
7% peptone+ dextrose	1.2×10^7	D*	8	16	130	173	215	542	419	263
		T	258	149	264	381	406	647	431	264
7% peptone + sucrose	1.9×10^7	D	6	25	71	130	97	189	300	226
		T	211	235	217	319	237	273	358	232
10% skimmed milk	4.7×10^7	D	2	4	6	5	14	50	65	121
		T	205	249	317	269	303	620	400	552
7.5% glucose serum	3.9×10^7	D	0	5	19	14	46	45	71	101
		T	113	222	338	256	443	415	366	407
7.5% glucose broth	3.9×10^7	D	3	30	58	99	82	280	337	289
		T	203	305	274	374	220	579	469	349
Distilled water	2.3×10^8	D	8	3	20	13	22	68	77	125
		T	320	313	414	234	345	477	338	491
10% glycerol	3.0×10^7	D	12	43	88	156	103	232	200	194
		T	119	208	218	457	205	380	272	229

Continued/

Appendix IV - 11 (Continued)

Medium	Concentration (spores/ml)		Period of storage (days)								
			0	4	14	21	33	63	130	133	257
3% gelatin + sucrose	4.1×10^7	D	3	3	9	9	9	34	114	216	167
		T	235	234	325	311	207	357	559	863	516
3% gelatin + dextrose	3.6×10^7	D	5	4	21	23	41	118	101	-	112
		T	271	265	251	338	427	783	408	-	332
Fresh Sabouraud Liquid Medium	3.7×10^7	D	4	8	99	167	199	206	215	-	373
		T	212	126	437	440	441	319	269	-	405
Horse serum	5.1×10^7	D	5	7	26	20	58	73	253	-	126
		T	276	234	351	253	471	327	482	-	233
5% lactose in 10% glycerol	2.2×10^7	D	12	19	53	109	46	142	163	-	188
		T	230	111	213	377	204	401	233	-	207
10% honey	1.5×10^7	D	9	8	33	106	78	143	130	-	263
		T	289	176	225	367	307	304	186	-	271
5% sodium glutamate	2.9×10^7	D	3	21	194	225	219	303	433	-	272
		T	168	228	406	380	321	331	443	-	273

* D = Dead spores; T = Total number of spores examined

- not examined

Appendix IV - 12

Survival of blastospores of Verticillium lecanii suspended in liquid media on 20.12.78 and stored at -20°C (Expt. 10)

Medium	Concentration (spores/ml)		Period of storage (days)						
			0	21	34	63	130	257	264
10% skimmed milk	5.8×10^7	D*	5	10	32	43	156	123	151
		T	182	249	301	468	635	532	413
7% peptone + dextrose	3.8×10^7	D	8	151	130	328	541	288	-
		T	208	300	221	441	634	308	-
5% inositol	4.8×10^7	D	25	135	193	343	268	232	-
		T	163	404	300	408	306	244	-
Distilled water	5.3×10^7	D	29	19	96	82	236	181	189
		T	460	308	872	482	642	412	403
3% gelatin + dextrose	6.4×10^7	D	8	33	35	64	111	124	-
		T	216	318	304	478	410	364	-
7% peptone + sucrose	3.5×10^7	D	9	180	172	337	389	312	-
		T	214	371	228	427	415	315	-
7.5% glucose broth	5.2×10^7	D	10	56	158	94	312	335	305
		T	157	300	559	289	445	491	411
7.5% glucose serum	8.1×10^7	D	10	15	84	68	106	91	116
		T	263	308	308	525	330	344	408
3% gelatin + sucrose	6.7×10^7	D	8	23	39	45	83	72	91
		T	208	252	402	412	411	506	424

* D = Dead spores; T = Total number of spores examined.

- not examined

Appendix IV - 13

Survival of blastospores of Verticillium lecanii suspended in liquid media on 28.12.78 and stored at -20°C (Expt. 11)

Medium	Concentration (spores/ml)		Period of storage (days)					
			0	13	32	62	130	256
Distilled water	1.3×10^8	D*	52	23	46	116	164	266
		T	349	279	300	582	408	408
7.5% glucose broth	5×10^7	D	41	75	100	213	268	251
		T	265	228	297	435	408	315
3% gelatin + dextrose	7.8×10^7	D	20	77	51	60	109	133
		T	206	415	335	351	402	406
7.5% glucose serum	5.5×10^7	D	28	50	18	78	119	105
		T	258	368	120	458	416	309
3% gelatin + sucrose	5.8×10^7	D	54	64	26	65	92	127
		T	457	443	204	504	409	421
7% peptone + sucrose	6.0×10^7	D	27	106	194	227	364	200
		T	262	225	285	304	404	209
10% skimmed milk	7.3×10^7	D	36	14	28	57	100	158
		T	273	256	228	412	400	529
7% peptone + dextrose	5.8×10^7	D	19	187	236	227	376	193
		T	208	374	366	327	410	205

* D = Dead spores; T = Total number of spores examined

Appendix IV - 14

Slopes of probit lines fitted individually to bioassays of *Verticillium lecanii*, conidia and blastospores on pupae of *Trialeurodes vaporariorum*

Assay batch	Slope ± S.E.		Differences of slopes (A) ± S.E. of differences (B)	$^{A}/_{B}$
	Conidia	Blasto-spores		
1	0.54 ± 0.12	0.75 ± 0.15	0.21 ± 0.20	1.10
2	0.87 ± 0.17	0.91 ± 0.07	0.04 ± 0.17	0.25
3	0.52 ± 0.25	0.78 ± 0.22	0.26 ± 0.33	0.79
4	0.77 ± 0.19	0.77 ± 0.16	0.004 ± 0.25	0.01
5	1.00 ± 0.17	1.06 ± 0.17	0.06 ± 0.24	0.23
6	0.99 ± 0.39	1.05 ± 0.04	0.06 ± 0.39	0.15
7	1.63 ± 0.43	4.00 ± 1.20	2.37 ± 1.27	1.87
8	0.79 ± 0.30	0.63 ± 0.19	0.15 ± 0.35	0.44
9	0.50 ± 0.25	0.90 ± 0.27	0.40 ± 0.37	1.09
10	0.51 ± 0.17	0.44 ± 0.09	0.07 ± 0.19	0.37

Appendix IV - 15

Mortality of whitefly scales caused by spraying conidia of *Verticillium lecanii* on cucumbers in glasshouses at low and high humidities on 30.9.77 (Expt. 1)

No. of days after spraying	Compartment C-4; Low temperature, High humidity				Compartment C-5; High temperature, Low humidity			
	Control plants		Treated plants		Control plants		Treated plants	
	Total scales counted	% dead	Total scales counted	% dead	Total scales counted	% dead	Total scales counted	% dead
10	595	5.7	908	90.2	554	14.3	777	62.3
12	665	9.3	876	86.0	566	9.2	715	69.4
14	518	6.2	875	92.0	590	5.1	1224	81.1
18	389	6.2	994	94.0	551	10.7	1141	82.1
20	389	16.2	1415	96.3	739	29.2	1338	81.7

Appendix IV - 16

Mortality of whitefly scales caused by spraying spores of *Verticillium lecanii* on 23.12.77 (Expt. 2)

Control Compartment C-3 No spores		Spore type	Spores/ml	Treated compartments C-4 High R.H., Low temp.		C-5 Low R.H., High temp.	
Total scales counted	% dead			Total scales counted	% dead	Total scales counted	% dead
			OBSERVED 14 DAYS AFTER SPRAYING				
96	0	Conidia	1.8×10^7	140	90.0	69	60.9
43	2.3			97	86.6	99	27.3
125	0.8			175	86.9	53	20.8
89	2.3	Conidia	3.6×10^6	41	34.2	163	40.0
86	0			107	46.7	112	33.0
104	0			173	45.1	68	42.7
115	1.7	Blastospores	1.76×10^7	54	85.9	101	50.5
112	0			78	88.5	95	62.1
57	0			133	88.7	136	68.4
129	0	Blastospores	3.52×10^6	67	68.7	56	25.0
81	0			95	49.5	71	40.9
135	1.5			108	73.2	70	51.4
			OBSERVED 17 DAYS AFTER SPRAYING				
97	1.0	Conidia	1.8×10^7	226	79.7	56	57.1
57	0			125	90.4	107	40.2
136	0			153	92.2	77	15.6
87	1.2	Conidia	3.6×10^6	40	57.5	101	36.6
138	2.9			112	57.1	88	44.3
116	2.6			158	63.3	56	55.4
98	3.1	Blastospores	1.76×10^7	121	83.5	78	61.5
102	1.0			128	90.6	115	66.1
56	1.8			96	92.7	91	65.9
89	1.2	Blastospores	3.52×10^6	112	71.4	68	48.5
103	1.0			67	59.7	73	32.9
104	2.9			122	82.1	67	52.2

Continued/

Appendix IV - 16 (Continued)

Control Compartment C-3 No spores		Spore type	Spores/ml	Treated compartments			
				C-4 High R.H., Low temp.		C-5 Low R.H., High temp.	
Total scales counted	% dead			Total scales counted	% dead	Total scales counted	% dead
OBSERVED 21 DAYS AFTER SPRAYING							
98	0	Conidia	1.8×10^7	211	89.1	48	60.4
60	0			160	88.1	59	33.9
157	5.7			201	89.6	72	36.1
86	1.2	Conidia	3.6×10^6	39	66.7	124	33.9
112	3.9			194	60.3	60	33.3
92	3.3			176	60.2	85	42.4
93	5.4	Blastospores	1.76×10^7	85	81.2	55	74.6
77	0			97	83.5	187	78.1
49	6.1			118	91.5	165	60.0
90	8.9	Blastospores	3.52×10^6	78	71.8	59	61.0
93	6.5			65	69.2	79	38.0
97	6.2			117	83.8	48	58.3

Appendix IV - 17

Mortality of whitefly scales caused by spraying spores of *Verticillium lecanii* on 20.1.78 (Expt. 2)

Control Compartment C-3 No spores		Spore type	Spores/ml	Treated compartment C-4 Humidified		C-5 Unhumidified	
Total scales counted	% dead			Total scales counted	% dead	Total scales counted	% dead
OBSERVED 14 DAYS AFTER SPRAYING							
188	1.6	Conidia	3.8×10^7	379	98.7	No scales	
163	0			592	91.1	390	100
207	0			370	95.4	434	94.7
291	0.3	Conidia	7.6×10^6	425	89.9	515	86.2
235	0			277	84.1	206	88.4
77	0			509	87.6	330	91.2
93	0	Blastospores	3.6×10^7	437	87.9	368	91.3
150	2.7			721	87.4	377	92.0
147	0			103	85.4	268	91.0
131	0.8	Blastospores	7.2×10^6	90	94.4	363	81.8
110	0.9			463	90.9	436	87.8
54	0			310	88.7	No scales	
OBSERVED 17 DAYS AFTER SPRAYING							
41	0	Conidia	3.8×10^7	229	99.2	74	91.9
108	0			370	99.2	274	98.2
-	-			372	97.6	373	97.3
191	7.3	Conidia	7.6×10^6	277	94.6	321	91.0
193	0			167	95.2	208	88.5
128	1.6			212	94.8	145	93.1
141	1.4	Blastospores	3.6×10^7	184	92.4	135	94.1
143	1.4			501	96.4	138	94.9
246	5.3			127	92.1	229	98.7
132	0	Blastospores	7.2×10^6	38	92.1	156	79.5
82	0			359	90.5	187	88.8
115	0			280	91.8	38	89.5

Continued/

Appendix IV - 17 (Continued)

Control Compartment C-3 No spores		Spore type	Spores/ml	Treated compartments C-4 Humidified		C-5 Unhumidified	
Total scales counted	% dead			Total scales counted	% dead	Total scales counted	% dead
OBSERVED 21 DAYS AFTER SPRAYING							
161	0	Conidia	3.8×10^7	166	90.4	No scales	
121	0			305	99.0	313	98.4
-	-			270	98.2	374	94.4
78	1.3	Conidia	7.6×10^6	224	83.9	321	96.6
102	1.0			179	90.5	278	79.9
104	0			216	91.7	156	93.6
-	-	Blastospores	3.6×10^7	164	84.2	203	93.6
175	5.1			182	95.6	323	96.3
101	2.0			93	86.0	338	90.0
139	9.4	Blastospores	7.2×10^6	75	80.0	243	80.3
172	1.7			542	89.1	177	95.5
173	0			131	87.8	No scales	
OBSERVED 24 DAYS AFTER SPRAYING							
137	5.8	Conidia	3.8×10^7	275	86.9	No scales	
89	4.5			357	98.6	317	99.0
-	-			159	98.7	284	99.7
167	1.8	Conidia	7.6×10^6	230	91.7	421	91.9
103	1.0			395	96.5	490	85.7
139	10.1			303	93.7	418	92.8
-	-	Blastospores	3.6×10^7	296	85.1	228	98.7
97	8.3			298	95.3	328	98.5
171	11.1			116	89.7	629	99.4
128	6.3	Blastospores	7.2×10^6	53	88.7	258	88.8
84	4.8			421	91.0	178	97.8
92	10.9			314	97.1	No scales	

- not examined

Appendix IV - 18

Mortality of whitefly scales on newly developed, unsprayed leaves on plants sprayed with Verticillium lecanii on 23.12.77 (a) and on 20.1.78 (b)

Control Compartment C-3 No spores		Spore type		Spores/ml	Treated compartments C-4 Humidified		C-5 Unhumidified	
Total scales counted	% dead				Total scales counted	% dead	Total scales counted	% dead
OBSERVED 28 DAYS AFTER 2ND SPRAYING								
112	1.1	Conidia	a	1.8×10^7	193	6.2	-	-
158	0		b	3.8×10^7	191	13.6	73	2.7
Plant dead					21	9.5	155	0
185	1.6	Conidia	a	3.6×10^6	65	0	62	4.8
232	1.3		b	7.6×10^6	146	4.8	466	26.4
289	0.7				-	-	-	-
Plant dead		Blasto-	a	1.76×10^7	12	50.0	-	-
191	0	spores	b	3.6×10^7	118	16.9	Many tiny scales	
122	11.5				95	31.6		
168	0	Blasto-	a	3.52×10^6	49	18.4	55	3.6
188	1.1	spores	b	7.2×10^6	46	19.6	10	70.0
154	0				-	-	175	1.7
OBSERVED 31 DAYS AFTER 2ND SPRAYING								
-	-	Conidia	a	1.8×10^7	78	0	9	11.1
-	-		b	3.8×10^7	142	6.3	Many tiny scales	
					57	1.8	227	1.3
-	-	Conidia	a	3.6×10^6	72	0	56	23.2
-	-		b	7.6×10^6	95	0	150	24.0
					250	0	Many tiny scales	
-	-	Blasto-	a	1.76×10^7	18	55.6	19	15.8
-	-	spores	b	3.6×10^7	203	11.3	Many tiny scales	
					72	1.4	31	19.4
-	-	Blasto-	a	3.52×10^6	103	1.0	37	10.8
-	-	spores	b	7.2×10^6	83	0	-	-
					52	1.9	58	0

/Continued

Appendix IV - 18 (Continued)

Control Compartment C-3		Spore type		Spores/ml	Treated compartments			
					C-4 Humidified		C-5 Unhumidified	
No spores								
Total scales counted	% dead				Total scales counted	% dead	Total scales counted	% dead
OBSERVED 35 DAYS AFTER SPRAYING								
243	2.1	Conidia	a	1.8×10^7	452	11.5	77	11.7
369	3.8		b	3.8×10^7	80	11.3	103	1.0
304	8.2				74	32.4	105	1.9
569	4.6	Conidia	a	3.6×10^6	80	0	56	0
218	0.5		b	7.6×10^6	71	1.4	126	0
Plant dead					-	-	73	0
Plant dead		Blasto-	a	1.76×10^7	17	0	46	13.0
260	0	spores	b	3.6×10^7	21	0	95	0
272	0.7				106	1.9	52	46.2
389	4.1	Blasto-	a	3.52×10^6	66	0	70	1.4
259	0.8	spores	b	7.2×10^6	190	1.1	-	-
310	0.7				102	30.4	146	0

- not examined

Appendix IV - 19

Mortality of whitefly scales caused by single spraying of blastospores of *Verticillium lecanii* on 5.4.78 on cucumber leaves at different levels on the main stem

15 days after spraying												27 days after spraying			
12th leaf				16th leaf				20th leaf				16th leaf (repeated)			
Concentration of spray (viable spores per ml)															
1×10^8		1.7×10^7		1×10^8		1.7×10^7		1×10^8		1.7×10^7		1×10^8		1.7×10^7	
T*	D*	T	D	T	D	T	D	T	D	T	D	T	D	T	D
Treated Compartment C-2															
150	91.3	69	82.6	80	96.3	125	94.4	108	79.6	102	56.9	207	89.1	181	93.6
124	96.8	97	89.7	173	97.7	121	96.7	102	76.5	115	64.3	365	95.8	419	95.2
Treated Compartment C-3															
127	97.6	60	95.0	68	98.5	115	87.0	91	94.5	53	81.1	232	98.2	294	92.5
113	99.1	51	84.3	100	98.0	103	93.2	76	98.7	50	68.0	344	98.2	91	90.4
Treated Compartment C-4															
119	98.3	78	83.3	143	96.5	60	85.0	85	94.1	50	78.0	332	98.4	131	88.8
98	99.0	52	94.2	191	99.5	39	74.4	67	85.1	33	39.4	210	99.7	110	74.1

Continued/

Appendix IV - 19 (Continued)

15 days after spraying												27 days after spraying			
12th leaf				16th leaf				20th leaf				16th leaf (repeated)			
Concentration of spray (viable spores per ml)															
1×10^8		1.7×10^7		1×10^8		1.7×10^7		1×10^8		1.7×10^7		1×10^8		1.7×10^7	
T*	D*	T	D	T	D	T	D	T	D	T	D	T	D	T	D
Treated Compartment C-5															
75	100	27	88.9	157	98.7	68	83.8	81	86.4	52	48.1	165	100	104	93.5
93	100	56	96.4	167	94.0	65	84.6	70	81.4	62	37.1	248	98.5	213	91.8
Control Compartment C-1															
No spores				No spores				No spores				No spores			
220	8.6			244	0.8			172	1.2			440	1.3		

* T = Total scales counted; D = % dead

Appendix IV - 20

Mortality of whitefly scales caused by spraying different concentrations of blastospores of *Verticillium lecanii* at different frequencies on cucumbers in glasshouses (April to June 1978)

22nd leaf on main stem				1st leaf on uppermost branch				4th leaf on uppermost branch				8th leaf on uppermost branch				New unsprayed leaves*			
Concentration of spray (viable spores per ml)																			
1.8×10^7		3.6×10^6		3×10^7		1.5×10^7		2.5×10^7		5×10^6		2×10^7		4×10^6		Top concentration		Diluted spray	
T+	D+	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D
Treated Compartment C-2																			
406	97.0	143	59.4	1995	92.8	1880	66.3	655	87.8	859	80.2	278	28.1	840	70.6	159	17.6	578	39.8
258	94.6	397	82.4	1621	92.0	2059	76.6	461	83.3	495	83.4	281	69.8	321	45.2	112	26.8	94	20.2
Treated Compartment C-3																			
113	90.3	247	94.3	332	87.7	681	93.1	196	96.9	158	93.7	325	79.1	158	84.8	9	0	4	0
151	98.0	127	80.3	175	90.9	503	80.5	151	94.0	211	91.0	138	94.9	137	92.0	24	0	57	40.4
Treated Comparment C-4																			
106	96.2	175	35.4	267	92.9	193	86.5	202	96.0	260	95.0	166	80.7	177	88.7	0	0	135	5.9
119	81.5	84	51.2	323	94.4	829	87.6	122	96.7	232	94.8	56	92.9	283	91.2	32	0	9	33.3

Continued/

Appendix IV - 20 (Continued)

22nd leaf on main stem				1st leaf on uppermost branch				4th leaf on uppermost branch				8th leaf on uppermost branch				New unsprayed leaves*			
Concentration of spray (viable spores per ml)																			
1.8×10^7		3.6×10^6		3×10^7		1.5×10^7		2.5×10^7		5×10^6		2×10^7		4×10^6		Top concentration		Diluted spray	
T+	D+	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D
Treated Compartment C-5																			
215	99.5	163	89.0	425	95.1	337	96.1	210	100.0	189	100.0	219	94.5	134	74.6	120	12.5	359	23.4
348	89.4	197	74.1	686	93.9	994	97.0	226	97.3	368	98.6	79	91.1	174	94.3	0	0	340	17.9
Control Compartment C-1																			
No spores				No spores				No spores				No spores				No spores			
516	2.1			5229	1.7			3368	8.8			4794	11.8			2550	5.6		

* Samples examined from plants treated with top concentration and diluated spray

+ T = Total scales counted; D = % dead

Appendix IV - 21

Temperature and relative humidity in the compartments of the glass-house (Fig. IV - 5) for Expts. 1, 2 and 3 on the use of *Verticillium lecanii* for the control of whiteflies on cucumbers

Sprayed date	Observed date	Compartment no.	Temperature (°C)			Relative humidity (%)		
			Max.	Min.	Approx. mean	Max.	Min.	Approx. mean
	1977		Experiment 1					
30.9.77	30.9-2.10	4	24.5	24	24.5	86	66	75
		5	27	26	26.5	84	40	66
	3.10-10.10	4	29	22	24	95	82	84
		5	29	23	26	88	68	75
	10.10-17.10	4	27	22.5	24	90	78	84
		5	29.5	25	27	89	60	70
			Experiment 2					
23.12.77	23.12-29.12	3	22	18	20	70	59	65
		4	24	22	23	84	63	75
		5	27	25	26	62	44	54
	1977-1978							
	29.12-3.1	3	23	20.5	21	67	60	64
		4	24.5	22.5	23.5	86	69	74
		5	31	29.5	29.5	60	46	54
	1978							
	3.1-9.1	3	23.5	18	22	70	56	60
		4	29	22.5	23.5	86	54	66
		5	30	27	28	70	43	55
20.1.78	16.1-23.1	3	26	20	22	88	78	82
		4	29	21	23	85	51	65
		5	24	20.5	22	81	41	55
	23.1-30.1	3	28	21	23	89	80	84
		4	29	22.5	23.5	88	55	66
		5	24.5	22.5	23	88	60	66
	30.1-6.2	3	28	21	22	90	80	84
		4	29	22	23	80	57	70
		5	25	22	23	89	56	68

Continued/

Appendix IV - 21 (Continued)

Sprayed date	Observed date	Compartment no.	Temperature (°C)			Relative humidity (%)		
			Max.	Min.	Approx. mean	Max.	Min.	Approx. mean
	1978		Experiment 3					
5.4.78	4.4.-10.4	1	29	21	23	82	50	60
		2	25	19	21	88	42	72
		3	27	20	22	88	52	72
		4	24	20	22	88	46	70
		5	30	19	23	94	36	68
	10.4-17.4	1	26	20	22	94	50	60
		2	26	20	22	94	60	68
		3	28	21	22	94	64	72
		4	28	22	23	94	64	70
		5	28	22	23	96	48	66
21.4.78	17.4-24.4	1	27	20	22	94	45	66
		2	30	20	22	88	48	70
		3	31	21	23	94	50	76
		4	31	22	23	96	46	76
		5	33	20	23	96	46	70
	24.4-1.5	1	28	23	23	92	54	68
		2	29	21	23	92	62	74
		3	28	22	23	92	58	80
		4	26	22	23	90	56	78
		5	29	20	22	92	48	76
5.5.78	1.5-8.5	1	25	20	22	92	54	70
		2	26	20	22	90	54	78
		3	26	22	22	94	60	80
		4	25	21	22	94	60	80
		5	27	19	22	94	52	74
	8.5-15.5	1	26	20	22	86	40	68
		2	26	22	22	88	42	76
		3	26	22	23	92	52	80
		4	25	22	23	92	46	76
		5	26	20	23	96	44	76

Continued/

Appendix IV - 21 (Continued)

Sprayed date	Observed date	Compartment no.	Temperature (°C)			Relative humidity (%)		
			Max.	Min.	Approx. mean	Max.	Min.	Approx. mean
	1978	Experiment 3 (Continued)						
17.5.78	15.5-22.5	1	28	20	23	92	50	70
		2	27	21	23	88	50	70
		3	26	21	22	94	56	70
		4	23	25	23	90	50	74
		5	27	20	23	96	50	70
	22.5-29.5	1	29	20	23	90	40	70
		2	30	20	23	92	42	78
		3	27	21	23	90	50	76
		4	26	23	23	92	50	74
		5	31	20	24	94	50	76
1.6.78	29.5-5.6	1	31	17	23	94	38	66
		2	31	15	23	98	42	72
		3	30	17	23	92	48	78
		4	28	19	23	98	54	80
		5	32	15	23	98	52	78
	5.6-12.6	1	25	20	22	90	52	74
		2	26	21	22	92	56	76
		3	26	22	23	90	58	75
		4	26	22	23	92	54	75
		5	28	20	23	92	54	75
	12.6-15.6	1	25	21	22	78	42	68
		2	25	21	22	78	46	70
		3	24	21	22	86	50	76
		4	25	24	24	94	50	76
		5	25	21	22	90	56	76

Appendix IV - 22

Temperature and relative humidity in the compartments of the glasshouse (Fig. IV - 5) for the experiment on integrating *Verticillium lecanii* and *Encarsia formosa* for the control of whiteflies on tomatoes

V. lecanii sprayed date or *E. formosa* released date	Observed date	Compartment no.	Temperature (°C)			Relative Humidity (%)		
			Max.	Min.	Approx. mean	Max.	Min.	Approx. mean
	1979							
9.2.79 (*V. lecanii*)	30.1-5.2	2	25	19	20	76	63	68
		3	23	17	18	74	60	66
		4	24	18	19	74	62	68
		5	24	20	23	64	50	56
	5.2-12.2	2	22	18	18	88	63	70
		3	21	17	18	78	54	64
		4	20	18	18	80	64	70
		5	24	21	23	64	48	52
	12.2-19.2	2	21	18	19	76	53	64
		3	18	17	18	78	46	60
		4	19	18	18	80	60	70
		5	24	19	23	60	42	50
	19.2-26.2	2	27	20	21	80	64	70
		3	26	18	19	76	60	66
		4	27	19	20	86	68	74
		5	27	23	24	68	54	58
27.2.79 (*V. lecanii*)	26.2-5.3	2	25	16	18	90	62	78
		3	24	17	19	74	52	66
		4	25	17	19	86	64	74
		5	30	21	24	68	48	60
	5.3-12.3	2	-	-	-	-	-	-
		3	26	19	19	80	62	66
		4	26	18	19	88	66	74
		5	30	23	23	74	56	64

Continued/

Appendix IV - 22 (Continued)

V. lecanii sprayed date or *E. formosa* released date	Observed date	Compartment no.	Temperature (°C)			Relative Humidity (%)		
			Max.	Min.	Approx. mean	Max.	Min.	Approx. mean
	1979							
15.3.79 (*E. formosa*)	12.3-19.3	2	-	-	-	-	-	-
		3	27	18	19	78	56	66
		4	29	18	19	88	68	76
		5	31	19	23	78	56	60
22.3.79 (*E. formosa*)	19.3-26.3	3	26	18	19	82	56	66
		4	28	18	20	90	62	78
		5	29	23	24	78	58	66
29.3.79 (*E. formosa*)	26.3-2.4	3	26	18	19	84	34	72
		4	29	18	19	94	66	82
		5	32	22	23	80	58	68
	2.4-9.4	3	23	18	19	84	60	74
		4	24	18	19	92	66	82
		5	28	22	24	80	56	68
	9.4-16.4	3	28	19	20	88	58	78
		4	30	19	21	94	64	78
		5	31	23	24	84	64	72
	16.4-23.4	3	25	18	19	88	52	78
		4	26	19	20	94	56	82
		5	20	23	23	82	54	70
	23.4-30.4	3	25	19	20	86	72	76
		4	26	18	20	94	70	82
		5	28	24	25	86	66	76

Continued/

Appendix IV - 22 (Continued)

V. lecanii sprayed date or *E. formosa* released date	Observed date	Compartment no.	Temperature (°C) Max.	Min.	Approx. mean	Relative Humidity (%) Max.	Min.	Approx. mean
	1979							
27.2.79	30.4-7.5	3	25	18	19	86	66	74
		4	26	18	20	92	70	80
		5	27	23	24	84	62	70
	7.5-14.5	3	24	19	20	92	58	80
		4	26	18	20	94	64	84
		5	30	23	24	88	60	76
	14.5-21.5	3	29	19	20	92	52	74
		4	30	18	20	94	58	80
		5	31	23	24	86	54	70
	21.5-28.5	3	27	19	20	86	62	76
		4	27	19	20	94	68	80
		5	29	23	24	80	60	70
	29.5-4.6	2	29	19	20	92	62	80
		3	28	19	20	94	64	80
		4	-	-	-	96	78	84
		5	26	23	24	88	66	80
	4.6-11.6	2	32	18	20	92	50	80
		3	29	19	20	88	54	76
		4	29	18	20	92	58	80
		5	29	22	24	90	58	74
	11.6-17.6	2	24	19	20	92	54	84
		3	26	19	21	92	54	80
		4	27	18	22	94	60	80
		5	29	23	24	88	54	74