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SECTION 1

INTRODUCTION

Puccinia horiana has been known in Japan since 1895 (Hiratsuka, 1957) but was first described taxonomically by Hennings (1901). It causes white rust or Japanese rust of chrysanthemum species and can lead to serious crop losses if left unchecked. Until 1963 it was confined to China and Japan but has since spread rapidly and has been reported on all continents (Table 1). It was first found in England in 1963 on stock originally imported from Japan (Baker, 1967) and, despite quarantine measures and a Ministry of Agriculture, Fisheries and Food (MAFF) statutory eradication campaign (Lelliott, 1984), it has continued to reappear. In 1989 the MAFF eradication campaign was ceased. This followed a five year test period when propiconazole proved to be an effective eradicant fungicide (Dickens, 1990) and procedures to reduce relative humidity in the crop were implemented, thereby reducing disease incidence and severity.

Recently, it has been demonstrated that the fungus *Verticillium lecanii* can be used to control aphids and thrips on chrysanthemum using a cultivation system which involves maintaining crops for extended periods at high relative humidity (Helyer & Chambers, 1991). The high humidity is required for optimum activity of the biocontrol fungus but has the inherent risk of increasing disease including white rust. Interestingly, *Verticillium lecanii* also has the ability to parasitise a range of rust fungi including *P. horiana* (Srivastava, Défago & Kern, 1985) and so the opportunity of a total integrated pest and disease management system for chrysanthemum exists. This review attempts to assess the feasibility of developing such a system drawing on information concerning the host-parasite relationship between *P. horiana* and chrysanthemum, current white rust control measures and, importantly, environmental and epidemiological studies of white rust disease.

SECTION 2

ETIOLOGY AND EPIDEMIOLOGY OF WHITE RUST DISEASE

Host range and disease symptoms

Puccinia horiana is a basidiomycetous rust fungus found only on chrysanthemum. It has no alternate hosts. It has been recorded to infect many chrysanthemum species including *C. articum*, *C. boreale*, *C. indicum*, *C. japonense*, *C. makinoi*, *C. marginatum*, *C. morifolium*, *C. nipponicum*, *C. pacificum*, *C. shiwogiku*, *C. sibiricum*, *C. yoshinaganthemum* and *C. zawadski* although there is some variation in susceptibility and symptom expression between species and cultivars (Yamada, 1956; Hiratsuka, 1957; Stark & Stach, 1965; Dickens, 1968; Cevat, 1979). The most important species from a commercial point of view is the florists chrysanthemum, *C. morifolium* var. *sinense* (*C. sinense* Sabine).

The first disease symptoms develop on the upper surface of leaves as pale green to yellow spots up to 5 mm in diameter. The centres of the spots become brown and necrotic and on the underside of the leaf raised buff, pinkish, waxy pustules, 2-4 mm across, also known as telia or sori, develop. Subsequently, as the spots on the upper surface become sunken, the pustules become whitish and prominent as sporidia (basidiospores) are produced directly from the teliospores in the pustule. With time, severely attacked leaves wilt, hang down the stem and dry out completely and can lead to complete crop loss. More rarely, pustules are produced on bracts, stems and flowers (Baker, 1967; Dickens, 1970; Water, 1981).

Life cycle of P. horiana

As the telia develop, bicellular, oblong to oblong-clavate teliospores, slightly constricted in the centre, 30-45 x 12-18 μm, with walls 1-2 μm thick and with pedicels up to 45 μm long are produced. These normally germinate *in situ*, without a period

of dormancy, to produce a promycelium on which the hyaline, slightly curved sporidia, 12-18 x 9-15 μm are formed. The sporidia are discharged and dispersed by air currents to infect other chrysanthemum leaves to complete the simple autoecious, microcyclic life cycle (Hennings, 1901; Firman & Martin, 1968).

Environmental factors and epidemiology

A series of detailed experiments concerned with elucidating environmental factors important in the epidemiology of *P. horiana* have been carried out (Yamada, 1956; Firman & Martin, 1968; Leu *et al.*, 1982). Although differing slightly in some observations, notably whether light influences spore biology, the key factors are clear and are summarised below.

High humidity (> 96% relative humidity (RH)) and a film of moisture on the leaf appear to be necessary for germination of both teliospores and sporidia which can take place between 4 and 23°C in the UK and between 6 and 36°C in Japan. At the optimum temperature of 17°C, discharge of sporidia from pustules starts within 3 hours. Once released from the pustule, maximum germination of sporidia can then occur within 3 hours over a temperature range of 0 to 30°C. Consequently the whole process of sporidia release and germination can be very fast. Further, germ tubes from sporidia can effectively penetrate either leaf surface between 4 and 24°C but within the optimum temperature range of 17 to 24°C, penetration can occur within 2 hours. This means that, given optimum humidity and temperature conditions, a total of 5 hours is sufficient to establish new infections on adjacent plants although this would be considerably longer as conditions deviate from the optimum.

Penetration and development of P. horiana *in leaves*

Microscopical details of fungal development in the leaf are scant and related to host resistance studies (Firman & Martin, 1968). In fully compatible interactions, sporidia germinate to produce a short germtube which penetrates the cuticle and epidermal

cell wall directly. A small vesicle is then produced in the host cell within 6 hours and by 48 hours this extends to form a septate, branched structure. Development continues with production of intercellular hyphae and formation of intracellular haustoria, the latter being important for nutrient uptake by the fungus. Symptom expression generally occurs within 9 days and pustule production within 17 days.

In some varieties, infection never proceeds past the early infection vesicle and a hypersensitive reaction occurs. Localised browning or necrosis takes place in the invaded cells. The mechanisms involved in such immune, completely resistant reactions are unknown.

Leaves on susceptible plants also become more resistant to infection with age but never become immune. This implies that in crops infected by wind-blown sporidia, the occurrence of greater numbers of infections on young leaves compared with old leaves probably results from a combination of greater susceptibility and greater sporidia interception on the top of the canopy (Zandvoort, 1968).

Disease arrival and carryover

The disease generally enters growers' holdings from infected propagating material and appears favoured under short day conditions and with moist, overcrowded cultivation. The period between infection and sporidial release varies from as short as 6 up to 60 days depending on conditions and may escape initial visual screening procedures in the symptomless stage. Once present, disease spread is efficient over short distances with the high sporidial production aided by dispersal from water splash, wind and movement of people through the crop. Zandvoort (1968) has reported wind dispersal of sporidia over 700 m or more, but as they are sensitive to desiccation at RHs below 90%, long distance spread would seem unlikely except during very wet periods.

The ability of the fungus to overwinter in the UK is unknown but Firman & Martin (1968) demonstrated that teliospores in pustules in detached leaves survived for 8

weeks at 50% RH, but at higher humidity or when buried in dry or moist compost, they survived only for 3 weeks or less. It would consequently seem that infected debris is not important for the carryover of this disease. However, it will overwinter on mother plants or all year round crops (Water, 1981).

SECTION 3

CURRENT CONTROL MEASURES FOR WHITE RUST

The control measures in current use are based on a variety of strategies including disease avoidance, chemical and cultural control and plant breeding. The primary measure involves avoiding the importation of infected stock. Strict quarantine procedures need to be carried out both on nurseries producing and receiving cuttings. This is also necessary with the importation of pot plants and cut flowers and should eliminate transport of stock exhibiting symptoms and quickly pick up those shipped with symptomless infection.

If the disease is subsequently found on established crops there are three control strategies available. Physical destruction of local infections is a rapid, ecologically sound approach, but although it may appear to work initially, there is a high risk that the infection has spread to otherwise symptomless plants nearby, allowing epidemic spread (Baker, 1967). Another physical method of control involves the use of heat. Treatment of individual plants to temperatures of 38-40°C for 24 h (Glaeser, 1966) or dipping cuttings in water at 45°C for 5 min (Coutin & Grouet, 1983) is reported to eradicate the fungus from some varieties resistant to the heat treatment. This may be useful for saving individual plants or cultivars but would appear to be impracticable on a large scale. A third option involves the use of fungicides. Numerous studies have been carried out around the world involving the use of benodanil, bitertanol, oxycarboxin, triadimefon and triforine for control of chrysanthemum white rust (Grouet & Allaire, 1973; Siegle, Maag & Dielsdorf, 1975; Leiber, 1977; Dirkse, 1980;

Leu, *et al.*, 1982; Krebs, 1985; Rattink, Zamorski & Dil, 1985; Dickens, 1990) but these have resulted in widely varying degrees of control and consequently different recommendations for use. In the UK, use of propiconazole as an eradicant is the current, acceptable option with off label approval to May 1991. Although propiconazole does have some growth retardant effect, it does not appear to have any significant phytotoxicity problems either as a spray or as soil residue in the UK. Benodanil, bitertanol, oxycarboxin, triadimefon and triforine were found to reduce disease but failed to eradicate *P. horiana* in the UK (Dickens, 1990). In contrast, in the Netherlands propiconazole was phytotoxic to chrysanthemums grown in rockwool when applied in the nutrient solution and this fungicide was the least effective compared with bitertanol and triforine (Rattink, Zamorski & Dil, 1985). The reasons for these variations are unknown.

In the UK, 3 sprays of propiconazole are required for eradication of *P. horiana* but prophylactic spraying is not advisable as this may result in fungicide resistance developing. Strains resistant to benodanil and oxycarboxin have already been reported in France, Japan and the Netherlands (Akiko, Kishi & Yoshioka, 1977; Grouet, Monfort & Leroux, 1981; Dirkse *et al.*, 1982).

Cultural control by reducing humidity in the crop has gradually become adopted since it became clear that high humidity was related to increased white rust (Hellmers, 1964). In the UK a series of recommendations were made to control humidity and foliage wetness (Dickens & Potter, 1983). These include night ventilation; spraying chemical treatments during the early part of the day to ensure the foliage is dry before night, especially if blackout is to be used; avoidance of thermal screens, particularly as in spring and autumn the saving is small; removal of the blackout at night while still ensuring a 13 h dark period; using portable fans under the blackout to increase air movement. Grouet (1984) also recommended avoiding the use of overhead watering and suggested using drip or subirrigation where possible. The adoption of permeable night screens rather than polyethylene has likely also helped minimise the development of the disease (J.T. Fletcher, personal communication) as

has the move to lower volume spray regimes.

A final measure continually under development involves the use of plant resistance. Considerable evidence exists that certain species or cultivars of chrysanthemum are more resistant to *P. horiana* than others (Baker, 1967; Dickens, 1968; Yamaguchi, 1981; Leu *et al.*, 1982; Water, Cevat & Riestra, 1984; Rademaker & de Jong, 1985, 1987; Hahn, 1989). In Holland complete resistance controlled by a single dominant gene has proved stable from 1975 to 1987 and is the breeding strategy of choice (de Jong & Rademaker, 1986; Rademaker & de Jong, 1987). Two other types of resistant reactions are known. With incomplete resistance, the fungus completes its life cycle more slowly; penetration needs high spore density and optimum environmental conditions and the pustules produced are smaller and take longer to mature. This has proved useful for some varieties such as Refour. In contrast, late hypersensitivity involves death of large numbers of plant cells associated with the developing pustule resulting in numerous small brown spots. Although this may restrict lesion development to some extent, it usually occurs too slowly and too late to prevent sporulation completely.

There appear to be no relationships between susceptibility and varietal characters such as flower size, obligate or facultative short day plants, varieties for pot or outdoor cultivation and early or late forms (Hahn, 1989). In addition there appear to be several races of *P. horiana* which differ in their ability to infect specific cultivars or groups of cultivars (Dickens, 1968; Yamaguchi, 1981; Grouet, 1984; Krebs, 1985) and suggests that resistance in any particular variety may be relatively shortlived. Fortunately, this has not proven to be the case in Holland so far. Nevertheless, many of the most desirable chrysanthemums are susceptible to at least some races of *P. horiana* and control must then rely on alternative cultural or chemical methods described earlier.

SECTION 4

POTENTIAL EFFECT ON WHITE RUST OF THE PROPOSED NEW INTEGRATED PEST MANAGEMENT SYSTEM USING *VERTICILLIUM LECANII*

Background

An integrated pest management (IPM) system for the control of the aphids *Myzus persicae* and *Aphis gossypii* on chrysanthemum was devised in the UK in the mid 1980s (Helyer & Wardlow, 1987; Scopes & Stables, 1989). This involved application of a low dosage ($^{1}/_{12}^{th}$) of Vertalec, a commercial preparation of *Verticillium lecanii*, twice weekly, from two weeks after planting. The frequent application enabled control of the more sedentary *A. gossypii* which was not obtained with the recommended single dose of Vertalec. This also had the advantage that it could be integrated with some pesticide and fungicide treatments, *Bacillus thuringiensis* for caterpillar control and natural predators such as *Phytoseiulus persimilis* for red spider mite control and *Dacnusa* sp. and/or *Diglyphus* sp. for leaf miner control. The crops were grown under otherwise commercial conditions including the use of polyethylene blackouts. Similarly on chrysanthemum in Canada, a single aqueous spray of Vertalec was also shown to control *M. persicae* but not the spatially restricted aphid, *Macrosiphoniella sanborni* but could be scheduled with benomyl (Gardner, Oetting & Storey, 1984).

At this time, three important events occurred which influenced the use of such IPM strategies for insect control on chrysanthemums in general. Firstly, western flower thrips (WFT) *Frankliniella occidentalis* became established in the UK and the existing IPM strategy could not be implemented as statutory destruction or chemical treatment of infested plants was required. Only in 1989 was the statutory eradication order for WFT rescinded. Secondly, the commercial preparations of *V. lecanii*, Mycotal and Vertalec, marketed by Tate and Lyle for control of whiteflies and aphids respectively, ceased to be available. Only recently has another commercial

preparation, Microgermin, marketed by Christian Hansen Biosystems A/S, containing both the whitefly and aphid strains of *V. lecanii* appeared on the market. Thirdly, woven screens became widely used for blackouts on chrysanthemums rather than polyethylene ones and led to a decrease in the relative humidity in the crops at night. With this background, a new IPM strategy for control of insects on chrysanthemums has been developed.

Development of the new IPM system for chrysanthemum

During the late 1980s MAFF investigated the possibility of using *V. lecanii* for the control of WFT (Helyer & Brobyn, 1988). In a series of direct applications on insects, strain 19-79 for whitefly control and 19-72 for aphid control were both tested on WFT and strain 19-79 was found to be the most pathogenic, attacking several stages of WFT. Consequently, a glasshouse trial on cucumber was carried out to assess the feasibility of using Microgermin (known now to contain the whitefly/WFT strain 19-79 and the aphid strain 19-72) to control WFT as part of another MAFF contract (Helyer & Brobyn, 1990). In this trial, only low levels of WFT occurred and although infected by *V. lecanii*, there was no effect on cucumber yield.

However, relative humidity rarely rose above 90% and it is known that the key to successful insect biocontrol with this fungus is the maintenance of high RH or free water on the leaf. For example, over 14 h at 100% RH at temperatures between 15-20°C were required for successful transmission, infection and mortality of whiteflies and aphids (Ekbom, 1981; Hall, 1981; Millner & Lutton, 1986). Little transmission occurred to aphids at 93% and none at 80% RH. Similarly, sporulation from *V. lecanii*-killed aphid cadavers was delayed and inhibited below 100% RH and there was virtually no sporulation at 80% RH. In other experiments, spray deposits of Vertalec required at least 36 h at 100% RH to become infective and the half life of spores on the leaves of chrysanthemum with RH varying between 65-90% was only 4 days, although some viable propagules could still be detected for up to 2 weeks after spraying (Gardner, Oetting & Storey, 1984).

In view of this information, a series of trials funded by HDC have been carried out to investigate the use of *V. lecanii* to control *A. gossypii*, *M. sanborni* and WFT on chrysanthemum, paying particular attention to the duration and timing of exposure of the crop to high relative humidity (Helyer & Chambers, 1991). The insects were added routinely and *V. lecanii* was applied every 14 days as a standard Microgermin preparation. Under woven blackout screens, the best and commercially acceptable control was obtained with an environmental regime of 4 consecutive nights with >98% RH (maintained with a computer-controlled low pressure misting system) for at least 11 h followed by 3 nights at ambient (low) RH. So far no disease problems such as *Botrytis* or white rust have been observed. The cuttings used for this trial were obviously white rust free and it may be that, at the moment, white rust is absent from the immediate experimental area.

SECTION 5

BIOLOGICAL CONTROL OF PLANT PATHOGENS BY *VERTICILLIUM LECANII*

Background

The Hyphomycete *Verticillium lecanii* (Zimm.) Viégas (synonyms *V. coccorum* Petch = *V. hemileiae* Bour Gams (1971)) was first described in 1939 attacking green scale of coffee in Brazil (Viégas, 1939). Subsequently, it was shown to attack a range of scale insects and aphids (Petch, 1948; Ganhão, 1956; Hall, 1976a,b) and has been found on other orders of insects (Gams, 1971; Barson, 1976) with a worldwide distribution (Kranz, 1981). In addition it has been shown to attack a wide range of fungi, such as leaf spots, powdery mildews and especially rusts, including *Puccinia horiana* (Table 2).

The observation that some *V. lecanii* strains from phytopathogenic fungi are also pathogenic to aphids and *vice versa* (Hall, 1980; Allen, 1982) led to the suggestion

that single strains could be used to control both glasshouse pests and diseases. Interestingly, strain R-7 (CBS 383-35) from 'rust on chrysanthemum' killed 100% of adult, apterous aphids (*Macrosiphoniella sanborni*) in two out of three tests after 6 days at 20°C when applied to aphids at 10^8 spores per ml (Hall, 1980). Although it is not clear whether the rust was *P. horiana* it clearly indicates that *V. lecanii* strains do exist that can attack both rusts and aphids on chrysanthemum. In addition, this study showed that *V. lecanii* strains differed in pathogenic activity towards insects. Such strain differences have also been observed in relation to mycoparasitic activity (Leal & Villanueva, 1962; Garcia Acha, Leal & Villanueva, 1965; Pfrommer, Sewify & Mendgen, 1988).

Mycoparasitism of rust fungi

V. lecanii is able to germinate and attack urediniospores and teliospores of a range of rust fungi but there are no studies investigating attack on sporidia (basidiospores).

Urediniospores of the coffee rust *Hemileia vastatrix* were penetrated within 24 h in water (Eskes, 1989) but the urediniospores of stripe rust of wheat (*P. striiformis*) germinated before spores of *V. lecanii* on agar and *P. striiformis* escaped infection as the germtubes of the rust were not subsequently attacked (Mendgen, 1981). Within pustules of stripe rust and phaseolus bean rust (*Uromyces appendiculatus*), urediniospores were rapidly invaded and the mycelium of *V. lecanii* spread through and between the urediniospores but did not invade the mycelium of the sporogenous layer or the surrounding plant tissue (Mendgen, 1981; Mendgen & Casper, 1980). This suggests that rust spores are more susceptible to attack than mycelia.

In pustules, teliospores of white rust of chrysanthemum (*P. horiana*), phaseolus bean rust (*U. appendiculatus*), *P. dianthi*, *P. malvacearum* and *P. glomerata* were all penetrated similarly, but teliospores of rusts which germinate quickly *in situ*, such as *P. horiana* and *P. dianthi*, were invaded more rapidly than others which germinate more slowly (Srivastava, Défago & Kern, 1985). Teliospores of rusts which

overwinter, such as *P. graminis*, were hardly ever penetrated. Pustules of *P. horiana* were completely covered with *V. lecanii* five days after inoculation and over 90% of teliospores were infected. Of these, about 90% were penetrated through the germ pore. Differential susceptibility of various regions of the spore wall may be a general phenomenon as the pellicle and spines of urediniospores of *P. striiformis* were all that remained following attack by *V. lecanii* (Mendgen, 1981).

The slimy conidia of *V. lecanii* adhere to surfaces through an amorphous mucous (Boucias & Latgé, 1986) with appressoria and rhizoid-like penetration structures occurring occasionally. From electron microscopical studies, penetration of spores by germ tubes or mycelia seems largely to involve enzymatic attack (Locci, Ferrante & Rodrigues, 1971; Spencer & Atkey, 1981; Hänssler, Hermanns & Reisener, 1982; Srivastava, Défago & Kern, 1985; Grabski & Mendgen, 1986; Uma & Taylor, 1987). In addition, although not a constant feature, there are *V. lecanii* strains which can lyse spores and germ tubes of rusts or inhibit their germination and germ tube growth in the absence of penetration (Hassebrauk, 1936; Kothoft, 1937; Garcia Acha, Leal & Vellanueva, 1965; Silveira & Rodriguez, 1972; Spencer, 1980; Allen, 1982; Uma & Taylor, 1987). This allows for the possibility of biocontrol of rust diseases before penetration occurs.

Protease, lipase, chitinase and low molecular weight, heat stable antifungal secondary metabolites have been found in culture filtrates of *V. lecanii* and may be important in the parasitic process on fungi (Silveira & Rodriguez, 1972; Hall, 1984; Jackson, Heale & Hall, 1985; Grabski & Mendgen, 1986; Mendgen, Pfrommer & Sewify, 1988; Eskes, 1989) but definitive evidence *in situ* is lacking. Large spore size, associated with rapid germination, was apparently correlated with better disease biocontrol (Pfrommer, Sewify & Mendgen, 1988) as with insect biocontrol (Hall, 1984) but no other positive correlation of physical or biochemical properties of *V. lecanii* with ability to parasitize fungi have been reported.

Influence of environmental factors on V. lecanii *epidemiology in relation to mycoparasites*

Numerous studies have indicated that the key factor in the development of *V. lecanii* on pustules of rust fungi is humidity. To obtain any infection, the relative humidity (RH) must be above 80% but for optimum activity the RH should be greater than 95% or with free water available on the leaves (Locci, Ferrante & Rodriguez, 1971; Casper & Mendgen, 1979; Mendgen, 1981; Grabski & Mendgen, 1985). Temperature requirements appear to vary with rust-host combination and experiment. For example, Castellani & Graniti (1949) and Bourriquet (1946) obtained optimal growth on pustules of *Cronartium asclepideum* and *Hemileia vastatrix* between 24-27°C whereas Locci, Ferrante & Rodriguez (1971) found optimum growth on *H. vastatrix* below 20°C. Temperatures between 12-18°C have been found to be optimal for growth on most other rust species including *P. horiana* (Casper & Mendgen, 1979; Mendgen, 1981; Grabski & Mendgen, 1985; Srivastava, Defago & Kern, 1985). Interestingly, on *P. striiformis*, *V. lecanii* appears to grow well at higher temperatures with lower relative humidities (Casper & Mendgen, 1981). Light has also been shown to improve development on pustules of *P. striiformis* (Mendgen, 1981).

Given optimum conditions, abundant mycelium of *V. lecanii* appears on the surface of pustules 2 days after infection, with conidiophores forming after 3-5 days, although up to 2 weeks may be necessary to occupy all the surface of the pustule in some rusts (Leal & Villannueva, 1962; Casper & Mendgen, 1979). With *H. vastatrix*-infected coffee and *P. striiformis*-infected wheat, if the high humidity conditions are maintained continuously, the mycoparasite tends to spread from colonized pustules to overgrow the whole leaf (Locci, Ferrante & Rodriguez, 1971; Casper & Mendgen, 1979). Consequently, spore release of *V. lecanii* can take less than 5 days and with wind or insect dispersal, is likely to rapidly decrease rust epidemics if temperature and humidity conditions remain optimal.

Biological control of rust diseases with V. lecanii

Despite the obvious potential of using *V. lecanii* as a biocontrol agent against rust diseases, experiments using this fungus in the field have always failed (Grabski & Mendgen, 1985; Eskes, 1989). There is evidence that *V. lecanii* reaches maximum levels on coffee rust at the end of the rainy season in Brazil and does limit rust epidemics on coffee in Ethiopia naturally each year (Kranz, 1981; Eskes, 1989) but frequently rust epidemics continue in the field despite the presence of *V. lecanii* (Bourriquet, 1946). However, in the glasshouse where humidity and temperature can be controlled, the prospect for use as a biological control agent looks more promising. When *V. lecanii* was applied as a spore suspension to *U. appendiculatus*-infected bean plants in the glasshouse, a 68% reduction in rust disease was observed (Grabski & Mendgen, 1985). Similarly, spore suspensions of *V. lecanii* applied to carnation plants 24 h before or 24 h after urediniospores of *Uromyces dianthi* or as the first pustules erupted or 1 week after the pustules erupted, resulted in significant reductions in disease when assessed three weeks after inoculation with the rust (Spencer, 1980). If applied 48 h before inoculation with *U. dianthi* urediniospores no control was obtained. This implies that the conidia of *V. lecanii* are either inherently short-lived or are sensitive to environmental stresses such as desiccation, a point also noted earlier in SECTION 4. Loss of viability on leaves of coffee infected with coffee rust has also been observed in the field in Brazil (Eskes, 1989). Nevertheless, *V. lecanii* has been recorded in dry plateau regions of Madagascar as well as humid areas (Bourriquet, 1946) which could indicate that more desiccation resistant strains exist.

Formulations to optimise survival and mycoparasitic activity on leaves have been attempted (Spencer & Ebben, 1983). Improved survival of *V. lecanii* was obtained on cucumber leaves when applied in a combination of 2% glycerol and 1% gelatin and a marked reduction in the incidence of powdery mildew compared with that on untreated plants was achieved. The commercial preparations Vertalec, Mycotal and Microgermin also contain materials which attempt to increase germination and survival of *V. lecanii*.

SECTION 6

CONCLUSIONS AND FUTURE WORK

With the environmental regimes necessary to obtain satisfactory control of aphids and thrips on chrysanthemum using *V. lecanii* there is a significant likelihood that white rust will become established and, if left unchecked, will reach epidemic proportions. This must be assessed.

Importantly, these same environmental conditions overlap those for biocontrol of rust fungi including *P. horiana* by *V. lecanii*. It is consequently difficult to predict whether white rust will become established in the presence of routine applications of *V. lecanii* suggested under the new regime for insect control. If infection by the rust was prevented by *V. lecanii* the control achieved would be excellent. A key issue here is the requirement for survival, spread and rapid growth of *V. lecanii* to attack sporidia of white rust when they arrive and to establish at high levels quickly and yet still survive periodic desiccation. These are similar requirements to those needed for prolonged insect biocontrol by *V. lecanii*. In this regard, strains of *V. lecanii* that lyse spores and germ tubes of rusts would be ideal candidates if they are active on the leaf (Uma & Taylor, 1987). Even if infection is not prevented, evidence exists that regular applications of *V. lecanii* can limit disease spread and pustule development (Spencer, 1980). This would be less preferable to preventing infection as control would still result in unsightly blemishes on the leaves unless the isolate of *V. lecanii* was extremely efficient. Nevertheless, one extremely encouraging observation is that strains of *V. lecanii* with activity against both insects and fungi are known (Hall, 1980; Allen, 1982). This adds credence to the idea that integrated insect and rust control could be achieved with a single strain of *V. lecanii*.

At the moment there is no evidence that the Mycotal, Vertalec or Microgermin strains of *V. lecanii* have any activity against white rust of chrysanthemum. This could rapidly and simply be tested in small glasshouse experiments as all the procedures for

maintaining the rust are known and the commercial *V. lecanii* preparations are in routine use. However, these isolates were chosen for their activity against aphids and whitefly and no matter what, if any, level of control of white rust is obtained, it is unlikely to reflect control under optimal conditions using a selected strain.

A detailed experimental programme to evaluate the use of *V. lecanii* to control white rust would seem appropriate. Initially a strain search involving collection from the field and use of existing culture collections should be carried out. The *V. lecanii* isolates would then be screened for the following attributes.

1) Ability for rapid conidial germination on leaves
2) Ability to survive periodic desiccation on leaves
3) Ability to lyse or invade rapidly sporidia and germtubes of *P. horiana*
4) Ability to invade teliospores and prevent sporidial release from pustules of *P. horiana*
5) Ability to attack both insects and *P. horiana* on chrysanthemum (or use mixtures of isolates if the cost is not prohibitive)

These screens would require experiments which investigate the interaction of sporidia of *P. horiana* and spores of *V. lecanii* on leaves as well as the survival of *V. lecanii* in relation to the environmental parameters found under the new insect control regime. Examination of other factors including inoculum type, food base, wetting agents and the use of white rust resistant and partially resistant cultivars would also be important and must be related to the development of the insect control programme. At an early stage, pesticide and fungicide compatibility tests of any new *V. lecanii* strains must also be carefully considered as chemicals would be the immediate response of a grower to any significant increase in pest or disease levels. Subsequently, larger glasshouse experiments under the new insect control environmental regime would be carried out with the newly selected isolates of *V. lecanii.*

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Table 1 Geographical occurrence of *Puccinia horiana* (compiled from Punithalingam, 1968; Water, 1981; Anonymous, 1982; Dordevic, 1983; Walker, 1983; Catley, 1987)

Continent	
Africa:	South Africa
Asia:	China, Hong Kong, Japan, Korea, Malaya and Taiwan
Australasia:	Australia and New Zealand
Europe:	Austria, Belgium, Denmark, Eire, Finland, France, Germany, Great Britain, Italy, Luxembourg, The Netherlands, Norway, Sweden, Switzerland and Yugoslavia
North America:	United States of America
South America:	Argentina, Brazil and Chile

Table 2 Examples of fungi attacked by *Verticillium lecanii**

Fungus	Host
Leaf Spots	
Cercospora arachidicola	Peanut
Cercosporidium personatum	Peanut
Powdery mildews	
Erysiphe graminis	Barley
Sphaerotheca fuliginea	Cucumber
Rusts	
Coleosporium domingense	*Plumeria rubra*
Cronartium asclepiadium	Maritime Pine
Hemileia vastatrix	Coffee
Puccinia allii	Leek
P. arachidis	Peanut
P. chrysanthemi	Chrysanthemum
P. dianthi	*Dianthus barbatus*
P. glomerata	*Senecio jacobea*
P. graminis f.sp. *tritici*	Wheat
P. horiana	Chrysanthemum
P. malvacearum	*Althaea rosea*
P. recondita	Wheat
P. striiformis	Wheat
Uromyces appendiculatus	Phaseolus bean
U. dianthi	Carnation
U. fabae	Faba bean

*Compiled from Kotthoff, 1937; Castellani & Graniti, 1949; McKenzie & Hudson, 1976; Spencer, 1980; Mendgen, 1981; Spencer & Atkey, 1981; Allen, 1982; Spencer & Ebben, 1983; McMillan, 1985; Srivastava, Défago & Kern, 1985; Zambettakis, Sankara & Metivier, 1985; Uma & Taylor, 1987; Mendgen, Pfrommer & Sewify, 1988; Subrahmanyam, Reddy & McDonald, 1990.