FLOWER BLIGHT OF CHRYSANTHEMUMS : THE CAUSAL FUNGI AND THEIR CONTROL

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by

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PREFACE

The commercial chrysanthemum commonly grown in New Zealand is botanically identified as *Chrysanthemum morifolium* Ramat. and is believed to have originated in China. As the family name Compositae suggests the individual flower ('flower head') is a composite arrangement of two types of florets, namely ray florets with well developed petals, and disc florets with tubular or poorly developed petals.

Production of commercial chrysanthemums basically comprises three categories:

(i) cut flowers;

(ii) cuttings;

(iii) container or pot plants.

Chrysanthemums are probably grown by more floriculturalists than any other flower crop. Much of its popularity is attributed to the wide range of colours and forms and the fact that it can be grown either as a pot plant or for cut flowers. Another important characteristic of the chrysanthemum is the long keeping quality of the flowers, a feature which is much appreciated by retailers and consumers. In the United States of America the chrysanthemum or 'mum' is the most popular flower, surpassing both the rose and carnation in total wholesale value.

A survey of the area in New Zealand used for production of flowering, bulbous, and softwooded plants was undertaken by the Department of Agriculture, covering the period from 1st September to 31st August 1967. The result of the survey as it applies to chrysanthemums is as follows:

(A) Outdoor plants

(i) Area for cut flowers = 24.71 hectares

(ii) Area for plant sale = 0.81 hectares

(B) Glasshouse plants

(i) Area for cut flowers = 8702 m^2

(ii) Area for plant sale = 741 m^2

According to this survey chrysanthemum is only surpassed by narcissus and gladiolus in importance as an outdoor plant, and second only to carnation as a glasshouse plant.

In New Zealand chrysanthemums flowering during June, July and August require protection from the weather and are generally grown under polythene or in glasshouses whereas during the remainder of the year they are grown outdoors. However, there is a trend to more all year-round growing under glass and this can be attributed to the response for chrysanthemums to environmental manipulation. Chrysanthemums are a 'short day plant' having a critical daylength requirement of 13½ hours for flower bud initiation and 14½ hours for flower development. Furthermore, the British early flowering varieties are temperature responsive in terms of flower initiation. Hence, by controlling temperature or daylength, year-round flowering under glass is now possible.

There are a number of diseases of chrysanthemums, some of the most important being those of the flower itself. Overseas the fungi which have been reported as pathogenic to chrysanthemum flowers are species of Alternaria, Mycosphaerella, Botrytis, Itersonilia, Stemphylium, Helminthosporium, Fusarium, and Puccinia. In New Zealand only the first five genera have been recorded as pathogens of chrysanthemum flowers. The fact that several different fungi can cause flower blight and that each fungus produces symptoms almost identical to the others has made specific identification and control of flower blight rather difficult. Various workers have indicated losses can be minimized by the adoption of favourable cultural practices, use of fungicidal sprays and resistant cultivars. Protectant fungicides with a broad spectrum of activity, such as mancozeb, zineb, chlorothalonil, captafol, and captan appear most likely to give control of a disease caused by such a taxonomically diverse group of fungi.

In view of the fact that very little experimental work has been conducted in New Zealand on the flower blight fungal complex of chrysanthemums a study was undertaken with the following objectives:

(1) To isolate and test pathogenicity of the fungi associated with flower blight.

(2) To study the morphological and cultural characteristics of the causal fungi relevant to their specific identification.

(3) To investigate the efficiency of certain available fungicides for disease control.

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SUMMARY

Five fungi, namely Alternaria alternata, Botrytis cinerea, Mycosphaerella ligulicola, Itersonilia perplexans, and Pleospora sp. (imperfect state Stemphylium vesicarium) were found to cause flower blight of chrysanthemums in the Manawatu district. This is the first record of a Pleospora sp. with a S. vesicarium imperfect state being associated with chrysanthemum flower blight. It is also the first report of pseudothecia of M. ligulicola occurring in New Zealand. The symptoms induced by all five fungi were very similar making specific identification on this basis difficult. Surface treatment of infected tissue pieces with 'Janola' 1:7 for 1 min followed by plating to antibiotic agar was the most satisfactory method of isolating the fungi and this in turn facilitated identification.

Both the sexual and asexual sporulation of Pleospora sp. on culture media was increased by light, although some sporulation did occur in the dark. In the laboratory protopseudothecia matured when exposed to constant low temperatures (8 - 16 C) for approximately 10 weeks, depending on the isolate and the temperature. Incubation temperature also had a considerable influence on conidial morphology, with the length/width ratio increasing as temperature was increased.

Pycnidiospores of M. ligulicola produced in culture were predominantly aseptate whereas the majority from the host were uniseptate. The percentage septate pycnidiospores produced in culture were not significantly affected by growth medium, light or

incubation temperature (20-32 C). Near-ultraviolet light was essential for the production of pseudothecia in the laboratory and 3 days was the minimum exposure required for their induction on 20% V-8 juice agar.

Captafol, chloroneb, mancozeb, thiram and carboxin were the most effective of fifteen fungicides tested against the five fungi by the laboratory poison food technique. Using the spore germination technique chlorothalonil appeared very promising. This was confirmed in a trial on chrysanthemums grown in field plots where chlorothalonil was outstanding against all five fungi in the complex, with mancozeb, captafol, and zineb also giving good control.

CHAPTER 1

DETERMINATION OF THE FUNGI CAUSING FLOWER BLIGHT OF CHRYSANTHEMUMS

1.1 ISOLATION TO CULTURE MEDIA

As indicated by several workers the identification of the fungi causing flower blight of chrysanthemums (*Chrysanthemum morifolium* Ramat.) on the basis of field symptoms is difficult (8, 18, 21, 51). Subjecting lesioned flower tissue to high humidity does not invariably result in sporulation of the causal fungi, particularly when the lesions are small.

Because isolation to culture media often assists identification of fungi and the latter is essential to enable appropriate disease control measures, a review of literature was made to identify the most suitable isolation technique. However, no details of isolation methods could be found relating to chrysanthemum flower blight. In fact in some cases no mention was made of isolation or evidence provided that pathogenicity had been proven (8). The assumption that the fungi associated with diseased flowers were the pathogens is a dangerous practice, particularly with fungi such as *Botrytis cinerea* Pers. ex Fr., *Alternaria alternata* (Fr.) Keissler and *Stemphylium* spp. which are known to be ubiquitous and effective saprophytes.

Preliminary attempts at isolating the component fungal pathogens from diseased petals by tissue plating indicated that the method of tissue treatment prior to plating had a profound influence on the number and species of fungi growing out and the extent of bacterial and fungal contamination. With a number of diseases, particularly root diseases, several tissue plating techniques have been devised including the addition of antibiotics to the agar medium (14, 15) and surface treatment of infected tissue pieces by dipping in chemicals such as mercuric chloride (2, 9, 21, 25, 36), sodium hypochlorite (14, 35), calcium hypochlorite (36, 39), and alcohol. Both concentration of chemical used for dipping and length of time of exposure of tissue to the chemical are critical with this latter technique. Treatment of tissue pieces by continuous washing in running tap water has also been widely used (21, 29).

In general it appears that surface treatment of infected tissue followed by plating to antibiotic agar is potentially the most effective means of minimizing the presence of associated fungal and bacterial contaminants.

1.1.1 MATERIALS AND METHODS

Infected tissue pieces for plating were either cut from the margins of lesions on ray florets, or when the lesions were only of pinhead size the entire lesion was removed. Thirty tissue pieces were prepared for each treatment, fifteen from 'Madonna' and the remainder from 'Fred Shoesmith'. All tissue pieces in each treatment were wrapped loosely in a muslin bag and then either surface treated by dipping in 'Janola',* or subjected to continuous water-washing.

'Janola' was used at concentrations of 1:7 (1.3% $^{w}/w$ chlorine) and 1:10 (0.94% $^{w}/w$ chlorine), and for each concentration four soaking periods were tested, namely 0.5 min, 1 min, 2 min and 5 min.

In the water-washing method tissue pieces were continuously washed for 1 hr, 2 hr or 3 hr.

With both the 'Janola' and the water-washing series tissue pieces were removed from the muslin bags and dried between sterile blotting paper before plating to agar. Half the tissue pieces in each treatment were plated to laboratory potato dextrose agar (PDA_L) and the remainder to PDA_L with penicillin and streptomycin sulphate, each added at the rate of 50 μg/ml. In each instance five tissue pieces were plated per petri plate, and the plates incubated in the dark at 24 C for four days. Details of all treatments used are given in Table 1.

1.1.2 RESULTS AND DISCUSSION

The results of the tissue plating experiment are presented in Table 1, and discussed in this section.

(a) <u>Antibiotic medium versus non-antibiotic medium.</u>

In all experiments where antibiotics were incorporated

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* A commercial preparation of sodium hypochlorite containing approximately 10.4% chlorine.

in PDA_L bacterial contamination was minimal. The benefits were particularly apparent in the series where tissue pieces had been water-washed. By contrast, and as anticipated, incorporation of the antibiotics in agar did not prevent the growth of saprophytic fungi.

(b) 'Janola' versus water-wash.

In the majority of cases 'Janola' was superior to water-wash as a surface treatment method. Most satisfactory results were obtained where 'Janola'-treated tissue pieces were plated to antibiotic agar.

(c) 'Janola' 1:7 versus 'Janola' 1:10.

As a generalization it may be stated that for any one treatment time, 'Janola' 1:7 was superior to 'Janola' 1:10 in suppressing both bacterial and fungal contaminants, irrespective of whether the tissue pieces were plated on PDA_L, with or without inclusion of antibiotics.

(d) Comparison of 'Janola' 1:7 using different soaking durations.

(i) Plating to antibiotic medium.

A soaking duration of 1 min (treatment 4) was most satisfactory in that bacterial and fungal contaminants were totally suppressed without affecting the associated pathogens (Fig. 1 B). Treatments 6 and 8 involving soaking durations of 2, and 5 min (Fig. 2 B) also totally eliminated bacterial and fungal contami-

FIGURES 1-2: Culture plates of PDA_L showing colonies of fungi and bacteria originating from diseased tissue pieces taken from chrysanthemum ray florets.

FIGURE 1A: Treatment 3 - tissue pieces treated with 'Janola' 1:7 (1 min) and plated to non-antibiotic agar.

FIGURE 1B: Treatment 4 - tissue pieces treated with 'Janola' 1:7 (1 min) and plated to antibiotic agar.

FIGURE 2A: Treatment 7 - tissue pieces treated with 'Janola' 1:7 (5 min) and plated to non-antibiotic agar.

FIGURE 2B: Treatment 8 - tissue pieces treated with 'Janola' 1:7 (5 min) and plated to antibiotic agar.

Key:

Alternaria alternata	A
Mycosphaerella ligulicola	M
Botrytis cinerea	B
Pleospora sp. (*Stemphylium vesicarium*)	P
Itersonilia perplexans	I
Contamination	C

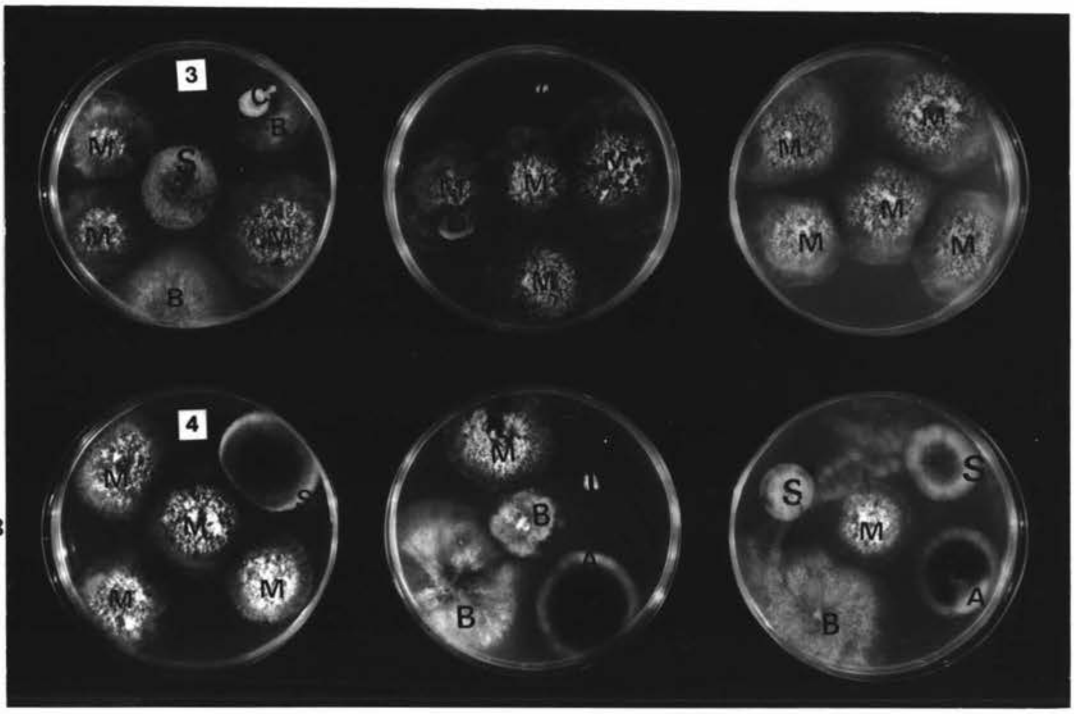


Fig. 1A

Fig. 1B

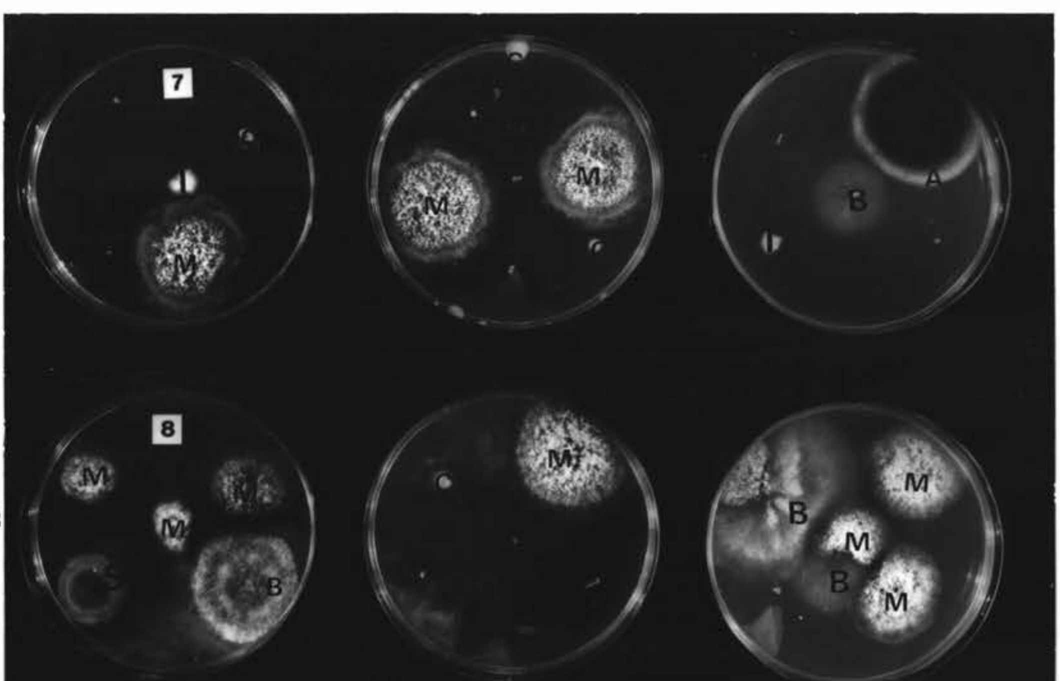


Fig. 2A

Fig. 2B

FIGURES 3-4: Culture plates of PDA_L showing colonies of fungi and bacteria originating from diseased tissue pieces taken from chrysanthemum ray florets.

FIGURE 3A: Treatment 15 - tissue pieces treated with 'Janola' 1:10 (5 min) and plated to antibiotic agar.

FIGURE 3B: Treatment 16 - tissue pieces treated with 'Janola' 1:10 (5 min) and plated to non-antibiotic agar.

FIGURE 4A: Treatment 17 - tissue pieces water-washed (1 hr) and plated to antibiotic agar.

FIGURE 4B: Treatment 18 - tissue pieces water-washed (1 hr) and plated to non-antibiotic agar.

Key:

Alternaria alternata	A
Mycosphaerella ligulicola	M
Botrytis cinerea	B
Pleospora sp. (*Stemphylium vesicarium*)	P
Itersonilia perplexans	I
Contamination	C

Fig. 3A

Fig. 3B

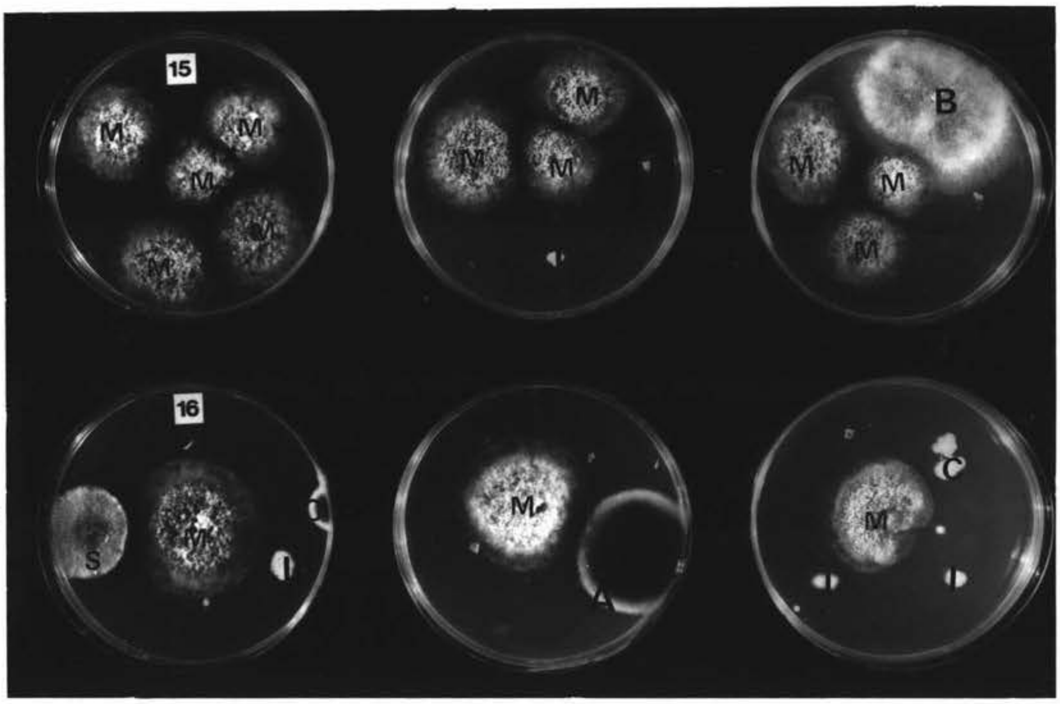
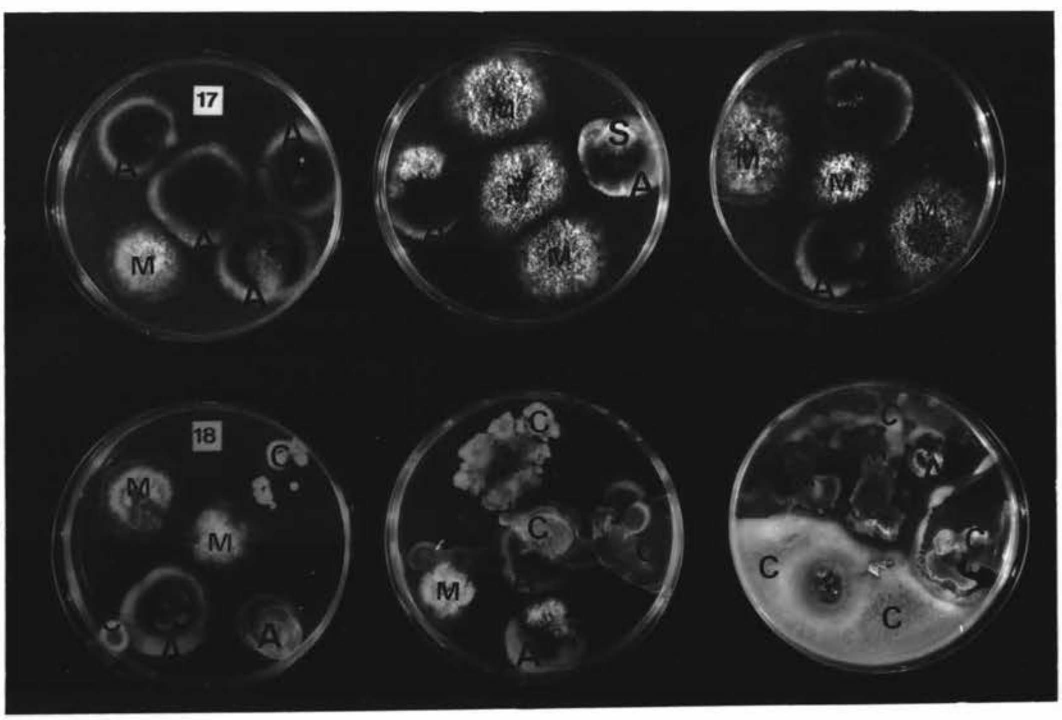


Fig. 4A

Fig. 4B



nants but to some extent also suppressed the component fungal pathogens.

(ii) Plating to non-antibiotic medium.

At all soaking durations tested there was some bacterial contamination. This indicated the ineffectiveness of the 'Janola' dip treatment in totally eliminating saprophytic bacteria, and emphasised the need for inclusion of antibiotics in the medium. Trace fungal contamination was present with all soaking durations of less than 2 min. Results for treatments 3 and 7 involving a 1 min and 5 min 'Janola' soak respectively are illustrated in Figure 1 A and 2 A.

(e) Comparison of 'Janola' 1:10 using different soaking durations.

(i) Plating to antibiotic medium.

The recovery rate of fungal pathogens was equally high at all treatment durations, but only at 5 min (treatment 15) was it associated with total suppression of the fungal and bacterial contaminants (Fig. 3 A).

(ii) Plating to non-antibiotic medium.

There was considerable bacterial and fungal contamination with all soaking durations, the extent decreasing with an increase in soaking duration. Treatment 16, involving a 5 min 'Janola' soak is illustrated in Fig. 3 B.

TABLE 1: Isolation of five fungi from chrysanthemum petals: the relative effectiveness of tissue treatment and addition of antibiotics to laboratory potato dextrose agar

Code No.	Treat-ment	Conc.	Time	Anti-biotics	Number of colonies & specific fungi associated					Total[b] colonies	Number of contaminants	
					Pleospora sp.[a]	*Botrytis cinerea*	*Mycosphaerella ligulicola*	*Itersonilia perplexans*	*Alternaria alternata*		Fungi	Bacteria
1	Janola[c]	1:7	0.5 min	-	1	3	18	0	1	26	2	5
2	Janola	1:7	0.5 min	+[d]	2	2	16	2	3	25	1	1
3	Janola	1:7	1 min	-[e]	1	2	21	0	2	26	1	3
4	Janola	1:7	1 min	+	5	5	12	0	6	28	0	0
5	Janola	1:7	2 min	-	2	2	13	0	3	20	0	3
6	Janola	1:7	2 min	+	4	3	6	2	2	17	0	0
7	Janola	1:7	5 min	-	1	3	8	2	2	17	0	3
8	Janola	1:7	5 min	+	2	6	10	0	2	21	0	0
9	Janola	1:10	0.5 min	-	1	2	12	0	5	20	2	8
10	Janola	1:10	0.5 min	+	3	4	5	4	3	20	0	2
11	Janola	1:10	1 min	-	3	2	18	0	4	27	2	6
12	Janola	1:10	1 min	+	4	2	8	2	4	20	0	2
13	Janola	1:10	2 min	-	1	2	16	1	4	24	2	4
14	Janola	1:10	2 min	+	2	3	18	0	2	25	1	2
15	Janola	1:10	5 min	+	3	1	16	2	3	25	0	0
16	Janola	1:10	5 min	-	1	2	10	4	2	20	3	4
17	Water		1 hr	+	2	1	14	0	9	26	0	10
18	Water		1 hr	-	1	3	13	0	7	24	4	30
19	Water		2 hr	+	2	2	16	3	3	26	1	2
20	Water		2 hr	-	2	4	10	0	5	21	2	30
21	Water		3 hr	+	2	6	9	2	7	26	1	1
22	Water		3 hr	-	2	3	7	0	8	20	2	30

[a] perfect state of *Stemphylium vesicarium*

[b] thirty tissue pieces per treatment

[c] commercial preparation of sodium hypochlorite containing approximately 10.4 per cent chlorine

[d] 50 µg/ml streptomycin and 50 µg/ml penicillin added to PDA_L

[e] streptomycin and penicillin not added to PDA_L.

(f) Water-wash.

(i) Plating to antibiotic medium.

This gave a significant decrease in the extent of fungal and bacterial contamination with an increase in the length of water-washing. Treatment 17 involving a 1 hr water-wash is illustrated in Fig. 4 A.

(ii) Plating to non-antibiotic medium.

Growth of fungal and bacterial contaminants was so intense with all washing times as to mask the presence of fungal pathogens as illustrated in Fig. 4 B, treatment 18.

1.1.3 CONCLUSION

From the above results it was concluded that surface treating tissue pieces with 'Janola' 1:7 for 1 min and plating to antibiotic PDA_L was the most satisfactory means tested of isolating the pathogens causing flower blight of chrysanthemums. Unless otherwise stated this method was followed in subsequent isolation studies. Although 'Janola' 1:10 for 5 min gave equally satisfactory results, it had the disadvantage of requiring a longer treatment time without providing additional benefit.

1.2 PROOF OF PATHOGENICITY

In order to confirm that the fungi associated with diseased chrysanthemum flowers were in fact pathogenic it was necessary that artificial inoculations be conducted.

1.2.1 PRODUCTION OF INOCULUM

An abundant supply of spores is of primary importance for the study of plant pathogenic fungi. With *A. alternata* and *B. cinerea*, which sporulate profusely in culture, no problems were encountered. However, with *Stemphylium vesicarium* (Wallr.) Simmons, *Mycosphaerella ligulicola* Baker, Dimock & Davis, and *Itersonilia perplexans* Derx a number of methods were investigated for improving sporulation on culture media and these involved either manipulation of the fungus or manipulation of the cultural environment.

(a) Manipulation of the fungus.

With species such as *Cercospora vitis* (Lev.) Sacc. (23) and *Septoria* sp. (38) multipoint inoculation of agar plates with either spores or mycelial fragments results in more rapid and intense sporulation compared with the conventional method of inoculating the centre of plates.

In the present investigation experiments were conducted to determine whether multipoint inoculation improved sporulation in the case of *S. vesicarium* and *I. perplexans*. *S. vesicarium* grows fairly rapidly in culture but sporulates poorly whereas the converse applies to *I. perplexans*.

(i) *S. vesicarium* - multipoint inoculation with mycelial slurry or spore suspension.

Mycelial fragments obtained by scraping the surface of three PDA_L cultures were shaken vigorously in a McCartney bottle containing a dozen glass beads and 10 ml of sterile distilled water. The resultant slurry was then poured onto V-8 juice agar plates and dispersed over the entire surface. Excess water was then removed and plates incubated in the dark at 22-28 C.

V-8 juice agar plates were inoculated with a spore suspension similarly prepared from 14 day old cultures.

Results of the above two experiments are presented in Table 2.

(ii) I. perplexans - multipoint inoculation.

Multipoint inoculation of plates with I. perplexans was readily achieved by flooding 12 day old cultures and using the resultant spore suspension to flood inoculate PDA_L plates. Further, to capitalize on the violent discharge of ballistospores a small agar plug from a 12 day old culture was inverted and attached to the lid of PDA_L plate. Within 24 hr ejected ballistospores were dispersed over the agar surface. Plugs were then removed from the lids and the plates incubated at 20 C for 7 days.

Both the above methods of culture inoculation proved to be effective in that there was rapid coverage over the total PDA_L surface and associated with this, greatly increased spore production.

(b) Manipulation of the cultural environment.

A number of factors are known to influence the sporulation of fungi in culture, including nutrition, light and temperature (7, 24).

Of the many media reported as suitable for inducing sporulation of 'difficult' fungi, V-8 juice agar has proved one of the most useful (11, 37).

Many reports examining the effects of light on fungi indicate that manipulation of the quality of light (wavelength) and duration of exposure can provide a means of inducing many fungi to sporulate in culture. Leach (31) demonstrated continuous exposure to near-ultraviolet (NUV) radiation induced or increased sporulation of 31 of 33 species of fungi tested, including species of *Mycosphaerella* and *Stemphylium*.

Temperature manipulations have also proved useful, with exposure to low temperatures being particularly effective for induction of perfect states in culture (5, 45, 47, 53). No doubt this is a reflection of an endeavour to simulate the field situations where many fungi produce perfect states under cooler conditions.

In this study these three environmental factors were examined either individually or in combinations in order to either increase sporulation, or to obtain a particular spore stage on culture media.

(i) *S. vesicarium* - V-8 juice agar and NUV radiation.

This experiment was conducted in conjunction with the investigation of multipoint inoculation of V-8 juice agar plates with either a mycelial slurry or a spore suspension. Plates were exposed to continuous NUV light in a 'light-box' for 11 days (the time taken for S. vesicarium to completely cover the agar surface) at 22-28 C. Results are presented in Table 2.

TABLE 2. The effect of inoculation method and near-ultraviolet radiation on sporulation of *Stemphylium vesicarium*

Inoculation Method	Light regime NUV	Dark	Spore conc. $(x10^4)^a$ Isolate S_3	Isolate S_4
Spore suspension	+		47.50	20.00
Mycelial slurry	+		25.00	15.00
Mycelial plug	+		247.50	225.50
Spore suspension		+	5.00	5.00
Mycelial slurry		+	2.50	2.75
Mycelial plug		+	12.50	15.00

[a] per culture plate

From the results in Table 2 it is apparent that both isolates of *S. vesicarium* gave improved sporulation after exposure to NUV compared with incubation in the dark. Furthermore, multipoint inoculation involving either a mycelial slurry or a spore suspension was not advantageous - in fact with both isolates used in this study it had a detrimental effect on sporulation compared to results obtained by inoculation with a mycelial plug. Colonies initiated by the multipoint method developed considerable tufty aerial mycelium, the latter possibly accounting for the poorer sporulation. Most abundant sporulation of *S. vesicarium* was obtained when V-8 juice agar plates were inoculated in the centre with a mycelial plug and exposed to NUV.

(ii) M. ligulicola - V-8 juice agar and NUV radiation.

Ascospores of M. ligulicola were obtained by growing the fungus in the dark on V-8 juice agar for 7 days at 24 C and then exposing cultures to NUV for a further 7 days at 22-28 C. This treatment also induced the production of a large number of fertile pycnidia.

(iii) S. vesicarium - exposure to low temperature.

In order to obtain ascosporic inoculum of Pleospora sp. (perfect state of S. vesicarium, Simmons (47) and personal correspondence) isolates were grown on PDA_L at 12 C in the dark. Observations at weekly intervals indicated that it required approximately 10 weeks at this cool temperature for the development of mature ascostromata in the cultures.

1.2.2 HOST INOCULATION

Chrysanthemums have the advantage that plants flowering in pots (pot mums), cut flowers, and detached petals are all suitable for inoculation purposes. In the majority of the pathogenicity trials 'Fred Shoesmith', 'White Anne', 'White Top' and 'Sunburst Meffo' were used mainly because the flower colour of these cultivars is either yellow or white and symptoms can be readily observed. Pot plants used were grown in a peat-perlite mixture containing nutrients. Three methods of inoculation were used.

(a) Spraying with an atomizer.

Inoculum was prepared by flooding sporulating plates of the fungi with sterile distilled water. A L-shaped rod was used to dislodge the spores. The spore suspension was filtered through cheesecloth to remove mycelial fragments, and the spore concentration appropriately adjusted. A hand atomizer was used to spray the flowers to run-off. Inoculated plants or cut flowers were incubated for 48 hrs in a mist cabinet set at a temperature of 24 C and near saturated atmosphere, after which they were removed to the glasshouse bench.

(b) Natural spore ejection method.

Since the ascospores of *M. ligulicola* and the ballistospores of *I. perplexans* are violently discharged, petri plates containing mature ascostromata of *M. ligulicola* and ballistospores of *I. perplexans* were suspended about 6-9 inches above flowers in the mist cabinet. Within 48-72 hr symptoms of the disease were present on the flowers.

(c) Inoculation of detached petals using drops of spore suspension.

This is an extremely convenient though less reliable method of inoculation, and was used to demonstrate pathogenicity with ascospores of *M. ligulicola* and *Pleospora* sp. (imperfect state *S. vesicarium*) and ballistospores of *I. perplexans*. It has been rather extensively used by Smith (44) in inoculation experiments with the latter species. In this case the outer petals were removed from the flowers and placed in a petri plate containing wet filter paper. These petals were then inoculated by placing a drop of spore suspension on the upper surface, and the plates incubated at 20 C (Fig. 5).

Ascosporic inoculum of M. ligulicola and Pleospora sp. was prepared by squashing approximately a dozen mature ascostromata in 5 ml of sterile distilled water. The asci were made to release the ascospores by gently agitating the suspension. To separate fragments of asci and ascostromata from ascospores the suspension was filtered through muslin cloth and the filterate retained for inoculation.

1.2.3 SYMPTOM PRODUCTION

Table 3 summarises the fungi recorded as pathogenic to chrysanthemums together with the symptoms or common names of the disease. Flower symptoms similar to those observed in the field were produced with all inoculation attempts made with the following:

(i) conidia of B. cinerea, A. alternata, Pleospora sp. (S. vesicarium) and M. ligulicola;

(ii) ballistospores of I. perplexans;

(iii) ascospores of M. ligulicola and Pleospora sp. (S. vesicarium).

(a) Field symptoms.

For two consecutive years a large number of diseased plants were observed critically for type of flower symptoms and isolations made by tissue plating to obtain a correlation between

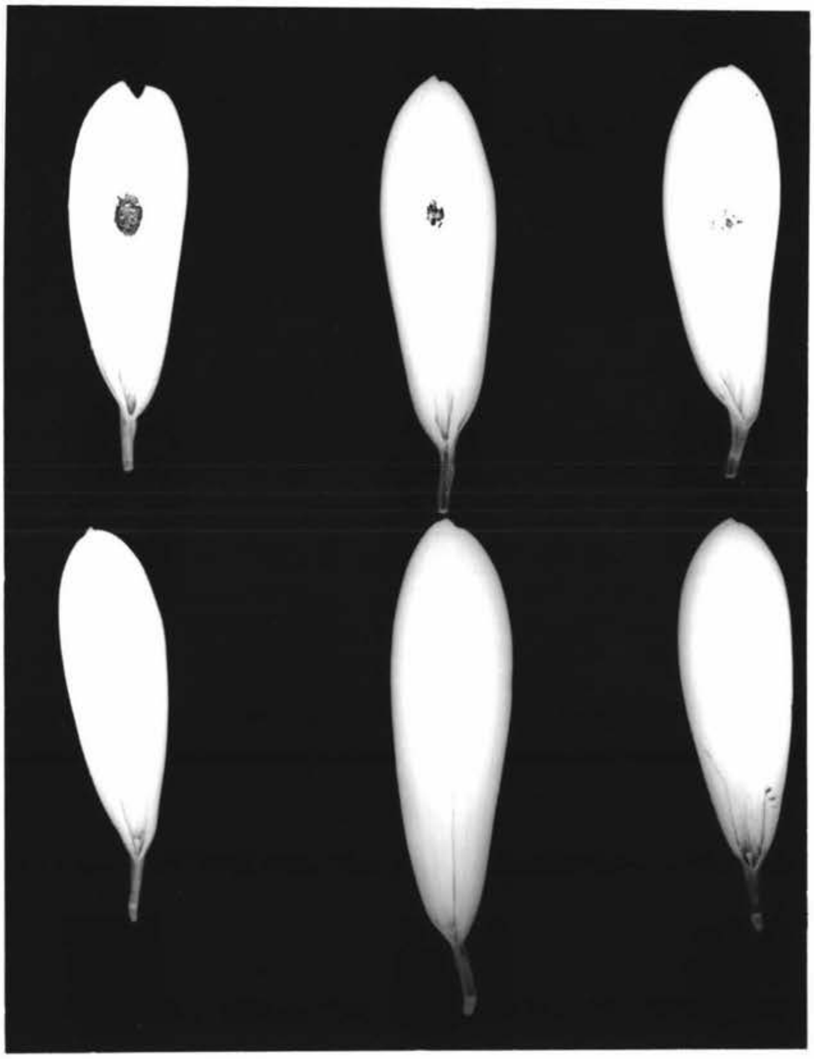


FIGURE 5: Spore drop (spore suspension) inoculation method.
Top row: symptoms on detached chrysanthemum ray florets inoculated with ascospores of Pleospora sp. (Stemphylium vesicarium).
Bottom row: control.

symptoms and causal agent. With all the five fungi it was observed that the first signs of infection were the development of numerous, small, reddish-brown or brick-red pinhead size flecks on the ray florets (Figs. 6, 7 & 8). Further progress of the lesions depended very much on the weather conditions. Under conditions of high humidity the lesions increased in size, coalesced together and became straw coloured. Lesions caused by B. cinerea, M. ligulicola and I. perplexans were occasionally water-soaked.

In addition to the above symptoms M. ligulicola caused bud blight (Fig. 9) and one-sided necrosis of the flower head (Fig. 10). In the latter case infection occurred only on one side of the flower, involved only a few florets and progressed from base upwards causing the petals to turn brown or tan in colour. When infection occurred at an early stage in the development of the flower, it invariably resulted in the distortion of the flower (Fig. 11). The bud blight phase of infection resembled bacterial bud blight with infected buds turning dark brown to black in colour. Infection of the leaves and stems was also fairly common. Leaf symptoms appeared as dark lesions generally starting from the margins, progressing inwards. Stem lesions were brown or brownish-black and had a girdling effect on the stem. Abundant pycnidia were produced on the infected stems, leaves and flowers (Fig. 12). Ascostromata were formed on the infected tissues later in the season (Fig. 13 & 14).

(b) Glasshouse symptoms.

All inoculated plants were placed in a glasshouse where symptom development was observed. The first signs of infection

FIGURE 6: Chrysanthemum flower showing petal flecking (field infection). Mycosphaerella ligulicola, Pleospora sp. (Stemphylium vesicarium), Alternaria alternata and Botrytis cinerea were isolated from this specimen.

FIGURE 7: Individual ray florets showing petal flecking (taken from flower in Figure 6).

FIGURE 8: Chrysanthemum bud 'showing colour' with petal flecking. *Mycosphaerella ligulicola*, *Pleospora* sp. (*Stemphylium vesicarium*), *Alternaria alternata* and *Botrytis cinerea* were isolated from this specimen.

FIGURE 9: Chrysanthemum flower buds infected with *Mycosphaerella ligulicola*.

(a) Blackening advancing down the stem

(b) Advanced stem blackening progressing from flower.

FIGURE 10: One-sided necrosis of a chrysanthemum flower caused by *Mycosphaerella ligulicola* (field infection).

FIGURE 11: Distortion of chrysanthemum flower caused by *Mycosphaerella ligulicola* (field infection).

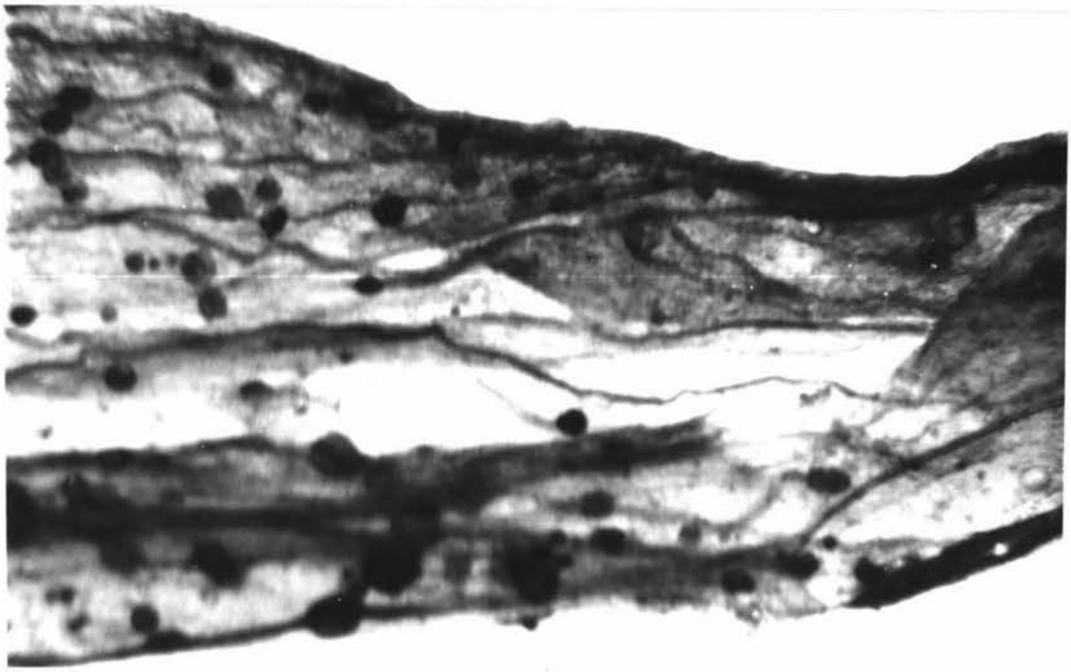


FIGURE 12: Pycnidia of Mycosphaerella ligulicola on a ray floret of chrysanthemum.

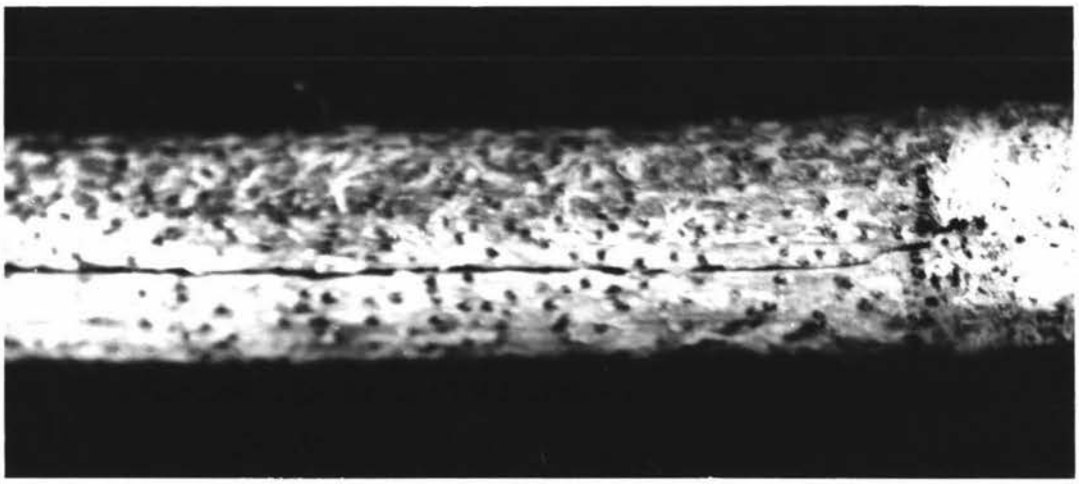


FIGURE 13: Pseudothecia of Mycosphaerella ligulicola on a stem of chrysanthemum.

FIGURE 14: Pseudothecia of Mycosphaerella ligulicola on a ray floret of chrysanthemum.

by all the five fungi were the development of numerous, small, pinhead size, brick-red to reddish-brown lesions (Figs. 15 & 17). These lesions increased in size with time and coalesced together to form larger lesions, sometimes covering the entire floret (Figs. 16 & 18). The larger lesions were normally straw or brown in colour. In the advanced stages of infection B. cinerea and M. ligulicola could be identified on the basis of sporulation on infected tissue. B. cinerea formed a gray moldy growth, whilst M. ligulicola produced pycnidia and later, ascostromata. Occasionally A. alternata too could be identified on the basis of sporulation on the host. M. ligulicola seldom produced the one-sided necrosis of flowers, a feature of the disease which is quite characteristic for the fungus (3, 28, 30). The failure to always produce this symptom with laboratory inoculations possibly may be attributed to the inoculum levels used and the application of spores over the whole flower.

(c) Conclusion.

Identification of the component flower blight fungi on the basis of symptoms is not possible, especially at the early stages of infection. The above is particularly true as far as field symptoms are concerned. Therefore it is recommended that for any accurate identification the fungi should be isolated to agar by tissue plating.

1.2.4 REISOLATION AND GENERAL CONCLUSION

The fungi were readily re-isolated from diseased tissues

FIGURE 15: Flecking on chrysanthemum ray florets artificially inoculated with Itersonilia perplexans.

FIGURE 16: Ray floret scorch of chrysanthemum flower inoculated with Itersonilia perplexans.

FIGURE 17: Symptoms on chrysanthemum ray florets caused by *Pleospora* sp. (*Stemphylium vesicarium*) (artificially inoculated).

FIGURE 18: Flecking (arrow) and tip necrosis on chrysanthemum ray florets artificially inoculated with *Alternaria alternata*.

of artificially inoculated plants, following the tissue plating method previously described. The colonies that developed were examined both macro- and micro-scopically and were found to be similar to the original cultures, thus fulfilling the requirements of Koch's Postulates and conclusively demonstrating that *A. alternata*, *B. cinerea*, *M. ligulicola*, *I. perlexans*, and *Pleospora* sp. (*S. vesicarium*) can individually cause blight of chrysanthemum flowers.

TABLE 3. Summary of flower diseases of florists' chrysanthemums[a] and the associated fungi

Fungus	Disease name or Symptoms.	Reference
Stemphylium floridanum Hannon & Weber	Flower rot	27
	Ray speck	28,48,50,51
Stemphylium sp.	Flower blight	8,21
Stemphylium sarcinaeforme (Cav.) Wiltsh[b]	Ray speck	51
Stemphylium loti Graham[b]	Ray speck	51
Stemphylium trifolii Graham[b]	Ray speck	51
Stemphylium callistephi Baker & Davis[b]	Ray speck	51
Pleospora herbarum (Pers. ex Fr.) Rabenh.	Ray floret blight	12
Botrytis cinerea (Pers. ex Fr.)	Grey mould	18,33,52
	Petal blight	30,34
	Petal spot	30,34
	Botrytis blight	12,28
	Water-soaking of petals	33
Alternaria alternata (Fr.) Keissler	Ray speck	51
	Alternaria blight	42
	Flower blight	8[c]
	Petal spotting	17
	Flower spotting	54
Alternaria solani Weber[b]	Flower blight	8[c]
Alternaria zinniae Pope[b]	Ray speck	51

continued over ...

Table 3. Summary of flower diseases of florists' chrysanthemums[a] and the associated fungi - continued.

Fungus	Disease name or Symptoms.	Reference
Alternaria sp.	Ray speck	51
	Petal lesioning	18
Alternaria chrysanthemi Simmon & Baker	-	16
Helminthosporium sp.	Petal spotting	18,19
Fusarium tricinctum f. poae (Pk.) Snyd. & Hans.	Flower rot	40
Puccinia horiana Henn.	Necrotic flecks on florets	10
Itersonilia perplexans Derx	Petal blight	13,22,44
	Flower scorch	12,22,41
Ascochyta chrysanthemi Stev.	Ascochyta blight	17,20,33
(Perfect st. Mycosphaerella ligulicola Baker, Dimock & Davis)	Ascochyta ray blight	3,6,28,30,49
	Ray blight	12
	Bud blight	20
	Systemic infection	20
	Ascochyta disease	1,4
Didymella ligulicola (Baker, Dimock & Davis) V. Arx (= M. ligulicola)	-	43

[a] Chrysanthemum morifolium Ramat.

[b] Not reported as naturally occurring; pathogenicity demonstrated by artificial inoculations.

[c] Pathogenicity not demonstrated.

TABLE 4. Method of inoculation of five fungi causing flower blight of chrysanthemums[a]

Fungus	Method of plant inoculation	Spore conc. (spores/ml)	Cultivar
Botrytis cinerea	Hand atomizer	100,000	Fred Shoesmith[1] White Anne[1] Sunburst Meffo[1]
Alternaria alternata	Hand atomizer	150,000	Fred Shoesmith[1] White Anne[1] Sunburst Meffo[1]
Mycosphaerella ligulicola	Natural spore ejection	Unknown	White Anne[2]
Ascochyta chrysanthemi (Perf. St. *M. ligulicola*)	Hand atomizer	120,000	Fred Shoesmith[1] White Anne[1] Sunburst Meffo[1]
Stemphylium vesicarium	Hand atomizer	50,000	White Anne[1] Fred Shoesmith[1] Maestro[1]
Pleospora sp. (Imp. St. *S. vesicarium*)	Drop of spore suspension	Unknown	Sunburst Meffo[1]
Itersonilia perplexans	Hand atomizer	150,000	Sunburst Meffo[1] White Anne[1] Maestro[1]
I. perplexans	Natural spore ejection	Unknown	White Anne[2]
I. perplexans	Drop of spore suspension	100,000	White Anne[3]

[1] cut flower; [2] flowers of pot plant; [3] detached petals.

[a] All five fungi pathogenic on flower tissue irrespective of inoculation method.

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CHAPTER 2

LABORATORY STUDIES ON THREE FUNGI CAUSING FLOWER BLIGHT

2.1 PLEOSPORA SP. (IMPERFECT STATE STEMPHYLIUM SP.)

The first record of a species of *Stemphylium* causing flower blight of chrysanthemums was by Tammen (34) in 1959, when isolations from necrotic specks on the ray florets yielded a species of *Stemphylium* resembling *Stemphylium floridanum* Hannon & Weber. Since then the only other species of *Stemphylium* recorded as causing a natural infection of chrysanthemum flowers (Table 3) is *Stemphylium botryosum* Wallr. [perfect state *Pleospora herbarum* (Pers. ex Fr.) Rabenh.]. However, Tammen (35) reported *Stemphylium callistephi* Baker & Davis, *Stemphylium loti* Graham, *Stemphylium trifolii* Graham, and *Stemphylium sarcinaeforme* (Cav.) Wiltsh. as capable of infecting chrysanthemum flowers following artificial inoculations (Table 3). In New Zealand *S. botryosum* is the only species that has been reported on this host (10). Findlay (13), while studying the flower blight fungal complex of chrysanthemums in the Manawatu district also isolated a *Stemphylium* sp. which according to him closely resembled *S. botryosum*. However, after referring to Simmons's (30) work on *Stemphylium* spp. it is questionable whether the conidia photographed by Findlay (13) were actually *S. botryosum* as was suggested. Furthermore, according to G.F. Laundon* (personal communication) more than 90% of

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Stemphylium spp. pathogenic to plants in New Zealand belong to the species *Stemphylium vesicarium* (Wallr.) Simmons.

It is also noteworthy that the only report of a *Stemphylium* sp. from chrysanthemum flowers being associated with an ascigerous stage is by Dingley (10) for *S. botryosum* (*Pleospora herbarum*). However, as this publication is a plant disease record index there is no indication as to whether the perfect state was found on diseased plants, in culture, or assigned on the basis of Dingley identifying the imperfect state as *S. botryosum*. Therefore, in view of Simmons's work and the observations of Laundon the specific identity of *Stemphylium* spp. recorded in New Zealand must be re-examined. It was for this reason that studies were made on the morphology of *Stemphylium* isolates obtained from diseased chrysanthemum flowers. Because of the confusion over the recording of *S. botryosum* in New Zealand and the similarity of conidia (chrysanthemum isolates) to *S. vesicarium* (*sensu* Simmons, 1969), the morphological characters of the sexual and asexual stages of *S. botryosum* and *S. vesicarium* are compared in Table 5. According to Simmons's the main diagnostic characters of *S. botryosum* and *S. vesicarium* are:

(i) maximum conidial dimensions;
(ii) length/width ratio of conidia;
(iii) contrictions of the conidia.

In order to examine the *Stemphylium* isolates from chrysanthemum flowers more closely a number of cultural studies were conducted, mainly with the objective of producing abundant conidia and if possible also inducing the formation of the perfect state.

TABLE 5. Reproductive features of *Stemphylium botryosum* and *Stemphylium vesicarium*[a]

	Stemphylium botryosum	*Stemphylium vesicarium*
A. Conidia		
Shape of mature conidia.	Subspherical, oblong, or broadly ovoid to subdoliiform.	Oblong or broadly oval, sometimes inequilateral.
Shape of juvenile conidia.	Spherical.	Oblong.
Constrictions	Median construction very conspicuous; very slight or no constrictions at other transverse septa.	Constrictions at 1 or more commonly 3 of the major transverse septa.
Septa	1-3 transverse septa; 1-3 (-4) complete or nearly complete longitudinal septa.	1-5 (-6) transverse septa; 1-2 (-3) complete or nearly complete longitudinal septa.
Colour	Pale to dark but translucent olive-brown.	Pale or medium golden-brown to olive-brown.
Size	Width 24 - 26 µ Length 33 - 35 µ.	Width 12 - 22 µ Length 25 - 42 (-48) µ.
Epispore sculpture	Warted or echinulate.	Verrucose.
Length/width ratio	1.0 - 1.5 (culture).	2.5 - 3.0 (culture) 1.5 - 2.7 (host).

continued over ...

Table 5. Reproductive features of Stemphylium botryosum and Stemphylium vesicarium[a] - continued

	Stemphylium botryosum	Stemphylium vesicarium
B. Pseudothecia		
Diameter	Up to 1000 µ.	500 - 1000 µ.
C. Asci		
Size	40 X 200 µ.	35 X 170 µ.
Shape	Narrowly cylindrical to clavate, tapering to a swollen or claw-like base.	Narrowly cylindrical to clavate, tapering to a swollen or claw-like base.
D. Ascospores		
Size	17 X 40 µ.	18 X 38 µ.
Shape	Young spores oblong with obtusely rounded ends and noticeably constricted at 1 or more of the initial three transverse septa when longitudinal septa are produced.	Young spores ellipsoidal, upper half narrowly tapered; noticeably tapered at the initial three transverse septa when longitudinal septa produced.
	Mature spores broadly rounded at the apex and with a flat base; seven transverse septa and one complete series of longitudinal septa plus a number of incomplete series.	Mature spores with rounded bases, apex obtusely pointed; seven transverse septa and numerous longitudinal septa rarely include an obviously complete series.

[a] summarised from Simmons's description of the two species (30).

2.1.1 INDUCTION OF SPORULATION

Stemphylium isolates obtained from diseased chrysanthemum flowers sporulated poorly on synthetic media when incubated in the dark at 25 C, thus necessitating an examination of methods to improve this so as to facilitate a specific identification of the fungus. Leach (20) reported that initiation of both the sexual and asexual stage (S. botryosum) of P. herbarum is very responsive to changes in environmental conditions, particularly light and temperature. Near-ultraviolet light (NUV) was shown to trigger the production of protopseudothecia* (22) in culture (20), whereas the conidial stage was produced sparsely or not at all in the dark but in profusion when cultures were exposed to alternating light and dark (19).

The objective of this investigation was to determine the effect of light on the production of conidia and the initiation of protopseudothecia.

(a) Effect of different light regimes on sexual (Pleospora sp.) and asexual (Stemphylium sp.) sporulation in culture.

MATERIALS AND METHODS

Cultures of a Pleospora sp. with a Stemphylium conidial stage were obtained by inoculating oxoid potato dextrose agar (PDA_o) plates in the centre with a 7 mm diameter mycelial plug and

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* Protopseudothecia - immature ascostromatic ascocarps; become pseudothecia when bitunicate asci form in locule(s) dissolved in the stroma.

then exposing these to the following conditions for 13 days:

(i) fluorescent light - provided by a 40 watt Phillips coolwhite fluorescent tube No. 33. Petri plates were placed at a distance of 41 cm from the tube. Temperature in the light-box ranged from 23 to 27 C;

(ii) near-ultraviolet light - provided by a 40 watt Phillips BLB, having a maximum wavelength of 350 nm. Petri plates were placed at a distance of 41 cm from the tube. Temperature ranged from 23 to 27 C;

(iii) dark at 25 $\pm$ 1 C;

(iv) daylight (cultures on laboratory windowsill). Temperature ranged from 4.6 to 25.2 C.

Three isolates, designated S_1, S_3, and S_4 were used in the study with each isolate being replicated four times per light treatment. At the conclusion of the experiment concentration of conidia was determined using a haemocytometer after first flooding cultures with 10 ml of water to obtain a spore suspension. Protopseudothecia production was assessed and rated on the basis of an arbitary scale of 0 - 5.

RESULTS AND DISCUSSION

The effect of the four light treatments are presented in Table 6. It is evident from the results that isolates varied in their response to the different light regimes.

TABLE 6. Effect of different light regimes on sexual (Pleospora sp.) and asexual (Stemphylium sp.) sporulation in PDA_o cultures, after 13 days

Light regime	Conidia/ml ($x10^4$)			Protopseudothecia Rating[a]		
	S_1	S_3	S_4	S_1	S_3	S_4[b]
Fluorescent light	52	180	245	3	5	5
Near-ultraviolet light	215	247	297	3	5	5
Dark	2	5	7	2	1	1
Daylight	132	70	107	2	4	4

[a] arbitary scale of 0 - 5

[b] isolate code.

Near-ultraviolet light gave the best overall result in terms of both production of conidia and protopseudothecia, although fluorescent light was also quite beneficial, particularly for conidial production of isolate S_4. The fact that all three isolates produced both protopseudothecia and conidia in continuous darkness indicates that light is not essential for the initiation of protopseudothecia or for production of conidia, although the three light regimes tested were obviously beneficial. The production of conidia by three isolates of this Stemphylium sp. in the dark is contrary to the findings of Leach (18) who reported a complete absence of conidiophores or conidia of S. botryosum under similar conditions. Although Leach concluded from his results that NUV stimulated the formation of conidiophores of S. botryosum, he went on to state that "radiation from the NUV lamps also strongly inhibits the formation of conidia at these same temperatures (above 25 C) unless NUV is followed by darkness at temperatures above 25 C, conidia will not form". Although temperatures in the fluorescent and NUV light treatments did fluctuate to 2 C above and below Leach's threshold temperature of 25 C the abundant asexual sporulation of these three *Stemphylium* isolates does not indicate a strong inhibitory effect of continuous light exposure. The results also suggest that although the conidia do bear some resemblance to those of *S. botryosum*, the isolates may in fact be a different species.

(b) Effect of duration of near-ultraviolet light on sexual (Pleospora sp.) and asexual (Stemphylium sp.) sporulation in culture.

Since earlier results indicated that NUV had the most beneficial influence on both initiation of protopseudothecia and production of conidia an experiment was conducted to determine the optimum exposure to NUV light.

MATERIALS AND METHODS

Colonies of the fungus were grown on plates of PDA_o at 23 - 27 C for three days in complete darkness and then exposed to NUV light for the following durations: 0, 0.5, 1, 3, 5, and 7 days. Following exposure to NUV light cultures were again incubated in the dark. Isolate S_3 and isolate S_4 were tested and each treatment was replicated four times. The results are presented in Table 7 and illustrated in Figure 19.

RESULTS AND DISCUSSION

The results in Table 7 indicate that both asexual sporulation and protopseudothecial production were increased as the exposure to NUV light was lengthened. The protopseudothecia were concentrated mainly in the region of new growth which occurred under NUV light (Figure 19) and any carryover was lacking although again, as in the previous experiment (Table 6), asexual sporulation and initiation of protopseudothecia occurred in continuous darkness. Although results were recorded two days earlier than in the previous experiment the number of conidia produced does not indicate that the return of cultures to the dark stimulated better sporulation than that achieved by incubating cultures in continuous NUV light, again disagreeing with the findings of Leach (18, 19) for P. herbarum.

TABLE 7. Effect of duration of near-ultraviolet light on sexual (*Pleospora* sp.) and asexual (*Stemphylium* sp.) sporulation in PDA_o cultures after 11 days.

Exposure (days)	Conidia/ml ($x10^4$) S_3	S_4	Protopseudothecia Rating[a] S_3	S_4[b]
0	5	7	1	1
0.5	10	20	2	2
1.0	25	30	2	3
3.0	32	100	3	4
5.0	77	120	4	5
7.0	147	125	5	5

[a] arbitary scale 0 - 5
[b] isolate code.

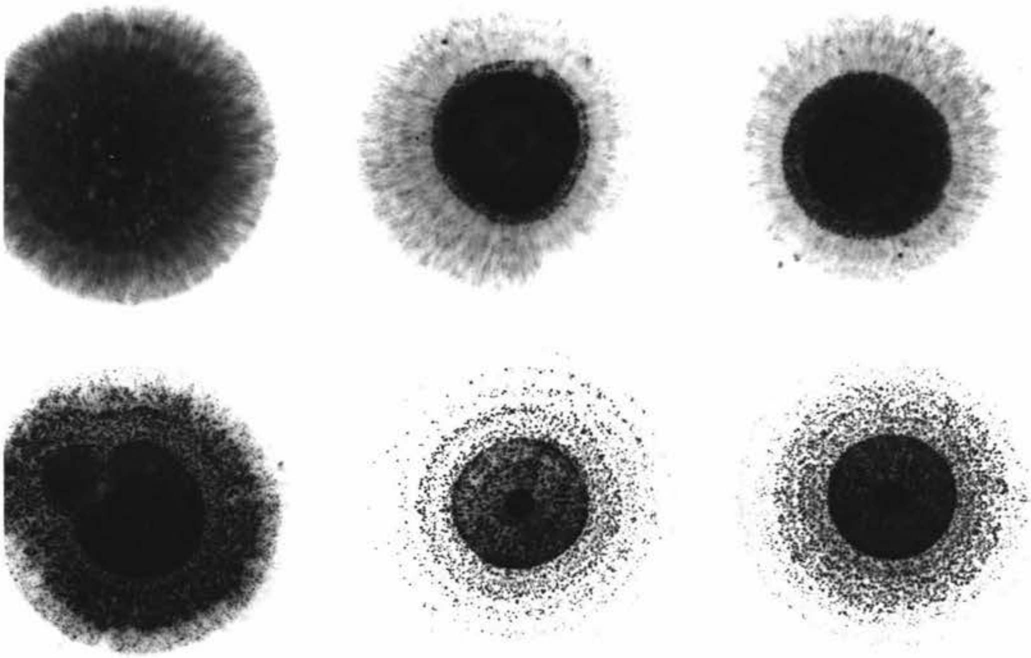

FIGURE 19: Production of Pseudothecia by Pleospora sp. (Stemphylium vesicarium) on PDA_L following different exposures to near-ultraviolet light.

Top row: (L to R) 0, 0.5, and 1 day exposure to near-ultraviolet light.

Bottom row: (L to R) 3, 5, and 7 days exposure to near-ultraviolet light.

(c) Maturation of protopseudothecia of *Pleospora* sp. in culture.

The morphological characters of the sexual stage of fungi are important for the determination of taxonomic relationships. Simmons (30) in his study of the perfect states of *Stemphylium* spp. observed that the morphology of the juvenile and mature ascospores were important in the delimitation of species, particularly the *Pleospora* state of *S. botryosum* and *S. vesicarium.* The main objective of conducting this experiment to produce the *Pleospora* state of the *Stemphylium* sp. pathogenic to chrysanthemum flowers was to facilitate a decision on its taxonomy.

Leach (20) reported that protopseudothecia of *P. herbarum* are induced by NUV light, but for them to mature the sterile pseudothecia require a long and continuous exposure to low temperatures (5 - 10 C). However, Rotem *et al.* achieved ascospore formation in *P. herbarum* by temperature alterations at bi-weekly intervals. Cultures maintained for two weeks at 10, 15, or 20 C produced ascospores when exposed for an additional 14 days to 15, 20, or 25 C respectively. Reverse temperature shifts also stimulated development of pseudothecia (29).

MATERIALS AND METHODS

For the production of mature pseudothecia two methods were investigated, namely temperature alterations at bi-weekly intervals, and exposure of colonies to constant low temperatures.

In both instances laboratory potato dextrose agar (PDA_L)

plates were inoculated at the centre with 4 mm diameter mycelial plugs cut from the periphery of actively growing colonies. For the fungus to establish itself and at the same time initiate protopseudothecia the plates were incubated for five days under near-ultraviolet light at a temperature ranging from 23 - 27 C. The temperatures used for the constant temperature experiment were at 4 C intervals ranging from 8 - 28 C. In the bi-weekly temperature alteration experiment the alternating temperatures used were 20 C and 25 C. Following the initial NUV light treatment the colonies were transferred to the appropriate temperature treatments.

Colonies were examined at weekly intervals for the presence of asci and ascospores.

RESULTS AND DISCUSSION

(a) Temperature alterations at bi-weekly intervals.

Bi-weekly temperature alterations from 20 C to 25 C failed to induce the maturation of protopseudothecia and production of ascospores. Gourley (14) working with *P. herbarum* reported similar negative results.

(b) Constant temperature experiment.

The influence of temperature on the production of ascospores is presented in Table 8.

TABLE 8. Influence of incubation temperature on the production of ascospores by *Pleospora* sp.

Temperature (°C)	Weeks for ascospore production S_1	S_3	S_4 [a]
8	11	10	9
12	10	8	12
16	12	9	-
20	-	-	-
24	-	-	-
28	-	-	-

[a] isolate code.

Exposure of protopseudothecia to continuous low temperatures significantly influenced their maturation with the lowest temperatures tested (8 and 12 C) being most beneficial, while no protopseudothecia matured at 20, 24, and 28 C. The shortest period for the production of mature ascospores was eight weeks which is considerably longer than the 24 days reported by Leach for *P. herbarum* (20). However it is within the range reported by Simmons (30) for *S. vesicarium* (3-6 months) and *S. botryosum* (2-12 months).

The slowness with which the *Pleospora* sp. from chrysanthemum flowers produce pseudothecia may well account for the failure of any reports of perfect states being associated with *Stemphylium* spp. on chrysanthemum flowers.

2.1.2 EFFECT OF TEMPERATURE ON CONIDIAL MORPHOLOGY

The effect of incubation temperature on spore morphology has been demonstrated by various workers (6, 24, 36, 38). Leach and Aragaki (21) observed that when Stemphylium floridanum was grown at temperatures ranging from 11.5 - 31.6 C the conidia produced at the higher temperatures were less divided, strongly pigmented and in many respects similar to spores of S. botryosum, whilst at lower temperatures the spores were increasingly more divided, longer, less pigmented and less verrucose.

MATERIALS AND METHODS

To determine whether the Stemphylium isolates from diseased chrysanthemum flowers exhibited such a variation in conidial morphology the fungus was cultured on 20% V-8 juice agar and incubated in the dark at the following temperatures: 8, 16, 20, 26, and 30 C. Isolate S_3 and isolate S_4 were used and each isolate was replicated four times at each temperature.

RESULTS AND DISCUSSION

The effect of different incubation temperatures on laboratory cultures of Stemphylium sp. from chrysanthemum flowers is clearly revealed in Table 9 and Figure 20. The increase in length/width ratio at higher temperatures is somewhat surprising considering the effect of different temperatures on S. floridanum

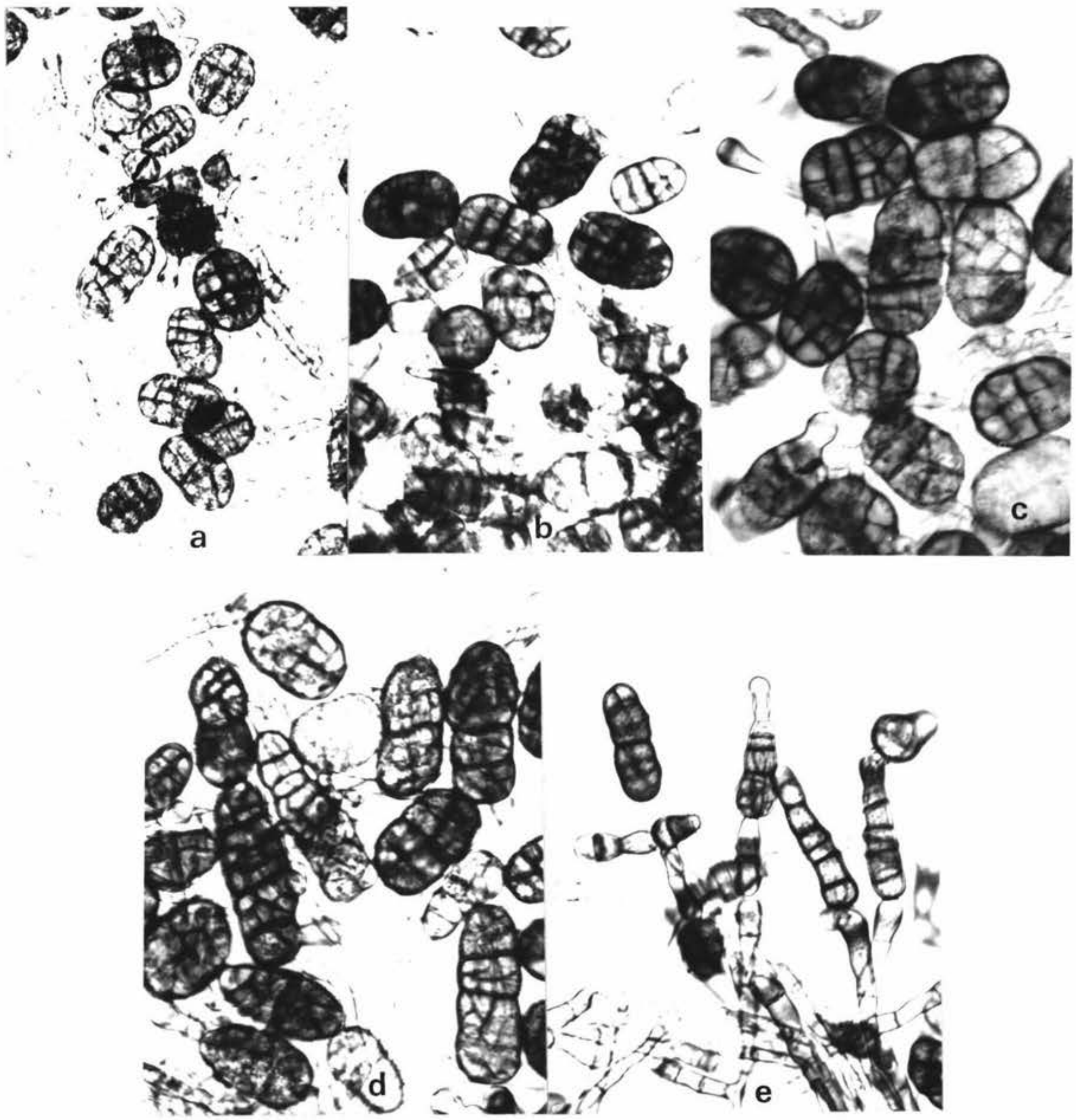

FIGURE 20: Effect of incubation temperature on conidial morphology of Pleospora sp. (Stemphylium vesicarium).
(a) spore formed at 8C; (b) spores formed at 12C;
(c) spores formed at 20C; (d) spores formed at 26C;
(e) spores formed at 30C.

TABLE 9. The effect of incubation temperature on conidial morphology of *Pleospora* sp. (*Stemphylium* sp.) isolate S_3 from diseased chrysanthemum flowers

Conidial characters	Incubation temperature (°C)				
	8	16	20	26	30
Size (average	32.4 X 24.5 μ	35.0 X 22.9 μ	35.0 X 21.9 μ	41.1 X 17.3 μ	39.3 X 12.9 μ
Length to width ratio	1.32 to 1	1.52 to 1	1.63 to 1	2.38 to 1	3.04 to 1
Shape					
(a) Young conidium	Spherical	Spherical	Spherical to oblong	Oblong	Oblong
(b) Mature conidium	Spherical to oval	Spherical, oval or oblong	Oblong to oval	Oblong to oval	Long, cylindrical and 'alternaria-like'
Colour	Golden-yellow to light brown	Light to dark brown	Dark brown	Olive-brown to greyish-brown	Olive-brown to greyish-brown
Constrictions	1 median	Mainly 1 median also 2 & 3	1,2,3 in equal proportions	Mainly 3; also 1 & 2	Mainly 3 or more; also 1 & 2
Epispore sculpture	Echinulate	Echinulate	Echinulate	Echinulate to verrucose	Echinulate to verrucose

and Ulocladium chartarum (Pr.) Simmons where Leach and Aragaki (21) found conidia produced at higher temperatures tended to be more compact. At 30 C the chrysanthemum isolate not only had a high length/width ratio but was also less divided, a temperature response similar to that reported for S. floridanum and U. chartarum.

Simmons (30) reported that S. botryosum has a length/width ratio approaching 1.0 whereas that of S. vesicarium is 2.0 or more, although he does not state at what temperature conidia were produced to obtain these figures.

The results obtained in this study in terms of length/width ratios, the oblong shape of the immature conidia and the several (often three) transverse septal constrictions (refer Table 9 and Figure 20) are more typical of S. vesicarium than S. botryosum (sensu Simmons, 1969). However it must be emphasised that conidia only had these features when cultures were incubated at 20, 26 and 30 C. Furthermore the results of this and the other studies referred to here clearly reveal the importance of making field collections at intervals during the year to determine the variability of an organism before making any taxonomic decisions. If this is not possible then certainly laboratory studies examining the effects of such factors as light and temperature on variability of the fungus are essential. All too often reports on the morphology of a fungus do not precisely state the cultural environment used during the study.

2.1.3 CONIDIUM ONTOGENY

Simmons (30) reported that the juvenile conidia of *S. vesicarium* are generally oblong, in contrast to those of *S. botryosum* which tend to be spherical. Consequently, to obtain further information on the identity of *Stemphylium* isolates from chrysanthemum flowers, experiments were conducted on the development of conidia.

MATERIALS AND METHODS

A technique similar to that described by Riddell (27) was used. A one centimeter square block of 20% V-8 juice agar was cut and transferred to a flamed microscope slide (Figure 21a) where it was inoculated at the centre of each edge with spores of *Stemphylium* sp., isolate S_3 (Figure 21b). A flamed cover slip was centrally placed upon the agar block and the slide transferred to a petri plate lined with moist blotting paper to maintain high humidity (Figure 21c). Plates were exposed to continuous NUV light at 25 - 27 C. After seven days incubation the mycelium of the fungus covered both upper and lower surfaces of the agar block and was adhering to the respective glass surfaces. The agar block was discarded, a drop of Shear's mounting fluid placed on the slide and the cover slip carefully lowered upon the slide (Figure 21d). The prepared slide was then examined with the aid of a compound microscope.

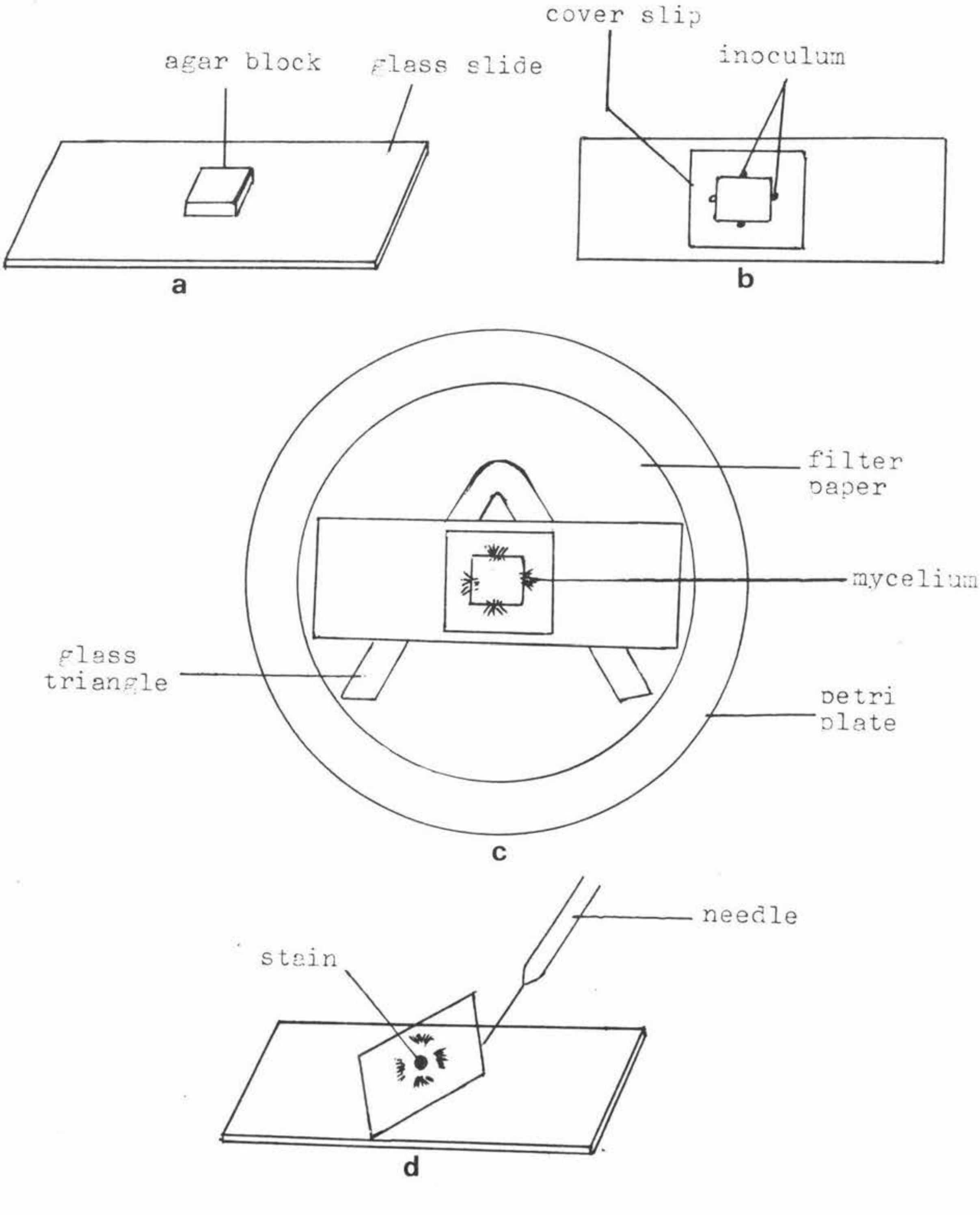

FIGURE 21. Technique for preparation of slide cultures.

RESULTS AND DISCUSSION

A single conidium was produced as a protoplasmic outgrowth through a large, discrete pore at the tip of the bulbous apical cell of the conidiophore (Figure 22a). Very young conidia are initially globose, aseptate and hyaline (Figures 22b and c) but as size increases (mainly lengthwise) they become oval even before the first septum is formed (Figures 22d and e). The first septum is laid transversely along the conidium dividing it into two approximately equal halves (Figure 22f). Subsequent transverse and longitudinal septa are laid down giving the conidium a muriform appearance typical of the genus Stemphylium (Figure 22g). At maturity conidia are oval to ovoid, olive-brown to olive-grey in colour, with 1-4 transverse septa and a number of longitudinal septa (Figure 22h). The majority of conidia observed had three transverse septal constrictions, although conidia with one and two constrictions were also observed. There was seldom a complete series of longitudinal septa from apex to base of the conidium.

After a conidium has fully matured there is renewed growth of the conidiophore through the terminal pore in a manner typical of the porosporaceae (16). The previously formed conidium is either dislodged or becomes lateral in position and another conidium is formed at the end of the newly proliferated conidiophore. In this way a number of apical condiophore proliferations may be formed, producing a monilioid effect.

Germination by repetition was also fairly common, being observed on both culture media and host material. Here conidia produced a short tube, stouter and darker than a normal germ tube.

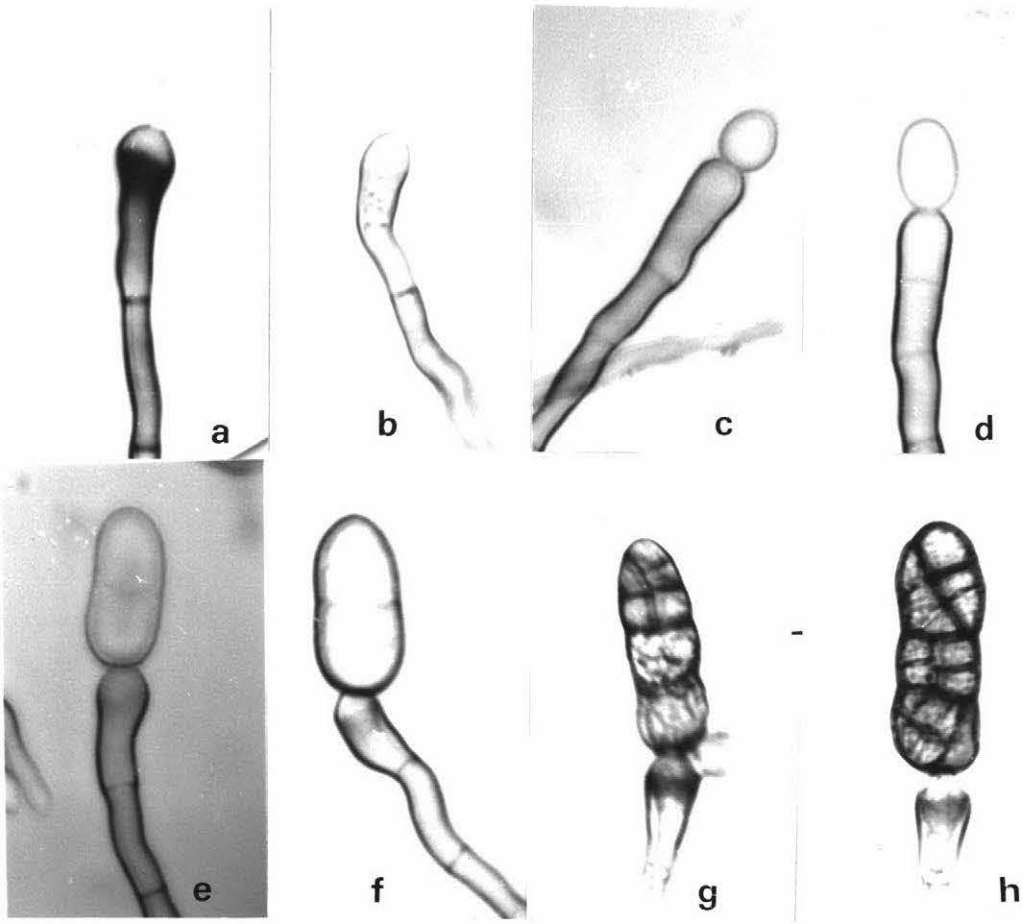

FIGURE 22: Conidium ontogeny of Pleospora sp. (Stemphylium vesicarium).

This in turn was quickly terminated by a second conidium (Figure 23).

Cultural studies on S. botryosum isolated from lucerne (P.D. Whitwell,* personal communication) indicated that conidia of this fungus virtually maintained a compact globose shape throughout their development whereas the conidial ontogeny of the Stemphylium isolate from chrysanthemums is more characteristic of that illustrated by Simmons for S. vesicarium (30).

2.1.4 MYCOLOGY

Unless otherwise stated the morphological features of Pleospora - Stemphylium isolates from chrysanthemum flowers are based on cultures grown on PDA_L and incubated at 25 C in the dark.

Colony	Greyish-green with abundant aerial mycelium.
Mycelium	Olivaceous-green, septate, vacuolate and branched.
Conidiophores	Olive-green to olive-brown and septate; apical cell of conidiophores swollen, bulbous and generally darker and rougher than the remaining body of the conidiophore; distinct pore present on the apical cell through which the conidium is blown out; conidiophores proliferated in close succession.
Conidia	Hyaline to golden yellow when young but olive-brown to greyish when mature;

* Masterate student; Department of Plant Pathology, Massey University, Palmerston North, New Zealand.

FIGURE 23: Production of a secondary conidium by Pleospora sp. (Stemphylium vesicarium) on PDA_L.

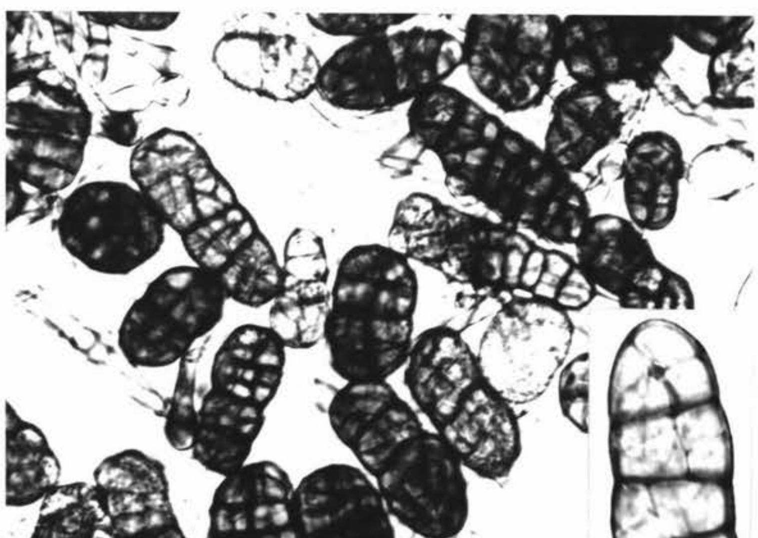

FIGURE 24: Conidia of Pleospora sp. (Stemphylium vesicarium) produced on V-8 juice agar cultures incubated for 11 days in the dark at 26C. Insert. Enlargement of a conidium showing 3 distinct transverse septal constrictions.

echinulate to verrucose; oblong to oval, muriform, base of conidium normally bluntly rounded; 1 - 6 transverse septa and a number of longitudinal septa but seldom with a complete series; 1 - 3, more commonly 3 constrictions at the transverse septa; when one-constricted, constriction is median, conspicuous and dark; basal pore conspicuous and dark; germination by repetition fairly common in culture and on the host; 30.8 - 49.6 x 15-21.2 μ (average 21.2 x 15 μ). (Figure 24).

<u>Pseudothecia</u>* Brownish-black to black; initially spherical or globose, at maturity flask-shaped with an ostiole & neck; submerged or erumpent in the medium; diameter 450 - 890 μ (average 674 μ). (Figure 25).

<u>Asci</u> Bitunicate; hyaline; cylindrical to clavate; tapering to a basal claw-like structure; 128 - 252.8 x 24.4 - 33.2 μ (average 198.7 x 31.6 μ). (Figure 27).

<u>Ascospores</u> Hyaline to lemon-yellow when young, golden-yellow or brownish-grey when fully mature; walls smooth; shape variable although predominantly ellipsoidal; apex of ascospores slightly pointed or rounded, base generally bluntly rounded; greatest width of ascospore one-third distance from apex; predominantly 7 transverse septa although 8 and 6 have also been noticed; numerous longitudinal septa but seldom any complete series; 28 - 45.8 x 12 - 18.4 μ (average 37 x 16 μ). (Figure 26).

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* Produced on PDA_L after 10 weeks incubation in the dark at 12 C.

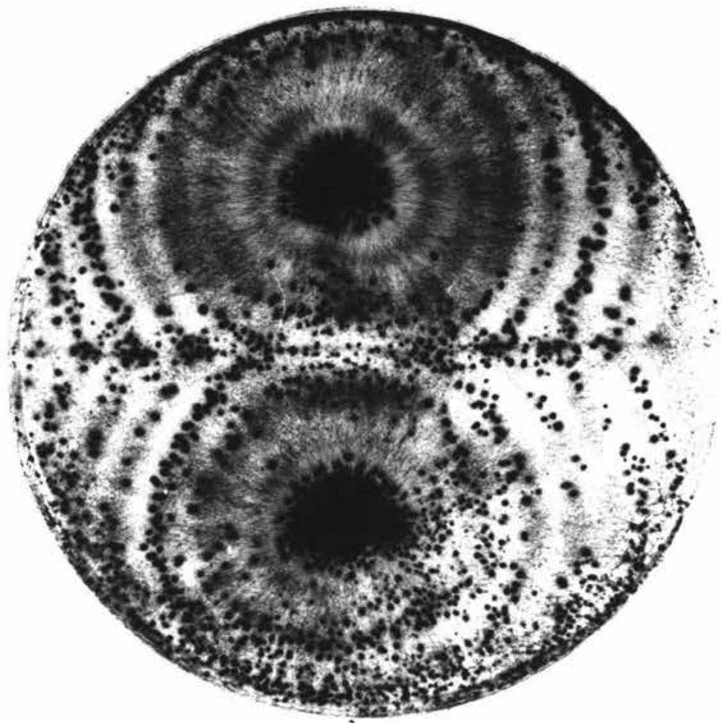

FIGURE 25: Protopseudothecia of Pleospora sp. (Stemphylium vesicarium) produced on PDA_L cultures incubated for 14 days under near-ultraviolet light.

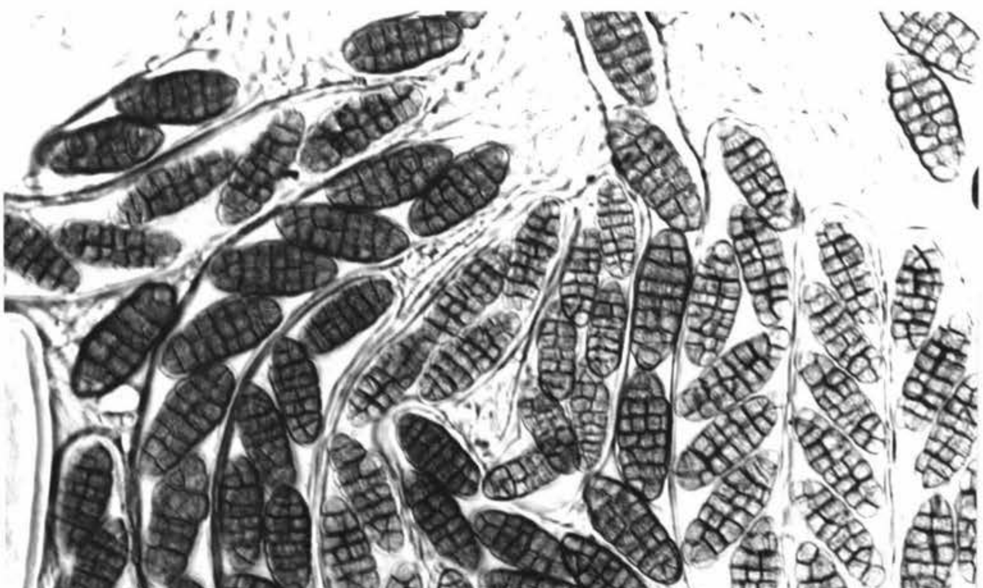

FIGURE 26: Asci and ascospores of Pleospora sp. (Stemphylium vesicarium) produced on PDA_L cultures incubated for 10 weeks in the dark at 12C.

FIGURE 27: Bitunicate ascus containing ascospores of *Pleospora* sp. (*Stemphylium vesicarium*). Produced on PDA_L cultures incubated for 10 weeks in the dark at 12C.

2.1.5 GENERAL CONCLUSION

On the basis of conidial development and the morphology of asexual and sexual stages of *Stemphylium* isolates from chrysanthemum flowers it is concluded that this organism is *Stemphylium vesicarium* (Wallr.) Simmons.

Because no decision has been made on a specific epithet for the perfect state of *S. vesicarium* (30) the imperfect name is used in this chapter after *Pleospora* sp. to designate the particular *Pleospora* in question.

2.2 MYCOSPHAERELLA LIGULICOLA (IMPERFECT STATE ASCOCHYTA CHRYSANTHEMI)

The first report of this fungus causing a flower blight disease of chrysanthemums was by Stevens in 1906 (33). Since then the fungus has been reported from most countries where the crop is grown. In New Zealand it was first reported from nurseries in Palmerston North and Auckland, although only the imperfect state was described (11).

Preliminary studies with *M. ligulicola* indicated that the conidia produced in culture differed in size and septation from those produced on the host tissue. Pycnidial production *in vitro* was erratic, with some isolates producing pycnidia and pycnidiospores abundantly whilst others only did so when exposed to daylight. It was further observed that pseudothecia of *M.*

ligulicola could be readily located on diseased chrysanthemums in the field but did not occur under normal laboratory conditions when culturing the fungus on synthetic media. A literature review indicated that the environment had a marked influence on reproductive development and growth of the fungus in the field and in culture (6, 24).

Accordingly, experiments were conducted to study the effect of media, light and temperature on the cultural and morphological characteristics of New Zealand isolates of M. ligulicola.

2.2.1 EFFECT OF MEDIA ON GROWTH AND SPORULATION

Hadley and Blakeman (15) observed that the pycnidia and pycnidiospores formed by M. ligulicola per unit area of culture were influenced by the type of medium and the nutrient level, with pycnidiospores smaller on media of low nutrient status. Stevens (33) reported cowpea agar as the best medium for growth and sporulation of the fungus.

MATERIALS AND METHODS

Mycelial plugs 4 mm in diameter obtained from the edges of actively growing colonies were used for inoculating the petri plates. The media used were laboratory potato dextrose agar (PDA_L), Difco prune agar (PrA), pea agar (PA), V-8 juice agar

(V-8 agar), malt agar (MA), nutrient agar (NA), oatmeal agar (OMA), cornmeal agar (CMA), water agar (WA), chrysanthemum leaf extract agar (CLA), and chrysanthemum petal extract agar (CPA). Details regarding the recipes and preparation are presented in Appendix I. Isolate M_1, isolate M_2, and isolate M_3 of the fungus were used in the study and each isolate was replicated four times on each medium. Following inoculation the plates were incubated at 25 C in the dark. To harvest the pycnidiospores from each medium the surface of two cultures of each isolate was scraped in a known volume of water and the resultant slurry left for 20 - 30 minutes to facilitate pycnidiospore discharge. The concentration of the resultant pycnidiospore suspension was estimated with a haemocytometer. It was observed that using this method only pycnidiospores from the erumpent or superficial pycnidia could be harvested, and that it was not possible to obtain the pycnidiospores from deeply submerged pycnidia.

RESULTS AND DISCUSSION

The influence of media on the growth and cultural characteristics of M. ligulicola are presented in Table 10a, 10b and 10c and illustrated in Figures 28, 29 and 30.

From Table 10a, 10b and 10c, and Figures 28 and 29 it is apparent that isolates behaved differently on the various media. The degree of variation between isolate M_1 and isolate M_2 was small and insignificant. In contrast and as discussed below isolate M_3 showed considerable variations from isolate M_1 and isolate M_2.

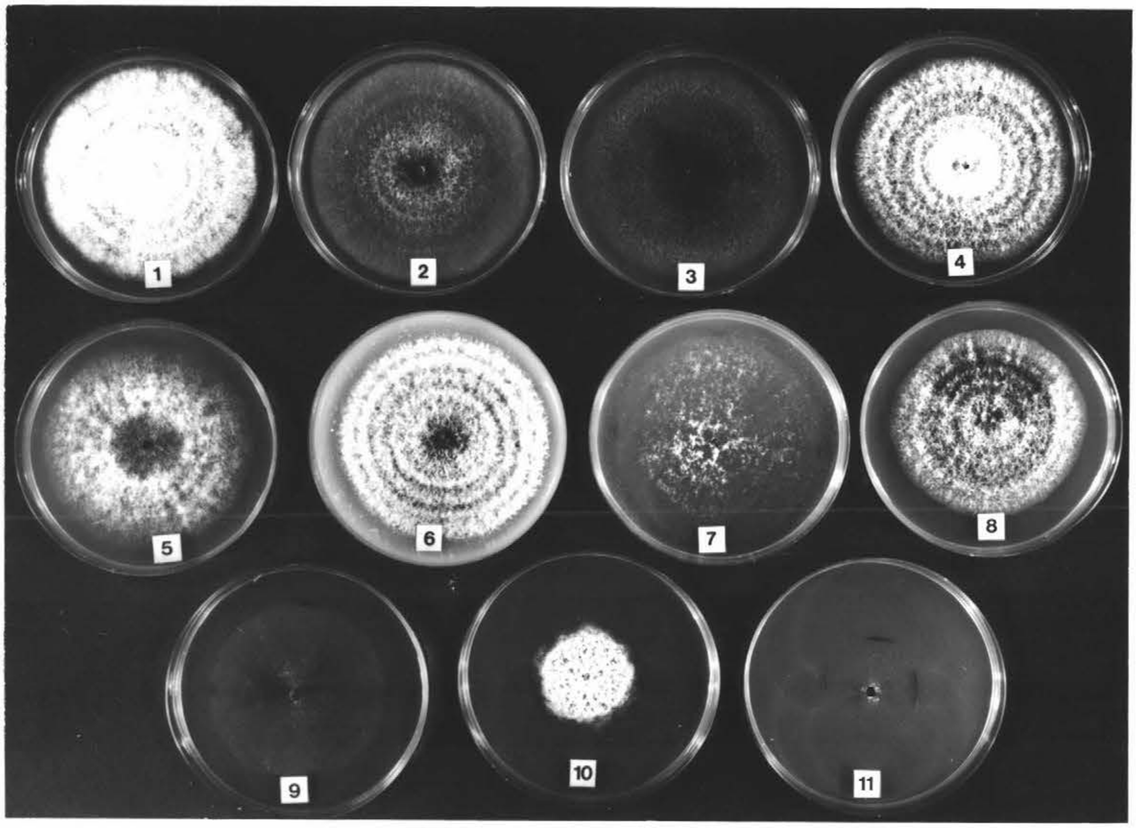

FIGURE 28: The effect of media on the growth of *Mycosphaerella ligulicola* after 7 days incubation at 24C. (1) pea agar; (2) V-8 juice agar; (3) chrysanthemum leaf extract agar; (4) laboratory potato dextrose agar; (5) chrysanthemum petal extract agar; (6) oatmeal agar; (7) cornmeal agar; (8) malt agar; (9) prune agar; (10) nutrient agar; and (11) water agar.

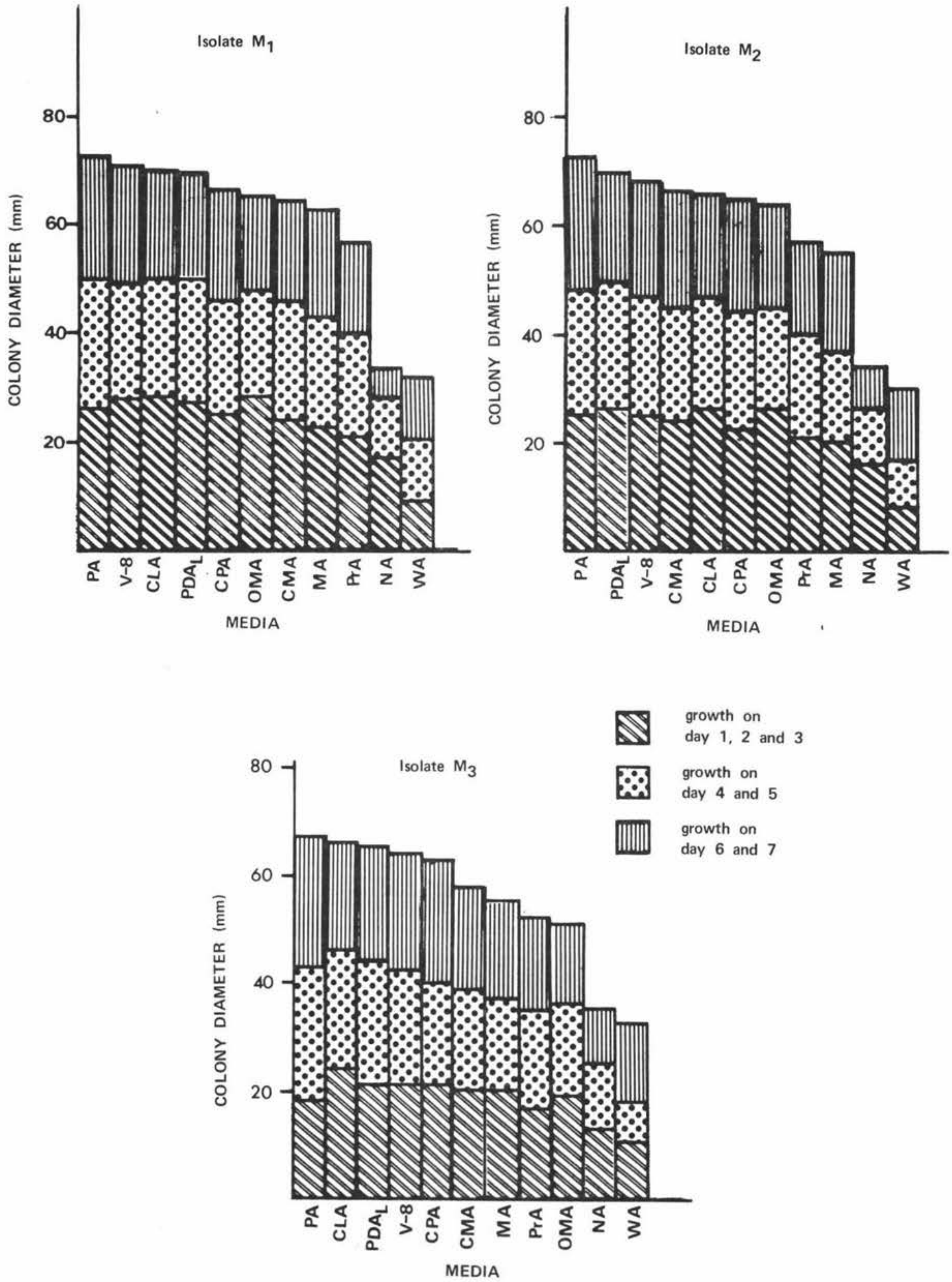

FIGURE 24 The effect of media type on the growth rate of Mycosphaerella ligulicola at 25C

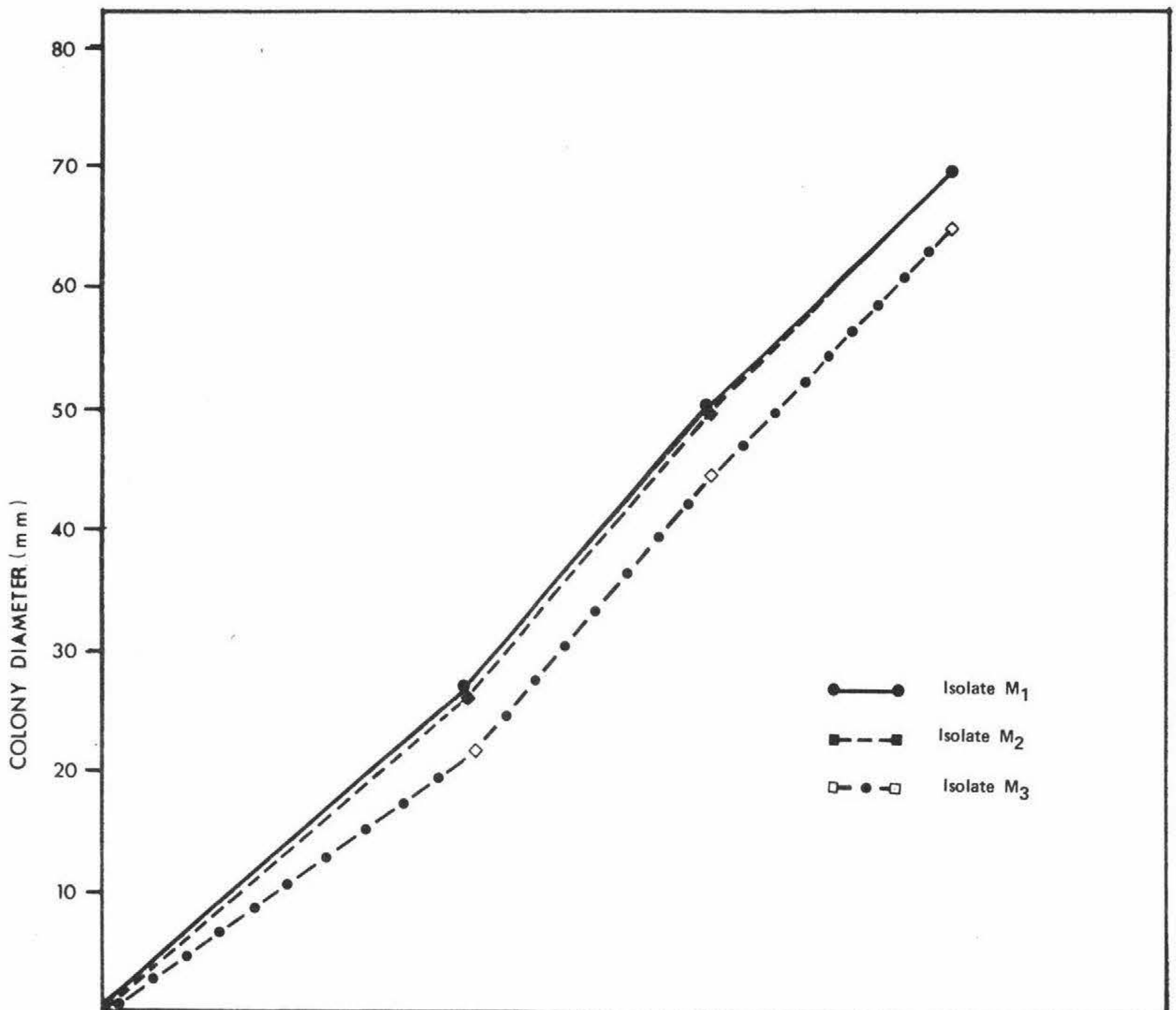

FIGURE 30 Growth rate of Mycosphaerella ligulicola on laboratory potato dextrose agar at 25C.

Colony colour varied with the medium and to some extent with the isolates. On most media the colonies comprised three distinct regions, namely fringe, intermediate and central. In most instances the fringe was either translucent or white, whereas intermediate and central regions varied in colour depending on media used. Colonies were predominantly circular in outline and zone formation was a characteristic feature of all three isolates on PDA_L, PA, OMA, and V-8 juice agar. The radial growth (Figure 29) of the three isolates was maximum on PA and least on WA. On PDA_L the growth rate of isolate M_3 was less than that of the other two isolates (Figure 30).

The aerial mycelium formed by the fungus was cottony or filamentous, and white in colour. It was absent on WA and most prevalent on PA. On all media tested isolate M_3 formed less aerial mycelium than isolate M_1 or isolate M_2.

Pycnidial production was most abundant on PA, V-8 juice agar, PDA_L and OMA. Pycnidia were generally submerged or erumpent although in a few cases they were even superficial. Isolate M_3 produced more pycnidia per plate than the other two isolates. The concentration of harvestable pycnidiospores was related to the pycnidial concentration and their horizontal distribution in the medium. With all three isolates the highest concentration of pycnidiospores per ml was obtained from PA. Here again isolates varied with isolate M_3 producing the largest number of pycnidiospores per ml.

On all media where sporulation occurred the pycnidiospores were predominantly aseptate confirming the observations of

TABLE 10 a. Effect of media type on the growth and cultural characteristics of *Mycosphaerella ligulicola* isolate M_1 at 25 C[a]

	PDA_L	MA	PrA	CMA	OMA	V-8	CLA	CPA	NA	WA	PA
COLONY COLOUR											
Fringe	W	T/W	T	T	CW	T	T	T	T	T	CW
Middle	OG	Gn/Gr	T	T	Gn/Gr	LBn	Gn/Gr	W	W	T	WGr
Centre	GrW	Gn/Gr	T	T/W	LBn	LBn	LBn	Gn/Gr	W	T	CW
SURFACE TOPOGRAPHY											
Shape	Cr	Cr	Cr	Cr	Cr	Cr	Cr	Cr	Cr	Ir	Cr
Elevation	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat
Zonation	Z	NZ	NZ	NZ	Z	Z/NZ	NZ	NZ	NZ	NZ	Z
AERIAL MYCELIUM											
Quantity	3	3	0-1	1	4	2-3	0-1	2	2	0	4
Location	G	G	G	C+M	C+M	C+M	G	G	C+M	-	G
Morphology	Cot	Cot	Fil	Fil	Cot	Cot/Fil	Cot/Fil	Cot	Cot	-	Cot
Colour	W	GrW	W	W	W	W	WGr	W/WGr	W	-	W
PYCNIDIA											
Quantity	2	1	1	1-2	2-3	1-2	0-1	0	0	0	4
Location	Er/Sub	Sub	Sub/Er	Sub	Sub	Sub/Er	Sub	-	-	-	Er/Sup
Colour	LBA/DBn	Bn/Bl	RBn/Bl	LBn/Bn	Bn/DBn	RBn/Bn	Bn/Bl	-	-	-	RBn
PYCNIDIOSPORES											
Conc/ml*	1480	15	65	100	80	100	0	-	-	-	406320
% septate	3.4	2.5	1.2	1.7	1.8	3.1	-	-	-	-	2
Colour	Hy	Hy	Hy	Hy	Hy	Hy	-	-	-	-	Hy
PSEUDOTHECIA	-	-	-	-	-	-	-	-	-	-	-

TABLE 10 b. Effect of media type on the growth and cultural characteristics of *Mycosphaerella ligulicola* isolate M_2 at 25 C[a]

	PDA_L	MA	PrA	CMA	OMA	V-8	CLA	CPA	NA	WA	PA
COLONY COLOUR											
Fringe	T/W	T	T	T	W	T	T	T/W	T	T	W
Middle	Gn/Gr	Gn/Gr	T	T	GrW	LBn	LBn	LBn	T	T	W
Centre	GrW	W	T	T	W	LBn/W	T/LBn	T/W	T	T	W
SURFACE TOPOGRAPHY											
Shape	Cr	Cr	Cr	Cr	Cr	Cr	Cr	Cr	Cr	Ir	Cr
Elevation	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat
Zonation	Z	NZ	NZ	NZ	Z/NZ	Z	NZ	Z	Z	NZ	Z
AERIAL MYCELIUM											
Quantity	3	2-3	0-1	0-1	4	2-3	1	2	2	0	4
Location	G	C+M	G	G	G	C+M	G	G	C+M	-	G
Morphology	Cot	Cot	Fil	Fil	Cot	Cot/Fil	Cot/Fil	Cot	Cot	-	Cot
Colour	W	W	W	W	W	W	W	W	W	-	W
PYCNIDIA											
Quantity	2	2	0	1-2	3-4	2	0-1	0	0	0	4
Location	Er/Sub	Er/Sup	-	Sub	Sub/Er	Sub/Er	Sub	-	-	-	Er
Colour	LBn/DBn	Bn/Bl	-	LBn/Bn	RBn/Bn	RBn/Bn	DBn/Bl	-	-	-	RBn/DBn
PYCNIDIOSPORES											
Conc/ml*	2120	105	-	45	510	25	0	-	-	-	216720
% septate	2.8	3.4	-	1.4	1.4	2	-	-	-	-	2
Colour	Hy	Hy	-	Hy	Hy	Hy	-	-	-	-	Hy
PSEUDOTHECIA	-	-	-	-	-	-	-	-	-	-	-

TABLE 10 c. Effect of media type on the growth and cultural characteristics of *Mycosphaerella ligulicola* isolate M_3 at 25 C [a]

	PDA_L	MA	PrA	CMA	OMA	V-8	CLA	CPA	NA	WA	PA
COLONY COLOUR											
Fringe	T	T	T	T	T/Gr	T	T/LBn	T	T	T	T/LBn
Middle	Gr	T/Gr	T	T	W/Gr	LBn/Gr	Bn	T/W	W	T	W/Gr
Centre	W/Gr	Gr	T	T/W	W/Gr	W/Bn	LBn	T/W	W	T	W
SURFACE TOPOGRAPHY											
Shape	Cr	Cr	Cr	Cr	Cr	Cr	Cr	Cr	Cr/Ir	Ir	Cr
Elevation	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat	Flat
Zonation	Z	Z	NZ	NZ	Z/NZ	Z	Z/NZ	NZ	Z/NZ	NZ	Z
AERIAL MYCELIUM											
Quantity	2	0-1	0-1	1	2	1	1	1	1-2	0	3
Location	C+M	C+M	G	C+M	C+M	C	C	C+M	C+M	-	C+M
Morphology	Cot	Fil	Fil	Fil	Cot	Cot	Cot/Fil	Cot	Cot	-	Cot
Colour	W/Gr	Gr	W	W/GrW	W	W	W	W	W	-	W
PYCNIDIA											
Quantity	3-4	3	1	1	3	1	0-1	1	0-1	0	4
Location	Sup/Er	Sup/Er	Sub	Sub/Er	Sub/Er	Sub/Er	Sub	Sub	Sup/Er	-	Er
Colour	Bn/Bl	RBn/Bn	RBn/Bn	DBn/Bl	RBn/Bn	DBn	DBn	Bn	LBn	-	Bn/Bl
PYCNIDIOSPORES											
Conc/ml*	4840	8950	3	55	9120	980	1.6	4	15	-	780000
% septate	1.03	0.8	1.2	1.7	0	2	5.8	0.8	1.6	-	
Colour	Hy	Hy	Hy	Hy	Hy	Hy	Hy	Hy	Hy	-	
PSEUDOTHECIA	-	-	-	-	-	-	-	-	-	-	-

[a] Key for Tables 10 a, 10 b and 10 c.

Colour

W = white
T = translucent
Bn = brown
Bl = black
OG = olive green
RB = reddish brown
Gn = green
Gr = grey
CW = cottony white
LBn = light brown
GrW = greyish white
RBn = reddish brown
DBn = dark brown
Hy = hyaline
WGr = whitish green

Quantity

Arbitary rating of 0 - 4

Shape

Cr = circular
Ir = irregular

Location

G = general
C = centre
M = middle
Er = erumpent
Sub = submerged
Sup = superficial

Zonation

Z = zoned
NZ = not zoned

Morphology

Cot = cottony
Fil = filamentous

* $X10^4$.

McCoy _et al._ (24) who reported that at temperatures greater than 24C only one-celled pycnidiospores were produced regardless of whether shredded chrysanthemum tissue or synthetic media were used for culturing the fungus. In the present study no pseudothecia developed on any medium under the incubation conditions used for this experiment. This also is in accordance with the observations of McCoy _et al._ (24) who did not obtain pseudothecial development at 25 C indicating some factor other than nutrition could be critical for the production of the perfect state.

2.2.2 EFFECT OF TEMPERATURE ON SPORULATION AND PYCNIDIOSPORE MORPHOLOGY

McCoy _et al._ (24), and Blakeman and Hadley (6) reported that asexual sporulation and pycnidiospore morphology of _M. ligulicola_ was affected by the incubation temperature. They observed that pycnidiospore size and percentage septate spores decreased with an increase in temperature, whereas the number of pycnidiospores produced increased with temperature. In another test using shredded chrysanthemum tissue as a natural substrate McCoy _et al._ found that the optimum temperature for pseudothecial development was 20 C and that no pseudothecia developed at 25 C. Barr (4) obtained mature pseudothecia of _Mycosphaerella tassiana_ (de Not.) Johans. by growing the fungus at 5 C for 6 weeks and then exposing cultures to room temperature for another two weeks.

Accordingly an experiment was conducted to study the effect of temperature on sexual and asexual reproduction and pycnidiospore morphology of _M. ligulicola_ in laboratory cultures.

MATERIALS AND METHODS

Cultures of the fungus were grown on 20% V-8 juice agar. Petri plates were inoculated with a 4 mm diameter mycelial plug and incubated in the dark at temperatures ranging from 4 - 32 C at 4 C intervals. Isolate M_2 and M_3 were used in the study and each isolate was replicated six times for each temperature. Half the plates at 4 C and 8 C were transferred to room temperature after 6 weeks incubation. A rating of the number of pycnidia was made after 21 days using a 0 - 5 arbitary scale. Observations for presence of pseudothecia were made at weekly intervals for 10 weeks.

RESULTS AND DISCUSSION

The effect of temperature on pycnidial production is presented in Table 11. This table indicates that no pycnidia were produced at 32 C or below 20 C. Pycnidiospores from cultures incubated at 20, 24 and 28 C were invariably aseptate. Although it has been reported that the percentage septate pycnidiospores produced by *M. ligulicola* increases with a decrease in temperature (6, 24), this phenomenon could not be investigated because of the lack of sporulation at the lower temperatures tested.

The optimum temperature for asexual sporulation of *M. ligulicola* was 24 C which was also the optimum temperature for growth. No pseudothecia were produced with any of the treatments.

TABLE 11. Effect of incubation temperature on pycnidial production by *Mycosphaerella ligulicola* on V-8 juice agar after 21 days.

Temperature (^{o}C)	Pycnidial Rating[a] M_1	M_2[b]
4	-	-
8	-	-
12	-	-
16	-	-
20	1	2
24	3	4
28	2	3
32	-	-

[a] arbitary scale of 0 - 5
[b] isolate code.

2.2.3 EFFECT OF LIGHT ON SEXUAL AND ASEXUAL SPORULATION

McCoy *et al.* (24) suggested that *M. ligulicola* may contain at least two light response groups. One group requires light for either sexual or asexual reproduction and the other group has no light requirements. He observed that the development of pycnidia was enhanced by near-ultraviolet light (280 to 380 nm) only with those isolates that were intrinsically sensitive to light stimulation. He obtained pseudothecia on shredded chrysanthemum tissue when incubated at 20 C under blue or green filters at intensities of 425 to 500 $\mu w/cm^2$ and 1400 to 15,000 $\mu w/cm^2$. However cultures under red or white light produced pseudothecia only at the higher light intensity. Blakeman and

Hadley (6) reported that pycnidial production by M. ligulicola was little influenced by illumination.

MATERIALS AND METHODS

To determine the effect of light on sporulation of M. ligulicola 20% V-8 juice agar plates were inoculated with a 4 mm diameter mycelial plug of the fungus and exposed to the following light regimes:

(i) fluorescent light - provided by a 40 watt Phillips cool white fluorescent tube No.33. Petri plates were placed at a distance of 41 cm from the tube. Temperature in the light-box ranged from 23 to 27 C;

(ii) near-ultraviolet light - provided by a 40 watt Phillips BLB, having a maximum wavelength of 350 nm. Petri plates were placed at a distance of 41 cm from the tube. Temperature ranged from 23 to 27 C;

(iii) dark - petri plates were placed in a light proof box and incubated in the fluorescent light-box in order to maintain similar temperatures to those in treatments 1 & 2;

(iv) daylight - petri plates were placed in a basket and suspended outside the laboratory window where temperatures ranged from 3.4 to 25.2 C.

Isolates M_1, M_2 and M_3 were used in the study and each isolate was replicated 6 times for each light regime.

RESULTS AND DISCUSSION

The effect of the different light regimes on pycnidial production are presented in Table 12.

TABLE 12. Pycnidial production by *Mycosphaerella ligulicola* on 20% V-8 juice agar after 12 days incubation in different light regimes.

Isolate	Dark	NUV	Fluorescent	Daylight
	Pycnidial Rating[a]			
M_1	1	3	4	2
M_2	2	5	5	4
M_3	2	5	5	4

[a] arbitary scale of 0 - 5

It is apparent from Table 12 that the intensity of pycnidial production was increased by exposing cultures to light, although some pycnidia were formed in total darkness. Exposure of cultures of *M. ligulicola* to continuous NUV or fluorescent light appeared the superior means of inducing abundant sporulation. With all the light regimes tested the pycnidiospores were aseptate. Pseudothecia were only formed in cultures exposed to

NUV light. They were initiated on the seventh day and by the tenth day they were mature.

2.2.4 EFFECT OF DURATION OF NEAR-ULTRAVIOLET LIGHT ON PSEUDOTHECIAL PRODUCTION

Since the earlier results indicated that only NUV light initiated the production of pseudothecia in culture an experiment was conducted to determine the minimum and/or optimum exposure to NUV light for pseudothecial production.

MATERIALS AND METHODS

Colonies of the fungus were grown on 20% V-8 juice agar for 7 days in complete darkness at 23 - 27 C and then exposed to NUV light for the following durations: 0, 1, 3, 5, 7, 10, and 14 days. Following exposure to NUV light the cultures were again incubated in the dark. Isolate M_1 and M_2 were used in the experiment and each isolate was replicated six times. The number of pseudothecia produced per plate were determined by counting the pseudothecia present in an area circumscribed by a 30° angle and then using this to estimate the total production per plate. The results are presented in Table 13.

RESULTS AND DISCUSSION

It is evident from the results in Table 13 that the minimum exposure to continuous NUV light necessary to induce pseudothecia of M. ligulicola was 3 days. Thereafter an extension of the NUV exposure time resulted in a progressive increase in the production of pseudothecia with maximum numbers being produced with the longest exposure tested, namely 14 days at which time they were mature.

TABLE 13. Effect of duration of near-ultraviolet light on pseudothecial production by Mycosphaerella ligulicola on V-8 juice agar

Exposure (days)	Number of pseudothecia per plate Isolate M_1	Isolate M_2
0	0	0
1	0	0
3	6	10
5	20	24
7	128	120
10	308	340
14	584	1368

2.2.5 MYCOLOGY

CULTURE

Unless otherwise stated the morphological features of M. ligulicola are described for cultures on PDA_L incubated at 25 C in the dark.

Colony Circular, flat, greenish-grey, abundant aerial mycelium; formation of annular growth zones characteristic of growth (Figure 31).

Mycelium Branched, septate and coarse.

Pycnidia Globose when young; flask-shaped at maturity with a distinct ostiole and neck; submerged, erumpent or superficial; brown or amber when young, at maturity shades of brown; ostiole darker than body; diameter 74 - 182 μ (average 128 μ).

Pycnidiospores Released as an ooze; guttulate or vacuolate; individually hyaline to greenish but *en masse* pinkish; oval to cylindrical, sides straight or slightly curved, ends bluntly rounded; predominantly aseptate, a few uniseptate; biguttulate or bivacuolate pycnidiospores appear uniseptate; accentuated by lactophenol acid fuchsin stain; 7.5 - 13.4 x 2.8 - 5.2 μ (average 11.2 x 4.3 μ).(Figure 32).

Pseudothecia* Globose to flask-shaped; superficial or erumpent; brownish-black to black; well defined neck at maturity; 87 - 158 μ in diameter (average 124 μ).

Asci Hyaline to greenish; fasciculate; bitunicate; clavate, ovoid-oblong or cylindrical with a basal-hook; 8 ascospores per ascus; asci embedded in interthecial tissue; 48 - 70 x 7 - 11 μ (average 57 x 10 μ).(Figure 34).

Ascospores Hyaline to greenish; fusiform or spindle-shaped and uniseptate; septal constriction prominent; upper cell slightly swollen immediately above

---

* Produced on V-8 juice agar after 5 days exposure to NUV light at 23 - 27 C.

septum, lower cell narrower; pointed at the upper end, bluntly rounded at base; 13.2 - 17.5 x 4.1 - 6.1 µ (average 14.9 x 5.1 µ). (Figure 34).

HOST

Pycnidia — Abundant on infected stems and flowers; formed below epidermis, later erumpent; diameter 80 - 194 µ (average 131.7 µ). (Figure 12).

Pycnidiospores — Either aseptate or uniseptate; when uniseptate, septum central or off centre; not normally constricted at septum, constriction develops with germination; 8.1 - 14.6 x 3.1 - 6.6 µ (average 11.5 x 4.7 µ). (Figure 33).

Pseudothecia — On stems, leaves and flowers; formed below epidermis, later erumpent; neck not pronounced; 92 - 182 µ in diameter (average 132 µ). (Figures 13 & 14).

Asci — 47.4 - 71.6 x 8.4 - 12.1 µ (average 60 x 10.4 µ); other characteristics as in culture. (Figure 35).

Ascospores — 13.5 - 18.9 x 4.4 - 6.4 µ (average 15.8 x 5.3 µ); other characteristics as in culture. (Figure 35).

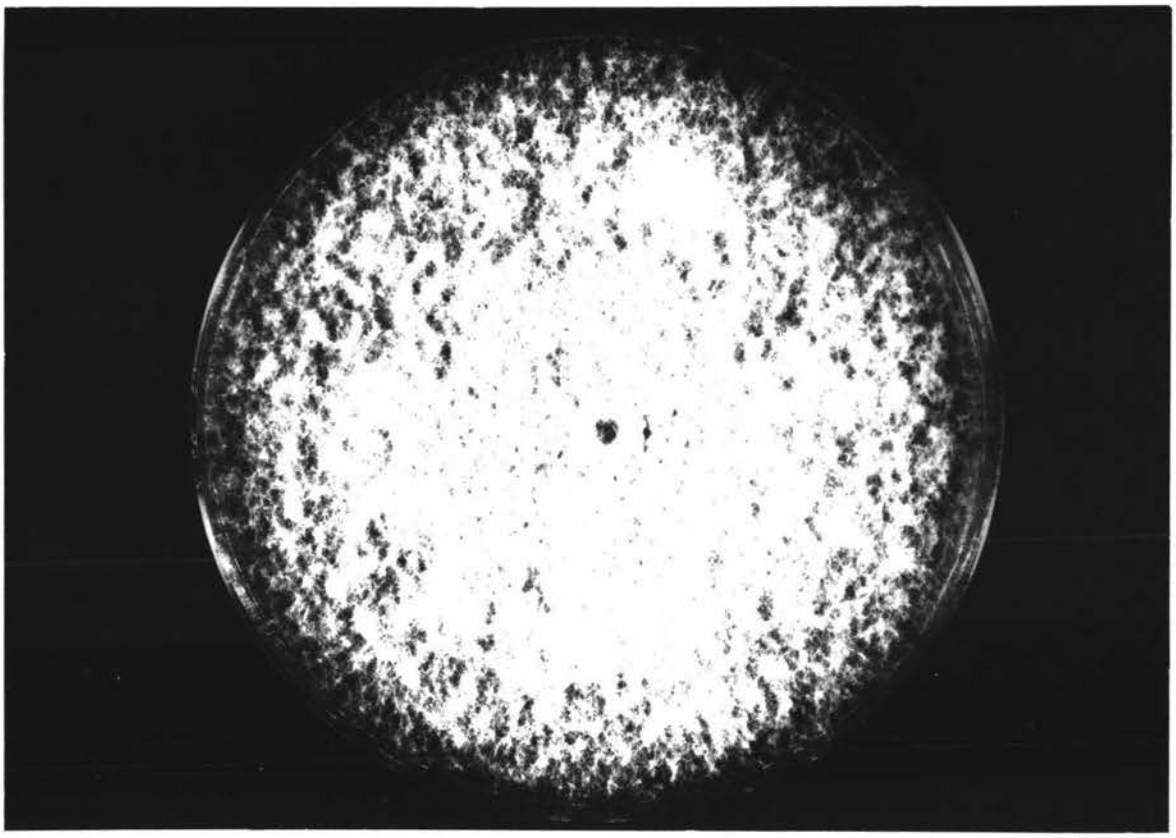

FIGURE 31: Colony of *Mycosphaerella ligulicola* on PDA_L after 12 days incubation in the dark at 24C.

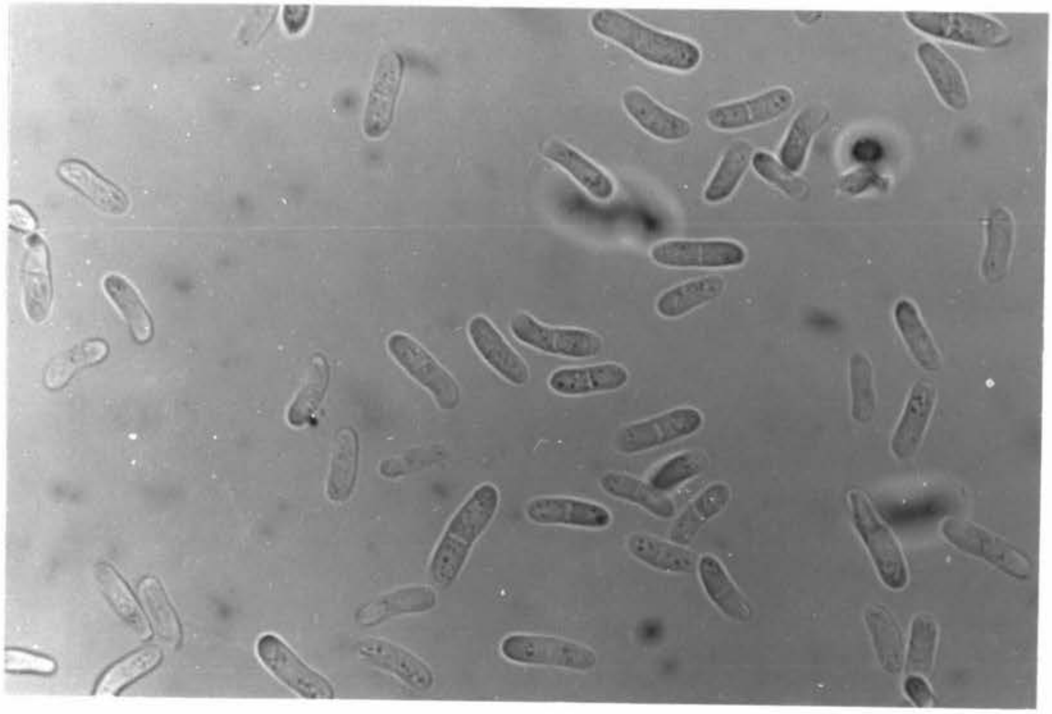

FIGURE 32: Pycnidiospores of Mycosphaerella ligulicola in Shear's mounting fluid. (ex naturally infected chrysanthemum flower).

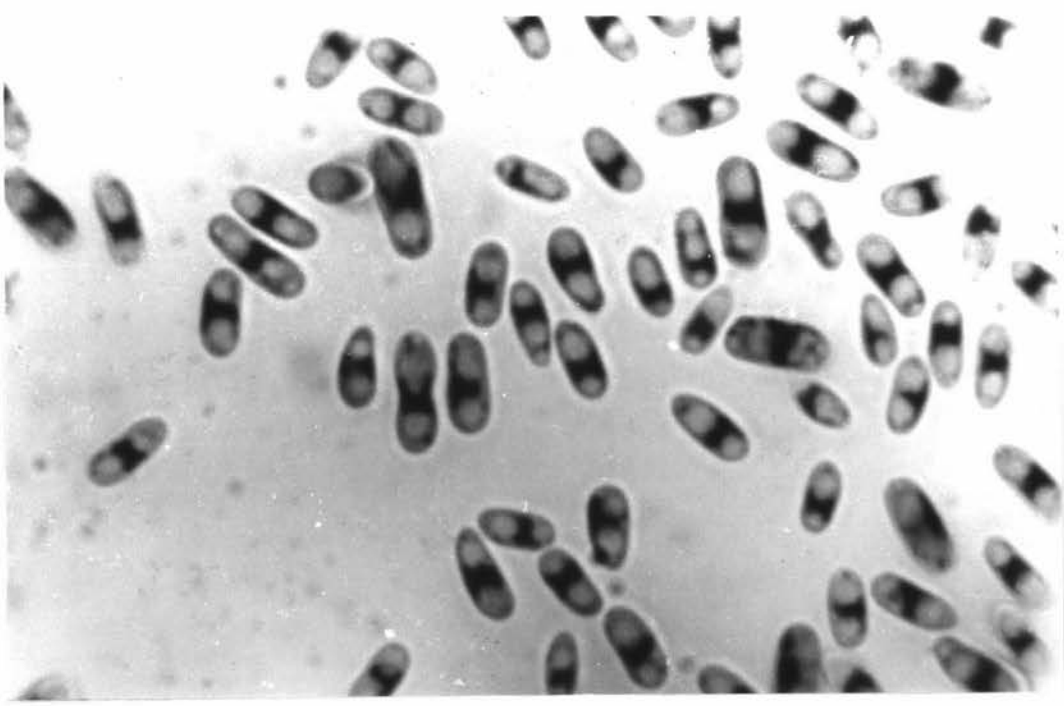

FIGURE 33: Pycnidiospores of Mycosphaerella ligulicola in lactophenol acid fuchsin (ex PDA$_L$ cultures after 21 days incubation in the dark at 25C). Note the illusion of a septum in each pycnidiospore.

FIGURE 34: Asci and ascospores of Mycosphaerella ligulicola on 20% V-8 juice agar.

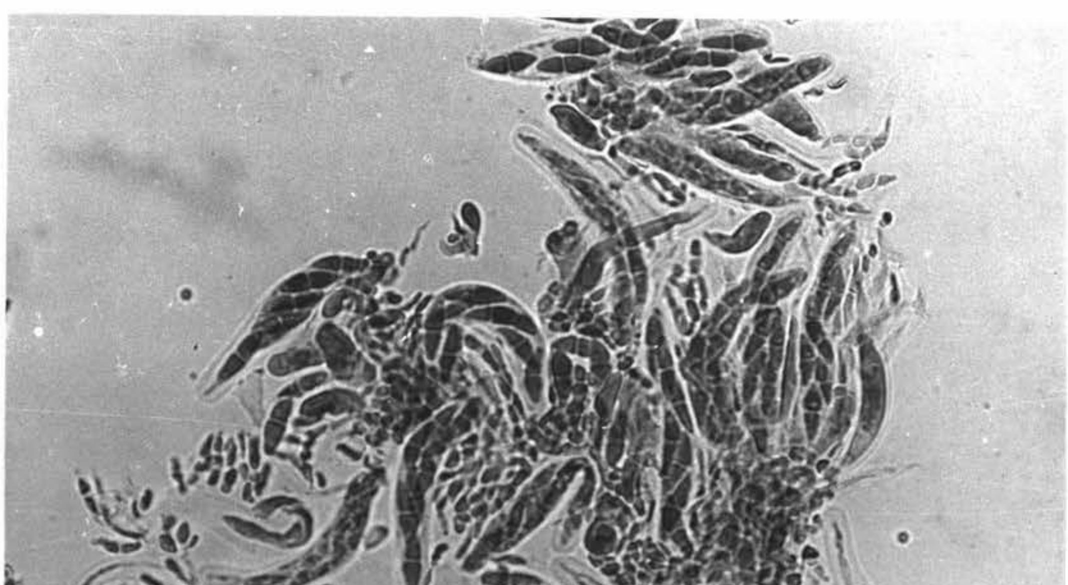

FIGURE 35: Asci and ascospores of Mycosphaerella ligulicola from pseudothecia on chrysanthemum stem.

TABLE 14. Characteristics of pycnidia and pseudothecia of *Mycosphaerella ligulicola* on culture media

Characters	Pycnidia	Psuedothecia
Colour	Amber to dark brown, rarely black	Brownish-black when young, black at maturity
Shape	Ampulliform	Obpyriform
Size (diameter)	74 - 182 μ (average 128 μ)	87 - 158 μ (average 124 μ)
Neck	Short	Long

Table 14 summarises the distinguishing features of pycnidia and pseudothecia on culture media. Pseudothecia are mainly confined to the margins of colonies and as they mature a clear drop of liquid appears in the ostiole. With the discharge of ascospores this droplet assumes a cloudy appearance thus providing a good indication of pseudothecial maturity.

2.2.6 GENERAL CONCLUSION

From the cultural and morphological studies it is concluded that the fungus conforms to the description of *M. ligulicola* by Baker *et al.* (2). Although the pycnidiospores produced in culture were predominantly aseptate there is ample evidence to indicate that the presence or absence of a septum is highly variable being influenced by cultural conditions (6, 24). Furthermore, in this study a large proportion of pycnidiospores from the host were uniseptate and typical of *Ascochyta chrysanthemi*. As reported by Baker *et al*. (2) the pseudothecia of this fungus are atypical of *Mycosphaerella* in that the pseudoparenchymatous mass of tissue present in the centre of a developing pseudothecium is only partially destroyed as the asci grow up into it and a large proportion remains as interthecial tissue in the mature pseudothecium. This type of ascocarp development has also been reported by Jenkins (17) for *Mycosphaerella berkeleyii* W.A. Jenkins.

The results of the experiments involving exposure of V-8 juice agar cultures of *M. ligulicola* to continuous NUV light provide a simple, rapid means of producing the sexual stage of this fungus in the laboratory. According to the author the discovery of pseudothecia of *M. ligulicola* on diseased chrysanthemum stems, leaves and flowers in the field is the first report of the occurrence of this stage in New Zealand.

2.3 ITERSONILIA PERPLEXANS

The genus *Itersonilia* was erected in 1948 by Derx (9) with *Itersonilia perplexans* Derx as the type species. He obtained the fungus by attaching a leaf of *Althaea rosea* Cav. bearing numerous sori of *Puccinia malvacearum* Mont. to the lid of a petri plate containing malt agar. Although he had observed this Basidiomycete earlier in 1925 it was not officially described by him until 1948. Derx believed that the same fungus was observed by Stempell (cited from Derx) in 1936, who mistook it for a stage in the development of Entyloma calendulae (Oud.) de Bary which he called "Myzel II".

Nyland (25) in 1949, while studying some unusual Heterobasidiomycetes from Washington State described a new species of Itersonilia from a dead leaf of Acer macrophyllum Pursh. This species, which he named Itersonilia pyriformis Nyland, differed from I. perplexans in that the chlamydospores (sporogenous cells) had no clamp connections associated with them, produced abundant aerial mycelium on malt agar and the ballistospores were slightly longer than those reported for I. perplexans.

An Itersonilia species causing root canker of parsnips was reported by Wilkinson in 1952 (37). Sowell and Korf (32) studied the dicaryophase and monocaryophase of the parsnip isolates and identified them as I. perplexans. Channon (8) however, although agreeing with Sowell and Korf's description of the fungus, considered it sufficiently different from the type species Itersonilia perplexans Derx to warrant a new specific name Itersonilia pastinaceae Channon. This fungus consistently produced abundant chlamydospores and was restricted to parsnips. It differed from I. pyriformis in that it produced abundant chlamydospores and had clamp connections associated with the bases of the terminal inflations.

Tubaki (cited from Sowell and Korf), and Sowell and Korf (32) considered I. pyriformis as a synonym of I. perplexans. Similarly Olive (26) believed that I. pyriformis was either closely related to I. perplexans or was a variant of it.

In 1955 Robertson (28) reported that a flower blight or 'scorch' of chrysanthemum had been present in Britain for many years. He observed that the fungus produced 'sporidia' and suggested it resembled E. calendulae. This is the first report of a Basidiomycete causing such a condition in chrysanthemums.

In the United States of America similar symptoms and associated mycelium and spores typical of Itersonilia were reported by Dosdall (12) in 1956 and he concluded in this case that the fungus was I. perplexans. Dosdall also concluded, on the basis of symptoms, sporidia measurements and description of mycelium, that Robertson was actually dealing with I. perplexans rather than E. calendulae.

Smith et al. (31) suggested that symptoms of I. perplexans were often confused with those produced by B. cinerea with the latter fungus often masking symptoms of I. perplexans (28). This could possibly account for the infrequency with which this fungus has been reported on chrysanthemums in New Zealand.

2.3.1 MYCOLOGY

In the present study I. perplexans was isolated from diseased chrysanthemum flowers by tissue plating. On PDA_L the fungus was slow-growing, forming a circular colony with feathery margins (Figure 36). Initially colonies were white but with age attained a slight tint of tan. The mycelium which was largely submerged in the agar was hyaline, septate and branched, and had

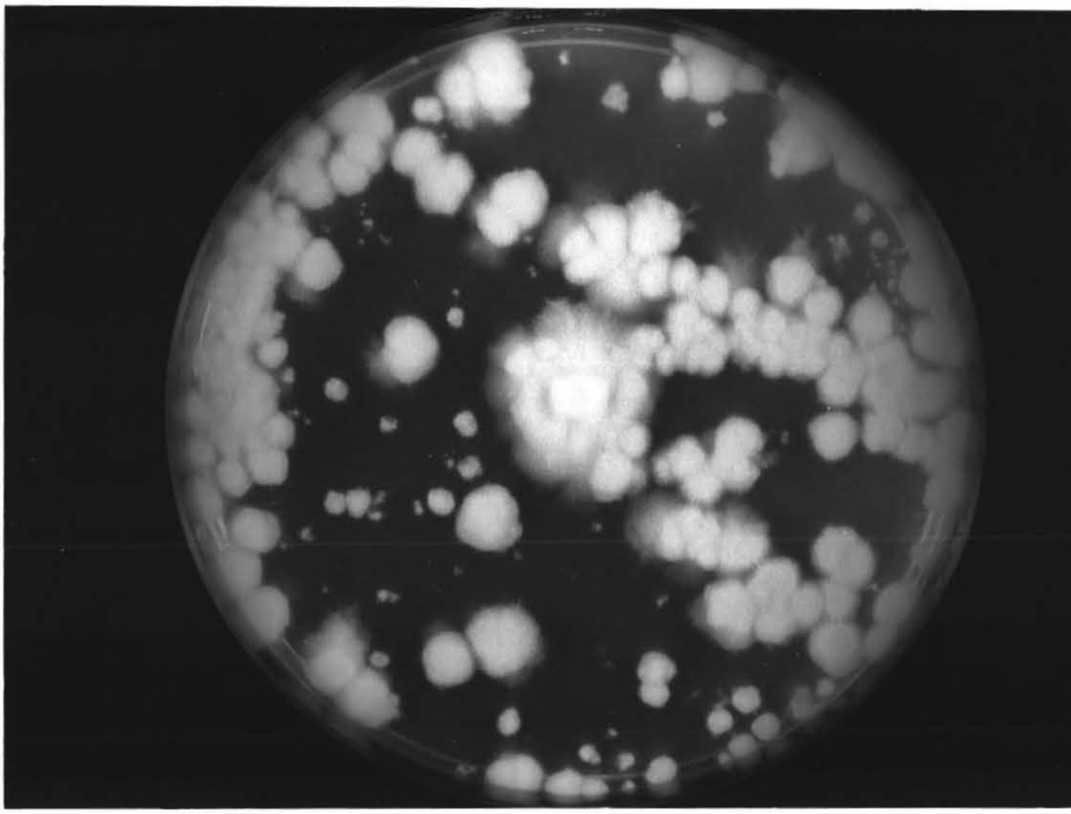

FIGURE 36: Culture of Itersonilia perplexans on PDA_L after 21 days at 24C. Note scattered colonies resulting from natural ballistospore ejection. Culture was originally inoculated in centre by mycelial plug.

clamp connections at almost every septum (Figure 37).

Ballistospores were produced singly at the ends of long, slender, apically tapered sterigmata-like structures called sporophores. The sporophores in turn arose from the sporogenous cells. The latter were inflated, had clamp connections at their bases and were either terminal or intercalary (Figure 38). Occasionally no distinct sporogenous cells were formed, but in such cases it is presumed that the terminal cells of hyphae act as sporogenous cells, at the ends of which the ballistospores form.

Ballistospores (14.4 x 9.3 μ) were hyaline, granular, semilunar, oval, spherical and at times even sickle-shaped (Figure 39). Spores devoid of cytoplasm were also observed. At maturity the ballistospores were violently discharged from the sporophores by the water drop mechanism (Figure 40). Ballistospore germination was either by the conventional method, simply involving the growth of a germ tube, or germination by repitition where a short germ tube was produced and quickly terminated by production of another ballistospore (Figure 41).

Chlamydospores, which have been reported for I. perplexans by Olive (26), were not observed in culture in this study. From the description given it is presumed that the chlamydospore-like structures reported by Dosdall (12) are actually the sporogenous cells.

Cells, which may represent conidia according to Olive (26) and Sowell and Korf (32), were obtained by flooding five day old cultures of the fungus growing on potato yeast marmite agar (PYMA). These conidia were hyaline, vacuolate and were very variable in

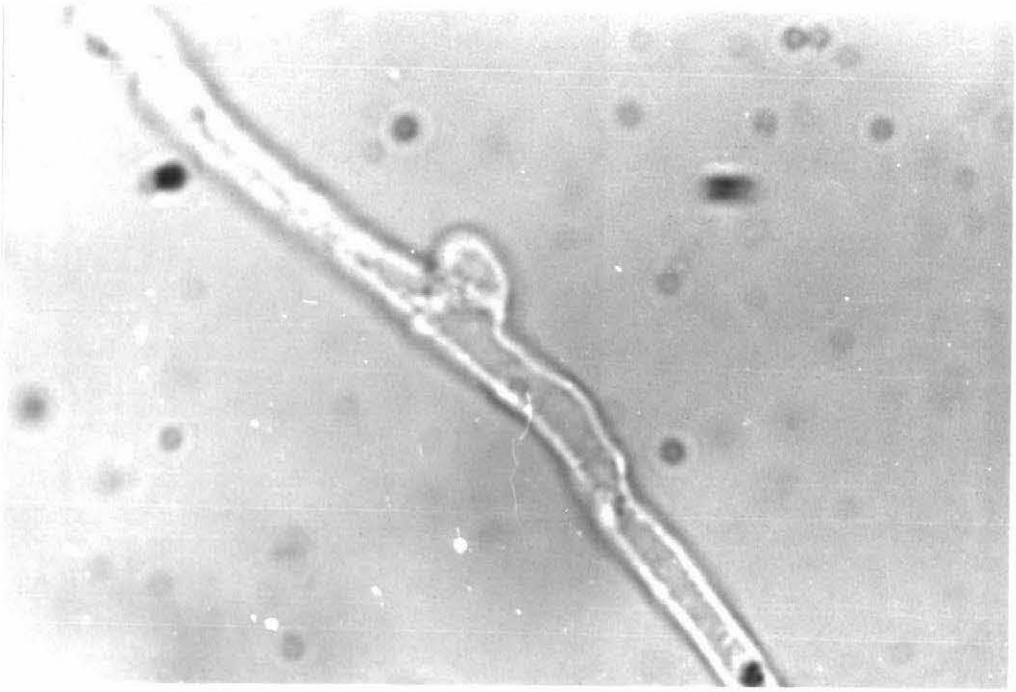

FIGURE 37: Clamp-connection of Itersonilia perplexans.

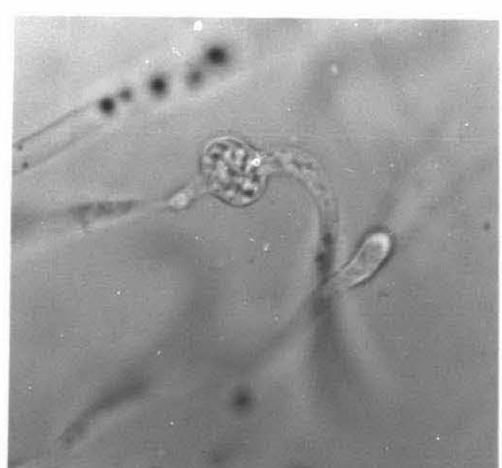

FIGURE 38: Sporogenous cell of Itersonilia perplexans on PDA_L culture.

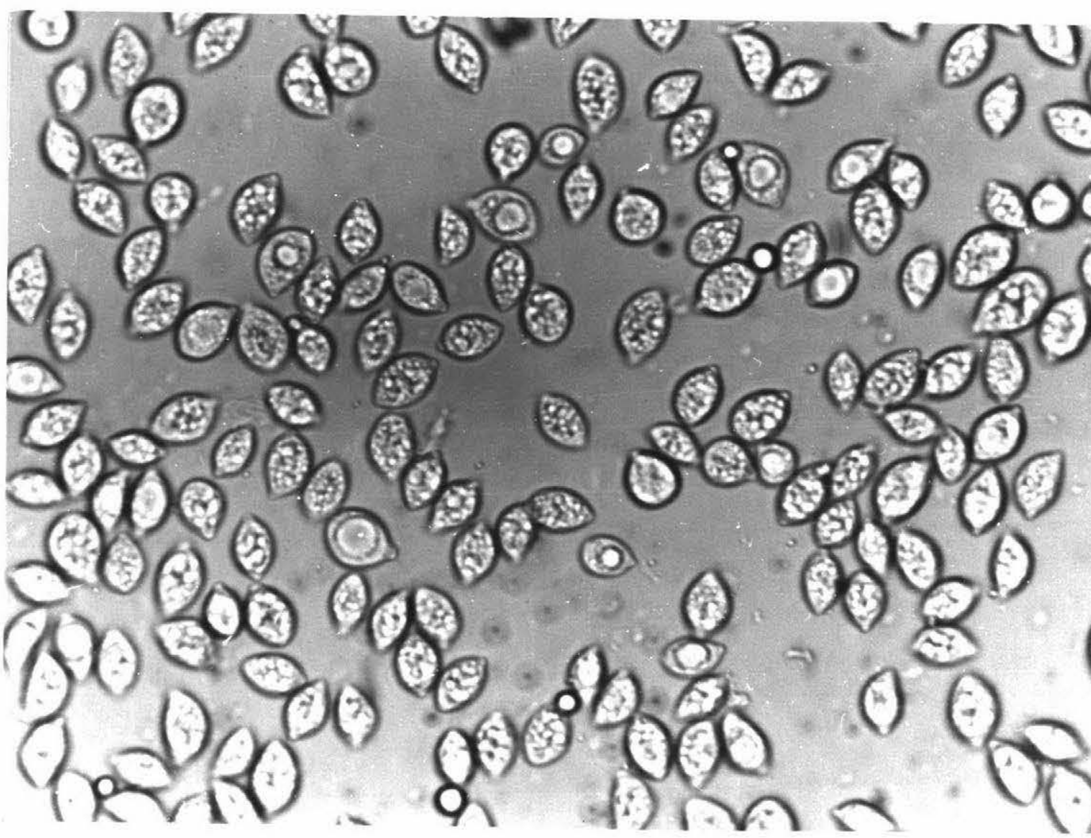

FIGURE 39: Ballistospores of Itersonilia perplexans produced on PDA_L after 10 days incubation at 20C.

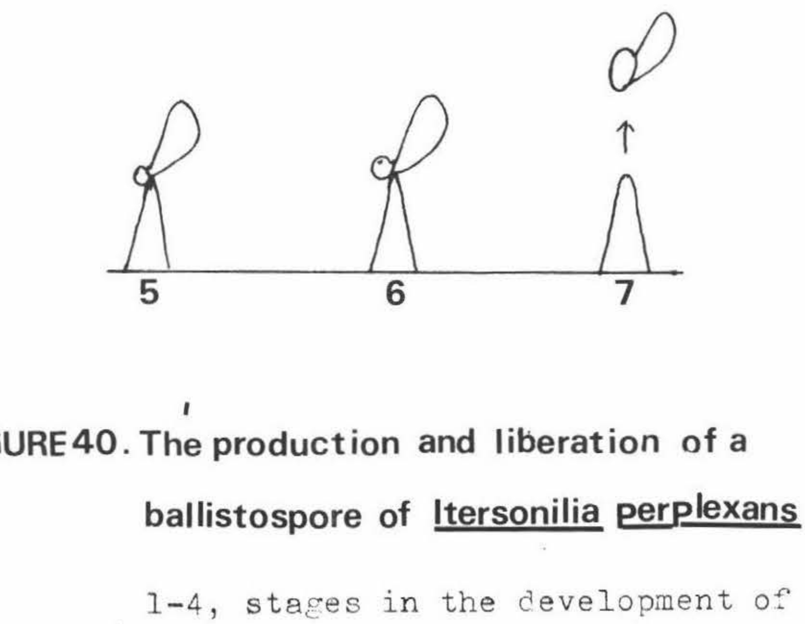

FIGURE 40. The production and liberation of a ballistospore of Itersonilia perplexans

1-4, stages in the development of the sterigma and the ballistospore

5-6, drop - excretion at the spore-hilum prior to spore discharge

7, discharge of ballistospore.

FIGURE 41 (a) Production of a secondary ballistospore by Itersonilia perplexans on PDA_L.
(b) Germination of secondary ballistospore while still attached to the primary ballistospore.

FIGURE 42: Conidia of Itersonilia perplexans from 7 day old potato yeast marmite culture.

shape often being elongate, cylindrical or even spindle shaped (Figure 42). They were formed at the tips of hyphae by budding. Unlike the ballistospores the conidia are not violently discharged. Occasionally a ballistospore produced a short germ tube at the end of which a conidium was budded off.

2.3.2 OBSERVATIONS ON THE STAGES IN THE DEVELOPMENT OF A CLAMP CONNECTION

The stages in the development of a clamp connection are photographically illustrated in Figure 32.

The clamp connection was initiated as a small protrusion or hook-like peg developing along the side of the terminal cell of the hypha (Figure 43 a). It gradually enlarged (Figure 43 b), curved backwards (Figure 43 c) and finally its free end touched the wall of the main hypha (Figure 43 d). The first septum was formed after 35 minutes at the base of the clamp connection (Figure 43 e) and the septum across the main hypha was formed 10 minutes later (Figure 43 f). The time lapse between the appearance of the clamp connection and the laying down of the septa across the main hypha was 45 minutes.

Unlike *Coprinus lagopus* Fr., and *Coprinus sterquilinus* Fr. (7) the first septum to be formed by *I. perplexans* was across the base of the clamp connection, whilst in the above two species the septum across the main hypha is laid down first.

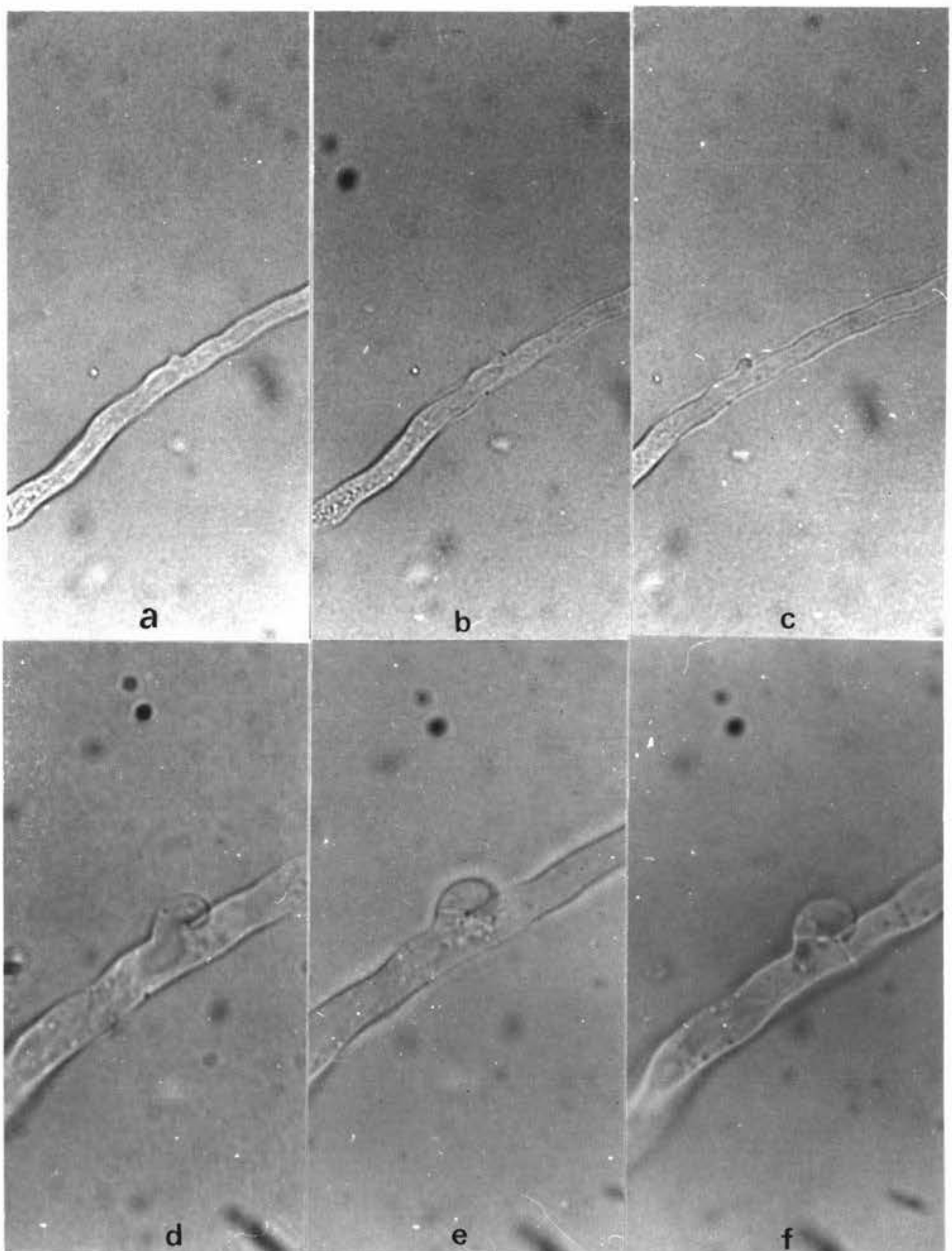

FIGURE 43: Stages in the formation of a clamp connection by Itersonilia perplexans.

2.3.3 GENERAL CONCLUSION

On the basis of the production of clamp connections and ballistospores it is concluded that this fungus is best classified in the Basidiomycetes. Furthermore, and despite the absence of chlamydospores, the author believes the specific identity of the *Itersonilia* isolates from chrysanthemum flowers is correctly *Itersonilia perplexans*. This decision is based on the morphology of the fungus from host and culture, spore dimensions and pathogenicity studies reported elsewhere.

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CHAPTER 3

CONTROL OF THE FLOWER BLIGHT FUNGAL COMPLEX OF CHRYSANTHEMUMS

The chrysanthemum flower is produced late in the life of the plant and thus represents the point at which maximum monetary investment has been made. Therefore, where the sale of flowers is the main source of income, it is imperative that all precautions be taken to preserve flower quality.

Several control measures aimed at reducing the incidence of flower blight of chrysanthemums have been reported, including the use of proper cultural practices (2, 4, 8, 23, 25, 37), resistant cultivars (15), and fungicides (17, 18, 23, 24).

By far the most popular and widely used method of controlling flower blight is the use of chemical sprays and dusts. Fungicides recommended are protectants, the presence of which either prevents the fungal spores from germinating or kills the spores upon germination.

Most investigations on chemical control of chrysanthemum flower blight have been made with control of a specific fungus in mind and on no occasion is the author aware of a report examining the effect of fungicides against the fungal complex as found in the Manawatu. Fungicides such as chlorothalonil (11, 17, 18), mancozeb (16, 17), zineb (8, 11, 18, 37), captafol (16, 17, 18), and captan (8, 17, 18, 35), which display a broad spectrum of activity, have been used extensively against the flower blight fungi. Since these protectant fungicides do not 'cure' infections they need to be applied rather frequently to maintain a protective residue on the flowers.

More recently benomyl, a fungicide which displays protective, eradicative and systemic activity has been used for control of *M. ligulicola* and *B. cinerea*. This fungicide has also been reported to be effective against verticillium and sclerotinia, diseases of chrysanthemums (6). In addition it is claimed that it displays mite ovicidal activity and activity against aphids (17).

Because, at least five taxonomically diverse fungi can be involved in the chrysanthemum flower blight complex in the Manawatu and cause symptoms which are indistinguishable from each other, a fungicide or fungicide mixture must have a broad spectrum of activity to be effective. Accordingly a variety of individual fungicide and fungicide combinations were tested.

3.1 LABORATORY SCREENING OF FUNGICIDES AGAINST THE CHRYSANTHEMUM FLOWER BLIGHT FUNGI

Several tests have been developed to evaluate the efficiency of fungicides in the laboratory. The literature concerning *in vitro* testing of fungicides has been reviewed by McCallan *et al.* (28), Torgeson (39) and more recently by Neely (30). Generally such tests measure either fungicide effect on spore germination or mycelial growth, the latter often being achieved by the poison food or poison plate method.

In vitro testing of fungicides has several advantages over *in vivo* methods including economy of time and space, usability and reliability. Their disadvantages mainly result from the absence of the complex interaction between host-parasite-fungicide-environment. For instance, a fungicide may be activated by the host, inactivated by the environment, or it may be phytotoxic - factors such as these must be evaluated before a final decision on the suitability of a fungicide can be made. However, as stated by Neely (30) "at present there are no practical substitutes for *in vitro* tests for the initial screening of candidate fungicides. Thus these tests are invaluable as one of a series of steps required in the evaluation of fungicides."

In the present study the 'so called' poison food technique and agar plate spore germination technique were both used for *in vitro* screening of candidate fungicides.

3.1.1 POISON FOOD TECHNIQUE

The poison food technique measures the radial growth of the fungus on substrates impregnated with different concentrations of fungicide and was initially developed for work with wood-rotting fungi (22). Since then it has been extensively used by many workers to evaluate the efficiency of fungicides *in vitro* (1, 7, 12, 21, 33). This technique is particularly useful for fungi which either do not sporulate or cannot readily be induced to do so in the laboratory (39).

MATERIALS AND METHODS

Tests were conducted with fifteen fungicides (Table 15) using three isolates each of Pleospora sp. (Stemphylium vesicarium), Alternaria alternata, Botrytis cinerea, Mycosphaerella ligulicola and one isolate of Itersonilia perplexans. All the isolates were obtained from lesions on the ray florets of chrysanthemum flowers and were pathogenic to chrysanthemum flowers.

Each fungicide was tested at concentrations of 0, 1, 10, 50 and 100 µg/ml active ingredient (a.i.) in laboratory potato dextrose agar (PDA_L).

The method of Hor (21), with minor modifications, was used for preparing the poison food plates. A 500 µg/ml a.i. stock solution of each of the fifteen fungicides was prepared by adding the appropriate weight of the fungicide to one litre of sterile distilled water. To screen all the fifteen fungicides against one fungus 90 ml of double strength PDA_L was poured into each of the required number of sterile 150 ml erlenmeyer flasks and maintained at 55 C in a waterbath. With the aid of a sterile pipette the required volume of each fungicidal stock solution was added to the sterile distilled water to obtain 90 ml aliquotes, each double the concentration to be tested. Each of these was then added to one of the flasks of double strength PDA_L maintained at 55 C, thus providing the required fungicide test concentration.

After mixing the agar and fungicide together approximately 15 ml was then poured into each of twelve petri plates. The plates were allowed to cool before inoculating the centre with a 4 mm diameter mycelial plug obtained from the periphery of actively growing fungal colonies. The controls consisted of PDA_L without fungicide. Four replicates were used for each treatment and the plates were incubated in the dark at 24 C. Diameters of the colonies were recorded when the controls had reached the margins of the plates.

Tests were also conducted to determine whether the action of fungicides were fungistatic or fungicidal. At the end of each experiment mycelial plugs, which showed no evidence of growth, were transferred to fresh PDA_L containing no fungicide. If the fungus resumed growth the fungicide was considered fungistatic; if, however, the fungus failed to grow out from the plugs then it was considered fungicidal (1, 21, 29).

RESULTS AND DISCUSSION

Results of the poison food fungicide tests are presented in Tables 16, 17 and 18, and illustrated in Figures 44 - 59.

No one fungicide was outstanding at low concentrations against all five fungi, although several performed well at higher concentrations. This result is not surprising in view of the taxonomic diversity of the fungi, including Ascomycetes, Deuteromycetes, and a Basidiomycete. Indications of the variation in fungus sensitivity that could be expected with the more specific benzimidazole and oxathiin compounds is now well documented (7, 14, 38). The relative ineffectiveness of the benzimidazoles against many dematiaceous fungi makes them unsuitable for use singly against a complex involving species of Alternaria and Stemphylium. The relatively poor total rating (Table 17) of benomyl, bavistin, thiabendazole and thiophanate-methyl is largely due to their ineffectiveness against these two fungi, and the poor performance of benomyl alone in the field tests confirms this conclusion. However, on the basis of poison food tests their high activity against the other three fungi in the complex (M. ligulicola, B. cinerea and I. perplexans) suggests a potential use of these compounds in fungicide combinations, particularly if no single fungicide can be found to give acceptable field control.

On the basis of total rating obtained by adding the ED_{50} group score for a fungicide against each fungus (Table 17), captafol appeared the most promising, with chlororeb, mancozeb and thiram also performing well. Mancozeb was the only compound tested which exhibited fungicidal activity at 100 µg/ml a.i. or less against all five fungi (Figure 54). With the exception of mancozeb all the fungicides were poorest in their action against A. alternata and Pleospora sp. (S. vesicarium).

One of the surprising results of the poison food tests was the activity (at 50 ug/ml a.i. or less) of carboxin against all five fungi (Table 16). Carboxin is most commonly cited for its activity against Basidiomycetes, particularly smut fungi and Rhizoctonia (9). The only reports the author is aware of involving the use of carboxin on chrysanthemums was in trials conducted in Holland against Japanese rust caused by *Puccinia horiana* P. Henn. (40). However, the material was not perservered with, mainly because of its poor solubility.

Numerous researchers have discussed the prediction of field performances of fungicides on the basis of *in vitro* fungitoxicity tests (30). Just as there are many instances of good correlations between field and laboratory performances (30) there are also examples of a fungicide performing well in the laboratory and not in the field (32), or conversely performing poorly in the laboratory but well in the field (27). However, it is fairly well accepted that *in vitro* tests can provide a useful guide to the potential of a fungicide, accepting good materials could be discarded when the decision is based purely on such tests.

3.1.2 AGAR PLATE SPORE GERMINATION METHOD

The spore-germination method of screening fungicides in the laboratory has been extensively used by various research workers for evaluating the fungistatic or spore inhibiting activity of protectant fungicides (19, 34, 35). Most of the modern methods in use are modifications of the conventional slide-germination technique. In view of the fact that the spore-germination method measures the chemical effect on spores, it was adopted in the present study together with the poison food technique as a laboratory screening technique. The method of Gattani (19) with minor modifications was used.

TABLE 15. Fungicides tested by the poison food technique against five fungi causing chrysanthemum flower blight

Trade Name & Formulation	Manufacturer	Common Name or Code No.	Chemical Name
Difolatan 80 W 75% W.P.	Chevron Chemical Company	captafol	N-(1,1,2,2-tetrachlorethylthio) cyclohex-4-ene-1,2-dicarboxyimide
Vitavax 75% W.P.	Uniroyal Inc.	carboxin	2,3-dihydro-6-methyl-5-phenylcarbomyl-1,4-oxathiin
Demosan 65% W.P.	E.I. du Pont de Nemours & Co. (Inc.)	chloroneb	1,4-dichloro-2,5-dimethoxybenzene
Allisan 50% W.P.	Upjohn Company	dicloran	2,6-dichloro-4-nitroaniline
Benlate 50% W.P.	E.I. du Pont de Nemours & Co. (Inc.)	benomyl	methyl N-[1-(butylcarbamoyl)-2-benzimidazole]carbamate
Orthocide 406 50% W.P.	Chevron Chemical Company	captan	N-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboxyimide
Mertect 10% dust	Merck Sharp & Dohme International	thiabendazole	2-(4-thiazolyl)benzimidazole

continued over ...

TABLE 15. Fungicides tested by the poison food technique against five fungi causing chrysanthemum flower blight - continued

Trade Name & Formulation		Manufacturer	Common Name or Code No.	Chemical Name
Parzate C	65% W.P.	E.I. du Pont de Nemours & Co. (Inc.)	zineb	zinc ethylene-1,2-bisdithiocarbamate
Voronit	50% W.P.	Farbenfabriken Bayer AG.	fuberidazole	2-(2-furyl)-benzimidazole
Dithane M-45	80% W.P.	Rohm & Haas Company	mancozeb	complex of zinc ion and manganese ethylene bis-dithiocarbamate
Daconil 2787	75% W.P.	Diamond Shamrock Chemical Company	chlorothalonil	2,4,5,6-tetrachloro-1,3-dicyanobenzene
Topsin M	50% W.P.	Nippon Soda Co. Ltd.	thiophanate-methyl (NF44)	1,2-di(3-methoxycarbonyl-2-thioureido)benzene
Arasan	80% W.P.	E.I. du Pont de Nemours & Co. (Inc.)	thiram	bis(dimethylthiocarbamyl) disulphide
Plantvax	75% W.P.	Uniroyal	oxycarboxin	5,6-dihydro-2methyl-1,4-oxathiin-3-carboxanilide-4,4-dioxide
Bavistin	50% W.P.	Badische Anilin & Soda -Fabrik AG.	BASF 3201F	1-([2-(methylthio)ethyl]carbamoyl)-2-(methoxyamino)benzimidazole

TABLE 16. Grouping according to ED_{50}[a] values of fifteen fungicides, based on effectiveness against five fungi causing flower blight of chrysanthemums

Fungus	ED_{50} (μg/ml active ingredient) group				
	1-5	6-10	11-50	51-100	101 and above
M. ligulicola	bavistin[b] thiabendazole	benomyl chloroneb dicloran fuberidazole thiophanate-methyl	captafol carboxin mancozeb thiram zineb	captan chlorothalonil	oxycarboxin
S. vesicarium	captafol chloroneb	carboxin dicloran mancozeb thiram	captan fuberidazole zineb	chlorothalonil	bavistin benomyl oxycarboxin thiabendazole thiophanate-methyl
A. alternata	captafol chloroneb	dicloran mancozeb thiram	captan carboxin fuberidazole zineb	chlorothalonil	bavistin benomyl oxycarboxin thiabendazole thiophanate-methyl
B. cinerea	bavistin benomyl captafol chlorothalonil fuberidazole thiabendazole thiophanate-methyl	captan chloroneb dicloran thiram	carboxin mancozeb	zineb	oxycarboxin

continued over ...

TABLE 16. Grouping according to ED_{50}[a] values of fifteen fungicides, based on effectiveness against five fungi causing flower blight of chrysanthemums - continued

Fungus	ED_{50} (μg/ml active ingredient) group				
	1-5	6-10	11-50	51-100	101 and above
I. perplexans	bavistin benomyl captafol carboxin mancozeb thiram zineb	chlorothalonil oxycarboxin thiabendazole thiophanate-methyl	captan chloroneb fuberidazole		dicloran

[a] concentration required to inhibit radial growth by 50%

[b] trade name

TABLE 17. Total rating of fifteen fungicides for effectiveness against five fungi[a] causing flower blight of chrysanthemums, based on poison food ED_{50} group score[b]

Fungicide	Total Rating[c]
captafol	23
chloroneb	21
mancozeb	20
thiram	20
carboxin	18
fuberidazole	18
bavistin	17
dicloran	17
benomyl	16
thiabendazole	16
captan	15
thiophanate-methyl	15
chlorothalonil	15
zineb	15
oxycarboxin	8

[a] fungi causing flower blight: *Pleospora* sp. (*Stemphylium vesicarium*); *Botrytis cinerea*; *Mycosphaerella ligulicola*; *Itersonilia perplexans*; *Alternaria alternata*.

[b]

Score	Poison food ED_{50} group (Table 16)
5	1-5
4	6-10
3	11-50
2	51-100
1	101 plus

[c] total rating obtained by adding the group score for each fungicide against all five fungi.

TABLE 18. Fungicides which were totally fungicidal or fungistatic at 1, 10, 50 or 100 µg/ml a.i. against five fungi causing flower blight of chrysanthemums

Fungus	Fungicide	Fungicidal				Fungistatic			
		Concentration (ug/ml a.i.)				Concentration (ug/ml a.i.)			
		1	10	50	100	1	10	50	100
M. ligulicola	bavistin						+	+	+
	benomyl						+	+	+
	fuberidazole							+	+
	mancozeb			+	+				
	thiabendazole		+	+					+
	thiram			+	+				
Pleospora sp. (S. vesicarium)	mancozeb				+				
	thiram								+
I. perplexans	carboxin			+	+				
	fuberidazole								+
	mancozeb				+				
	thiram			+	+				
B. cinerea	bavistin	+	+	+	+				
	benomyl		+	+	+	+			
	captan			+	+				
	chlorothalonil							+	+
	mancozeb			+	+	+			
	thiophanate-methyl		+	+	+				
	thiram			+	+				
A. alternata	mancozeb				+				

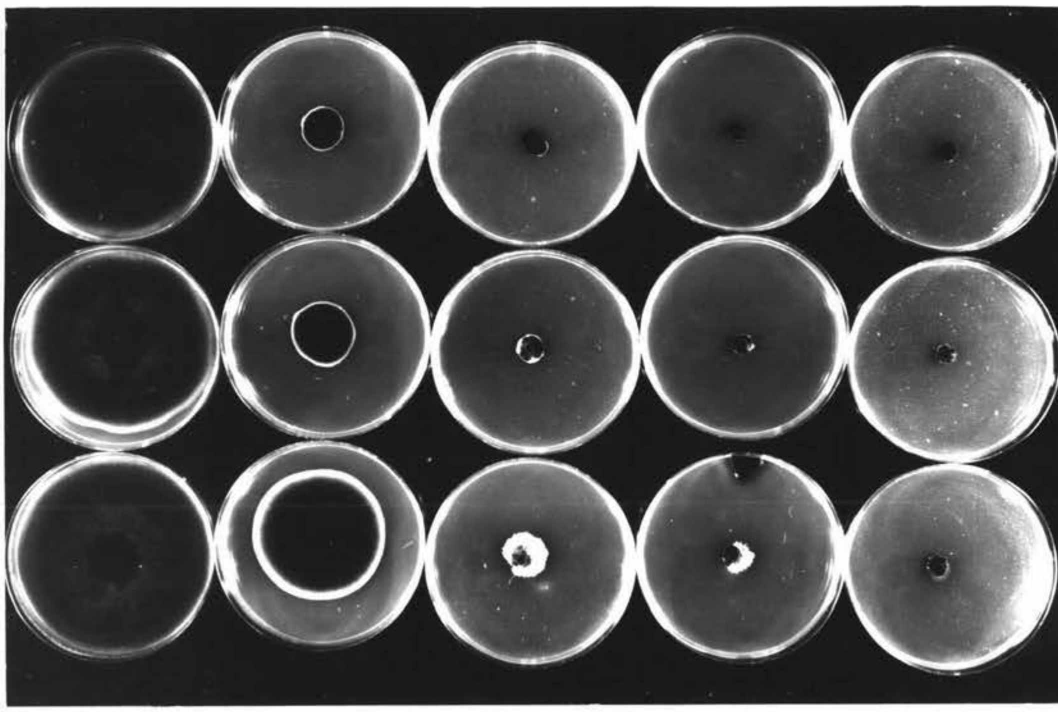

FIGURE 44: Effect of different concentrations of captafol incorporated in PDA_L on the radial growth of Alternaria alternata.

Top row (isolate A), L to R, 0, 1, 10, 50, and 100 µg/ml
Middle row (isolate A_2), L to R, 0, 1, 10, 50, and 100 µg/ml
Bottom row (isolate A_3), L to R, 0, 1, 10, 50, and 100 µg/ml

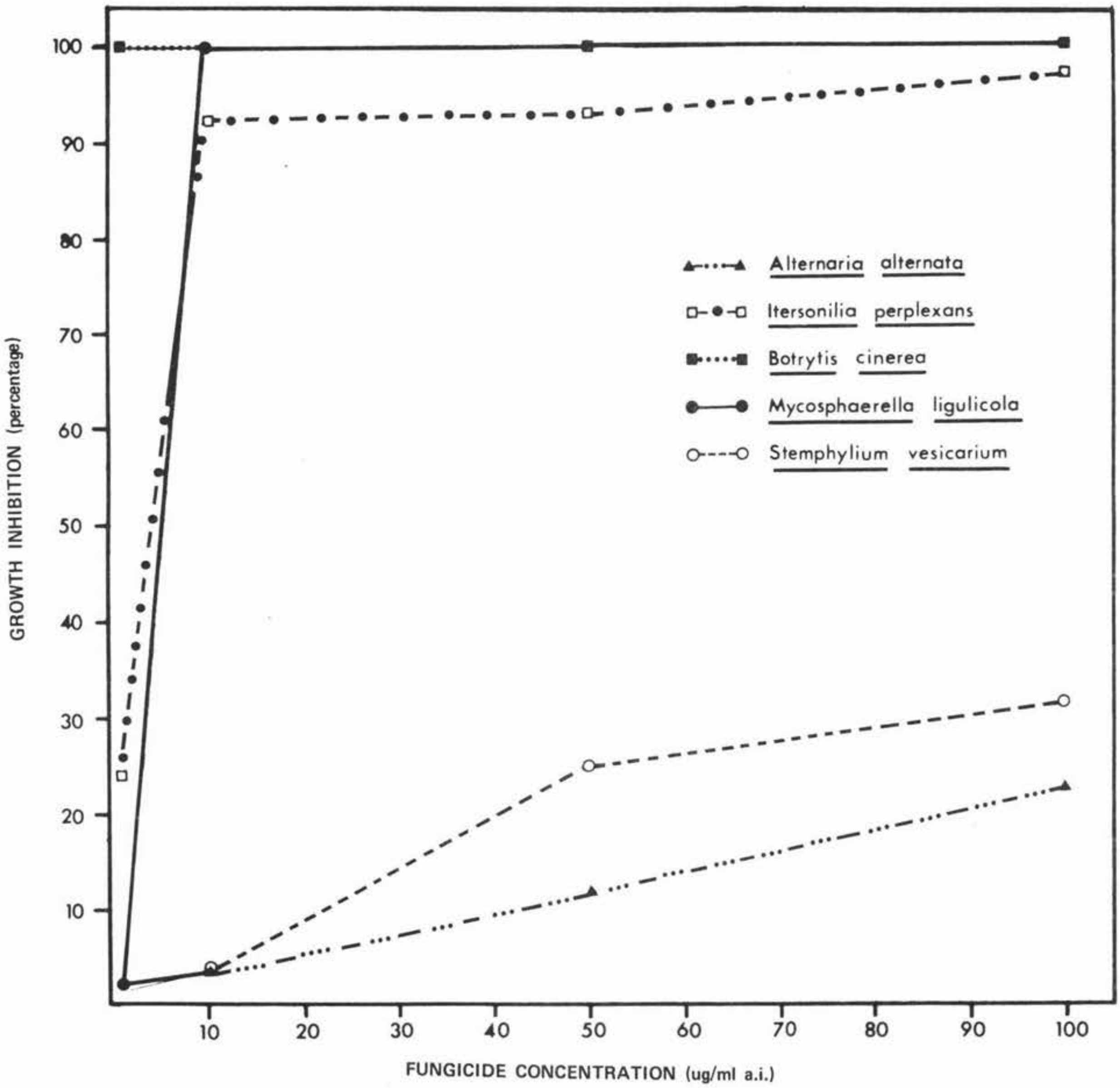

FIGURE 45 Effect of benomyl, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

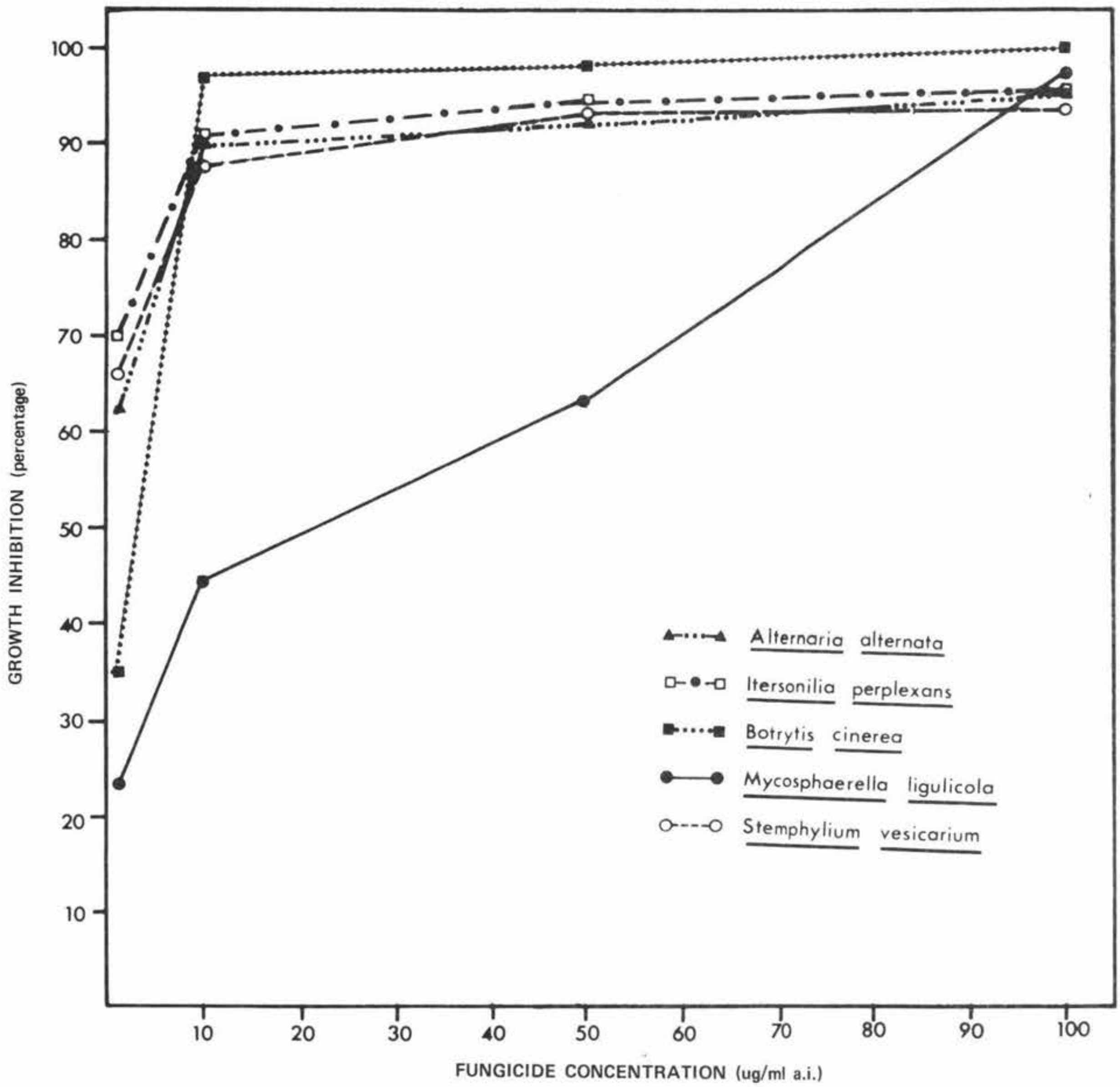

FIGURE 46 Effect of captafol, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

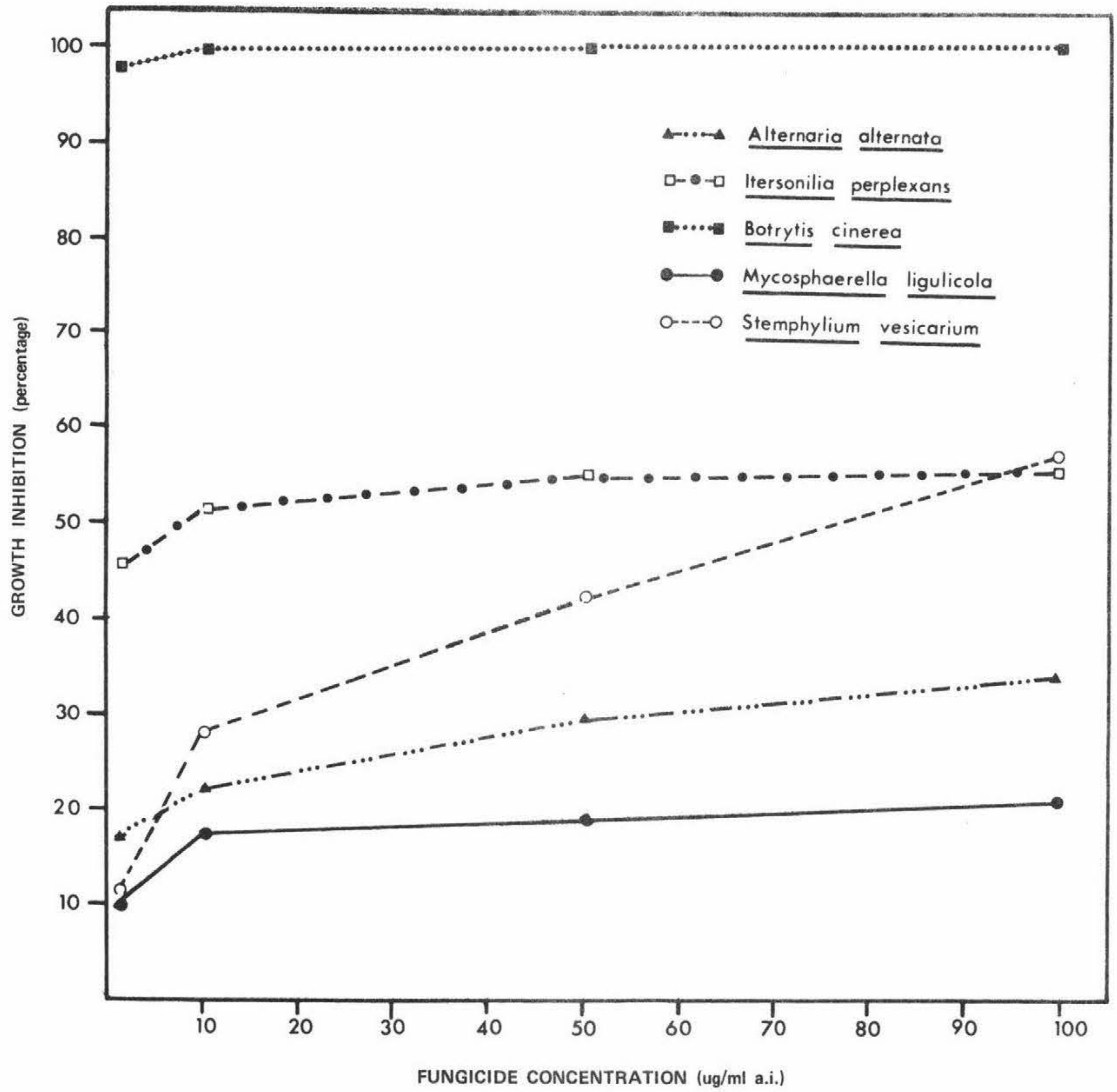

FIGURE 47 Effect of chlorothalonil, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

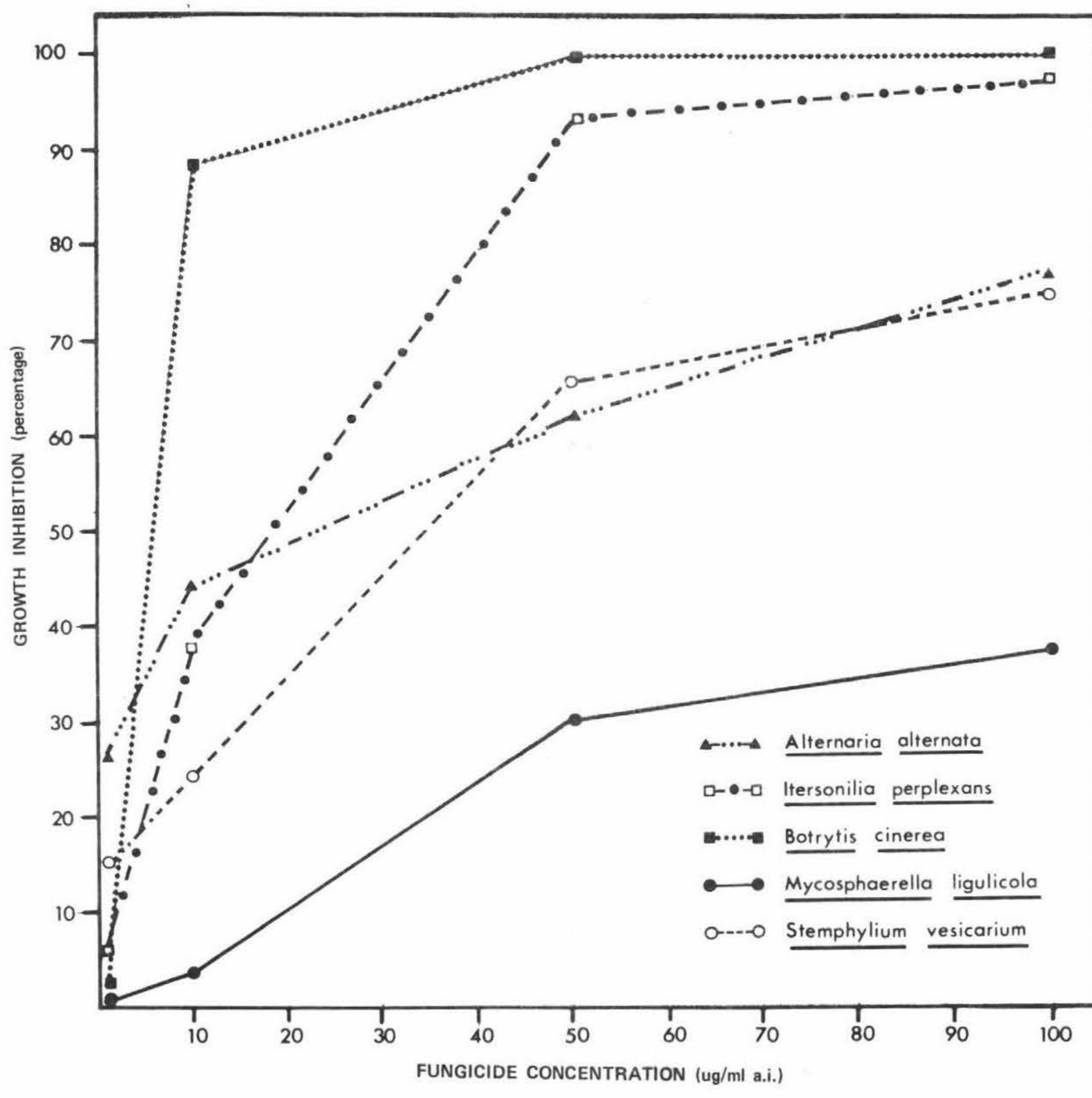

FIGURE 48 Effect of captan, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

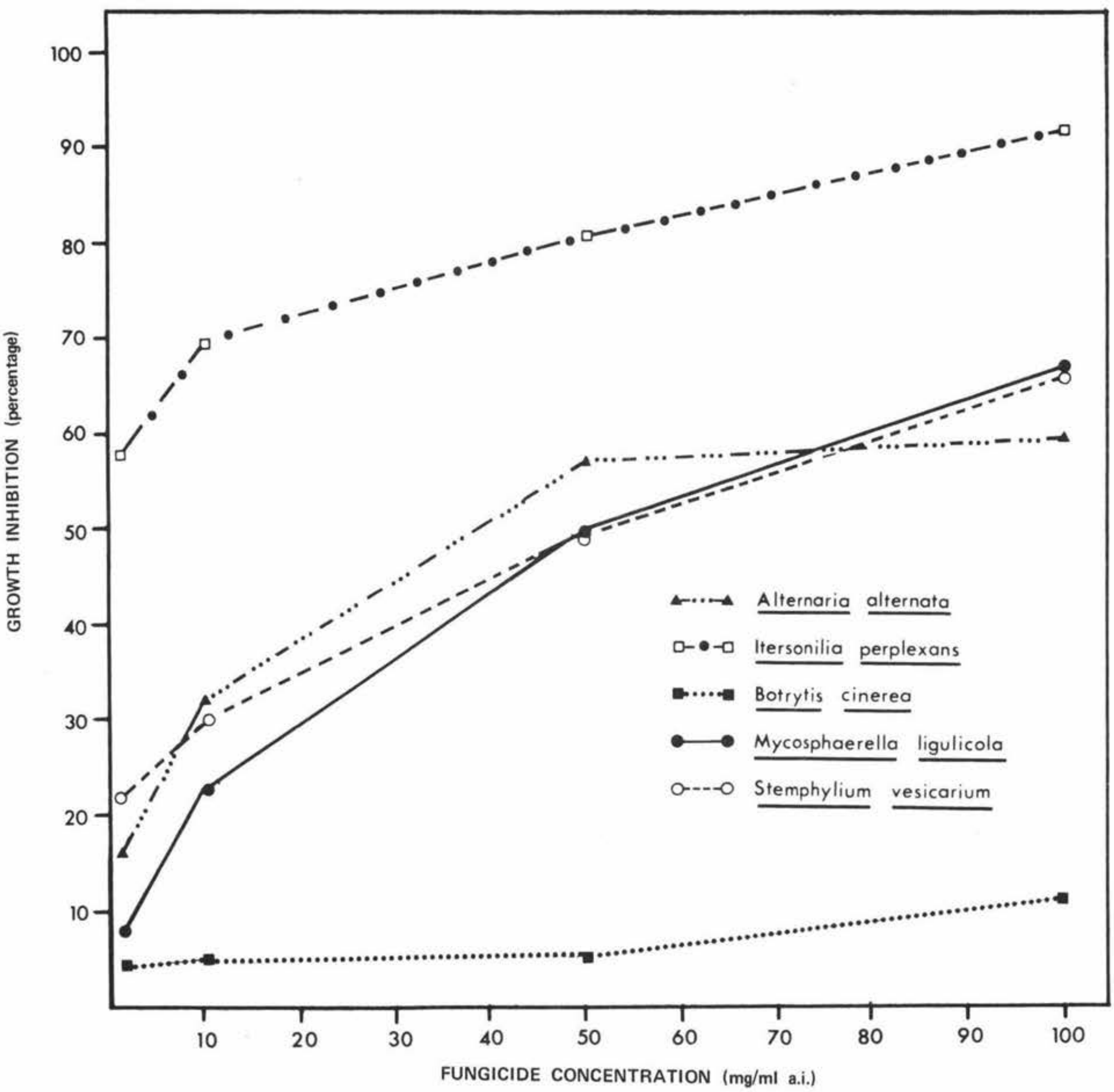

FIGURE 49 Effect of zineb, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

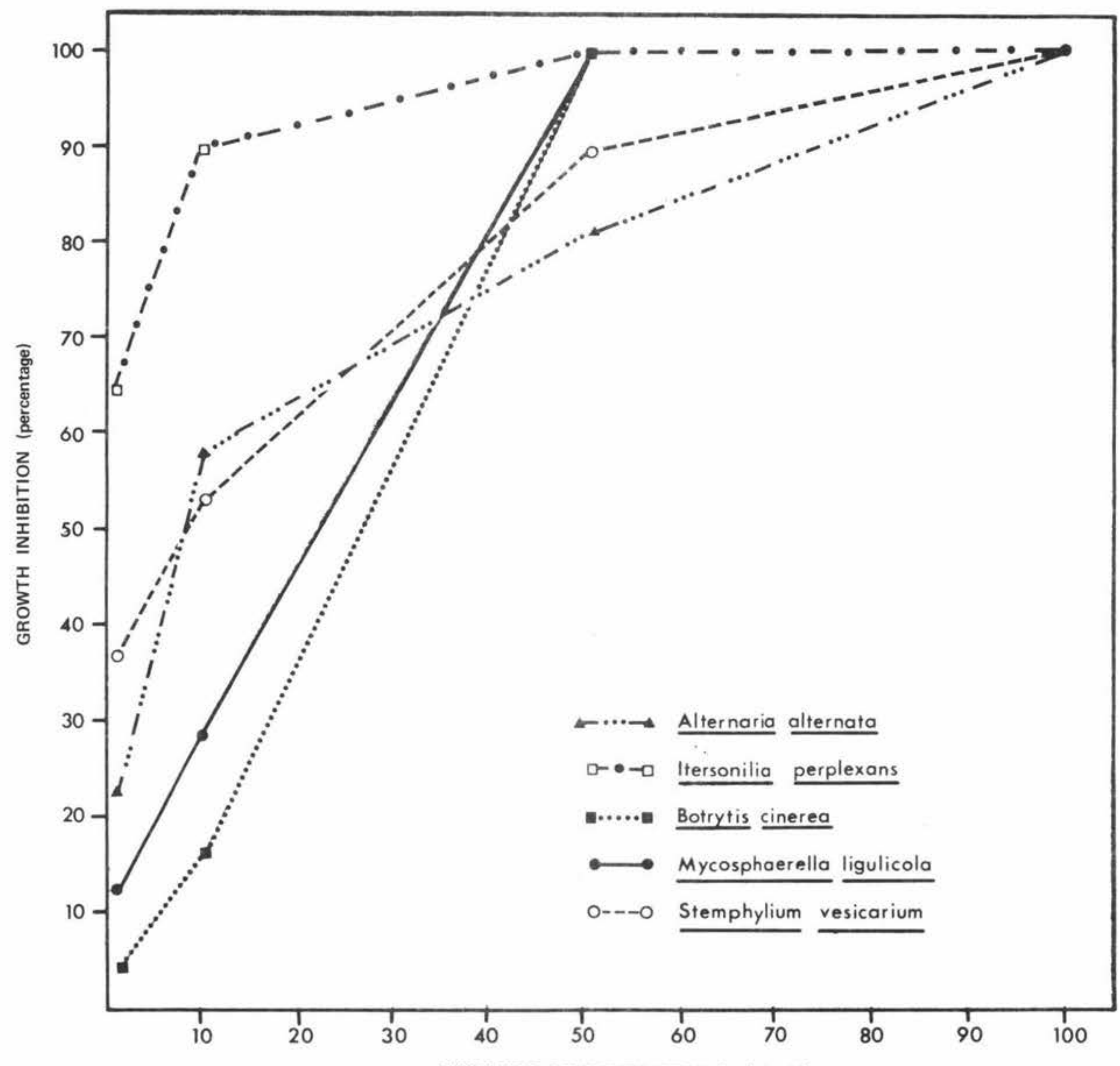

FIGURE 50 Effect of mancozeb, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

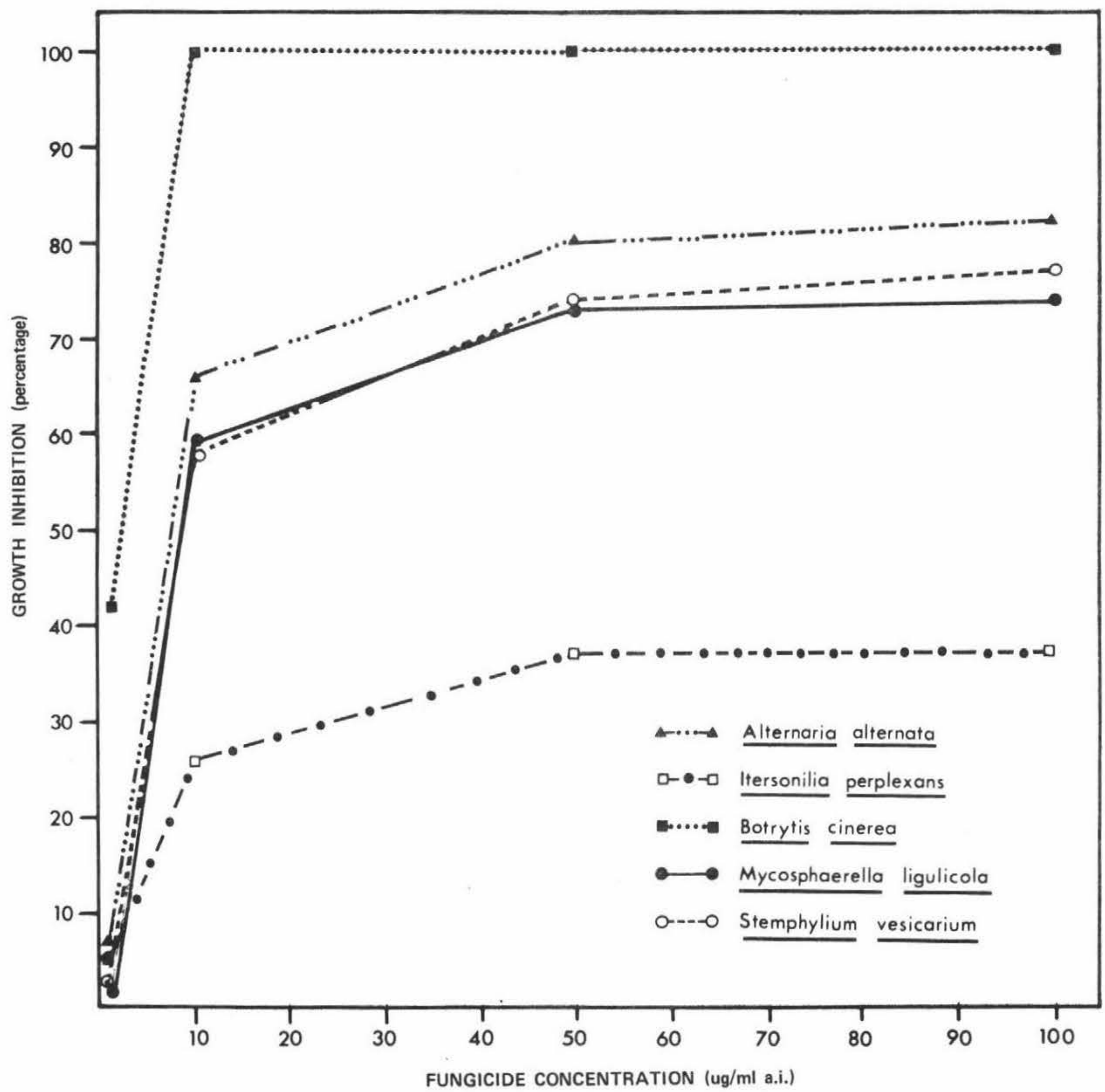

FIGURE 51 Effect of dicloran, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

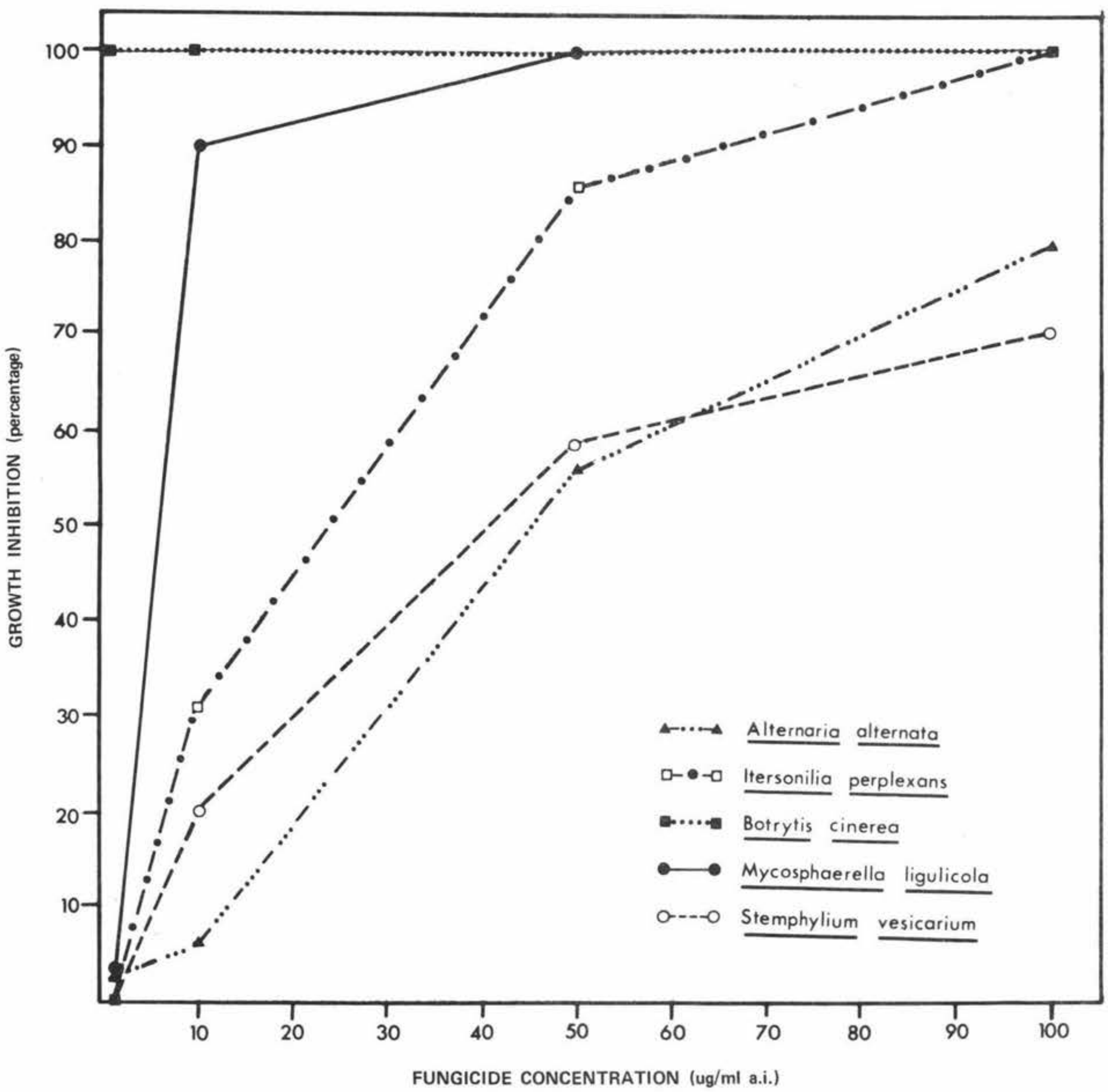

FIGURE 52 Effect of fuberidazole, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

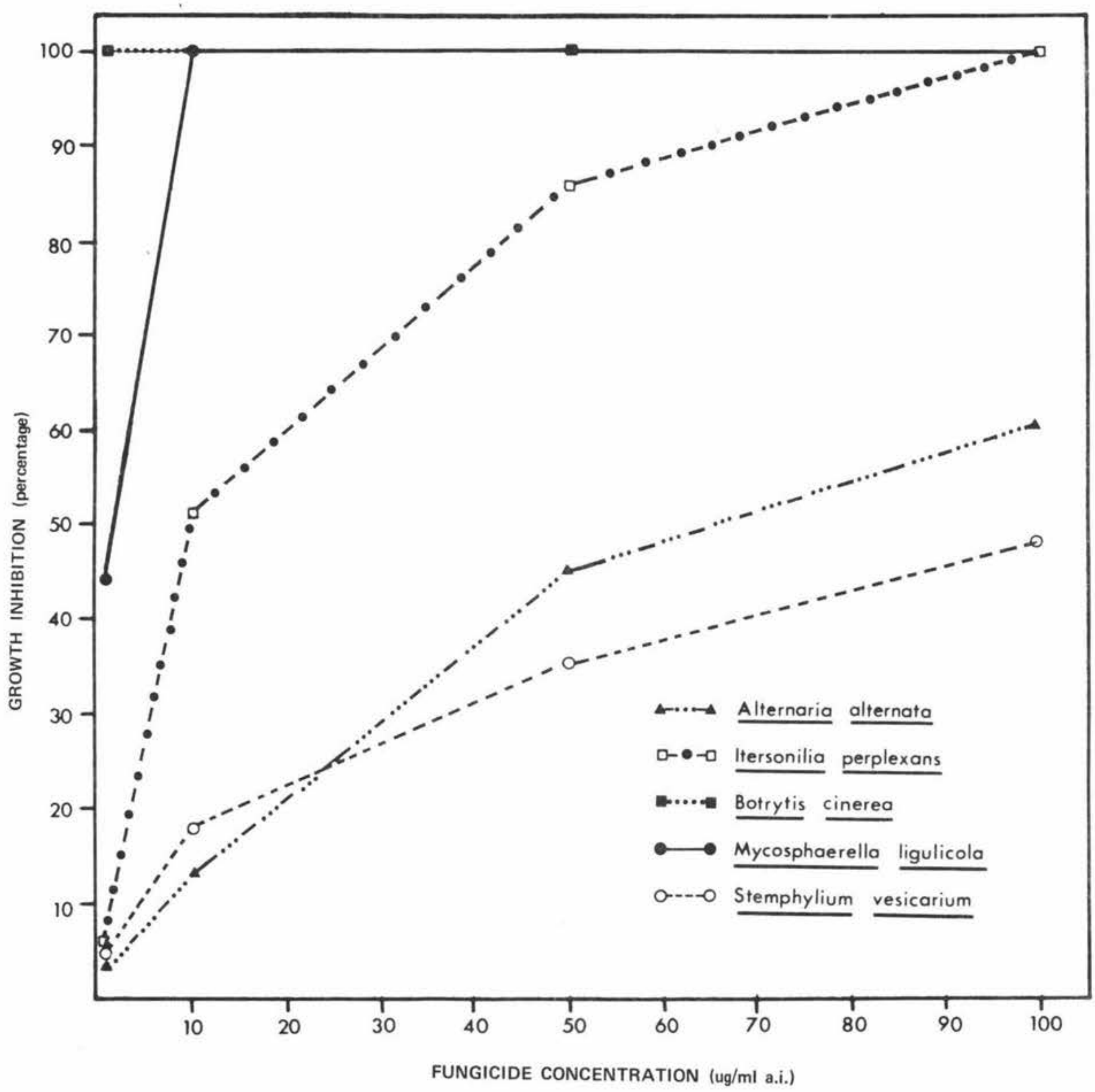

FIGURE 53 Effect of thiabendazole, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

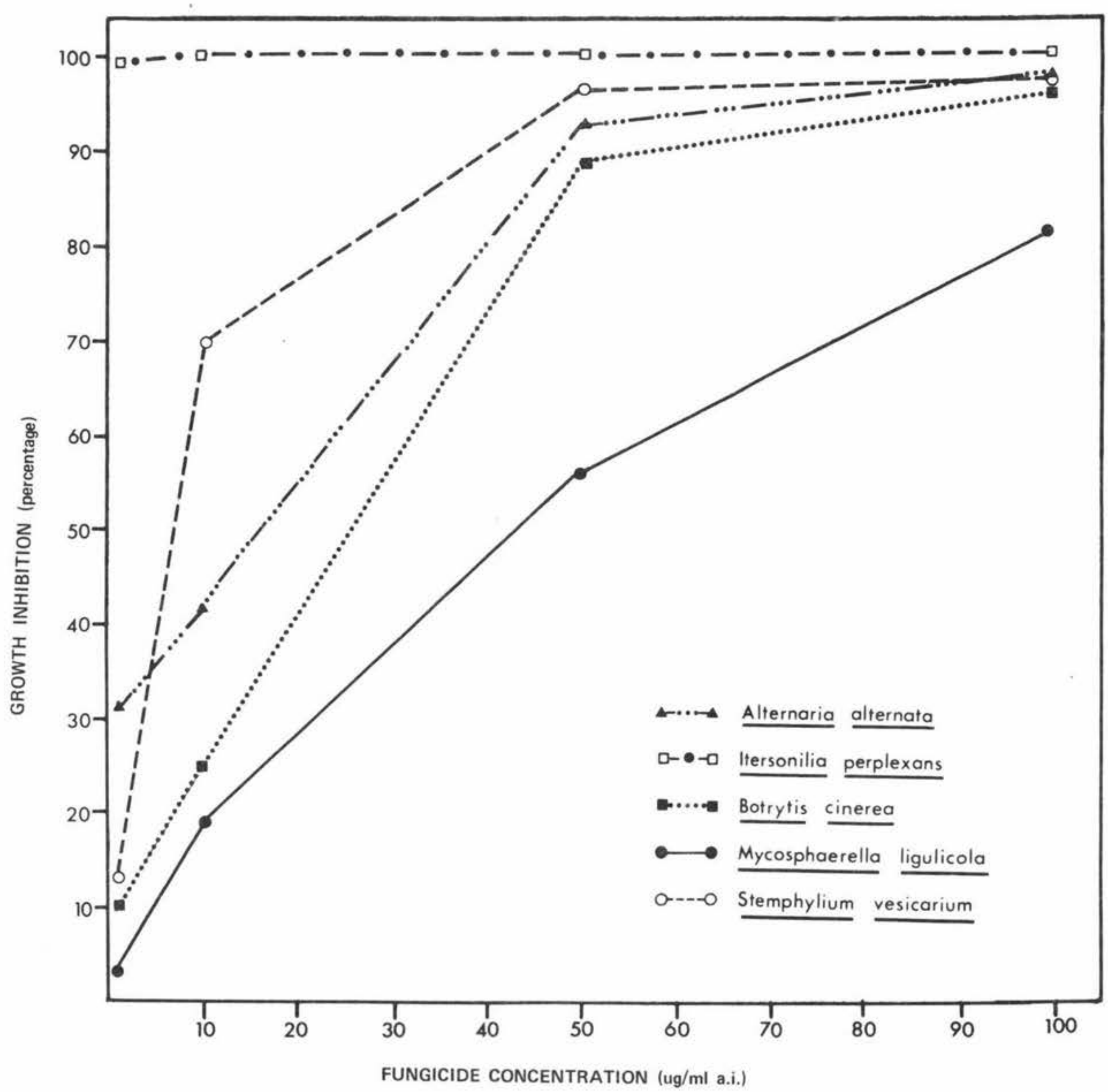

FIGURE 54 Effect of carboxin, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

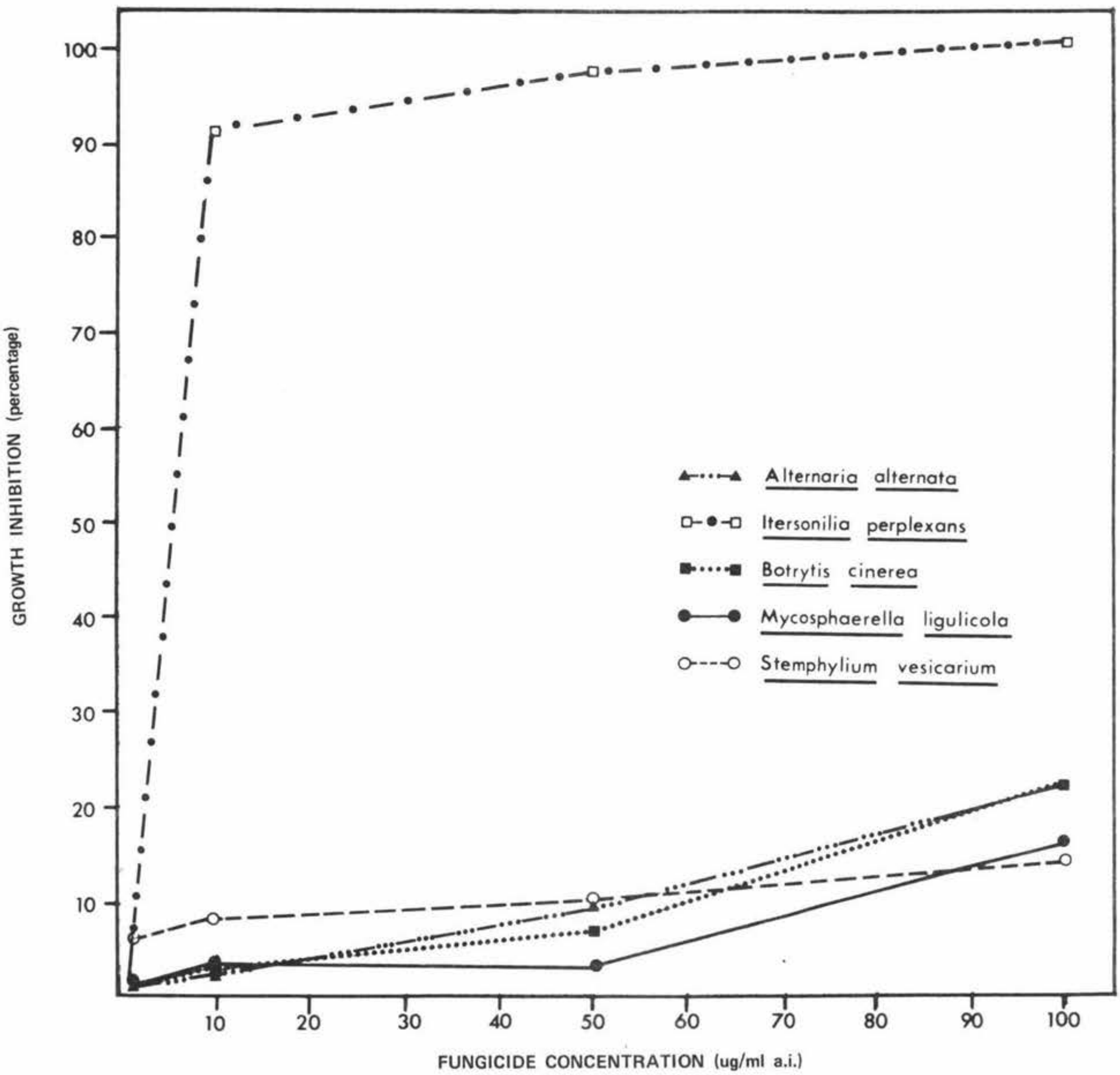

FIGURE 55 Effect of oxycarboxin, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

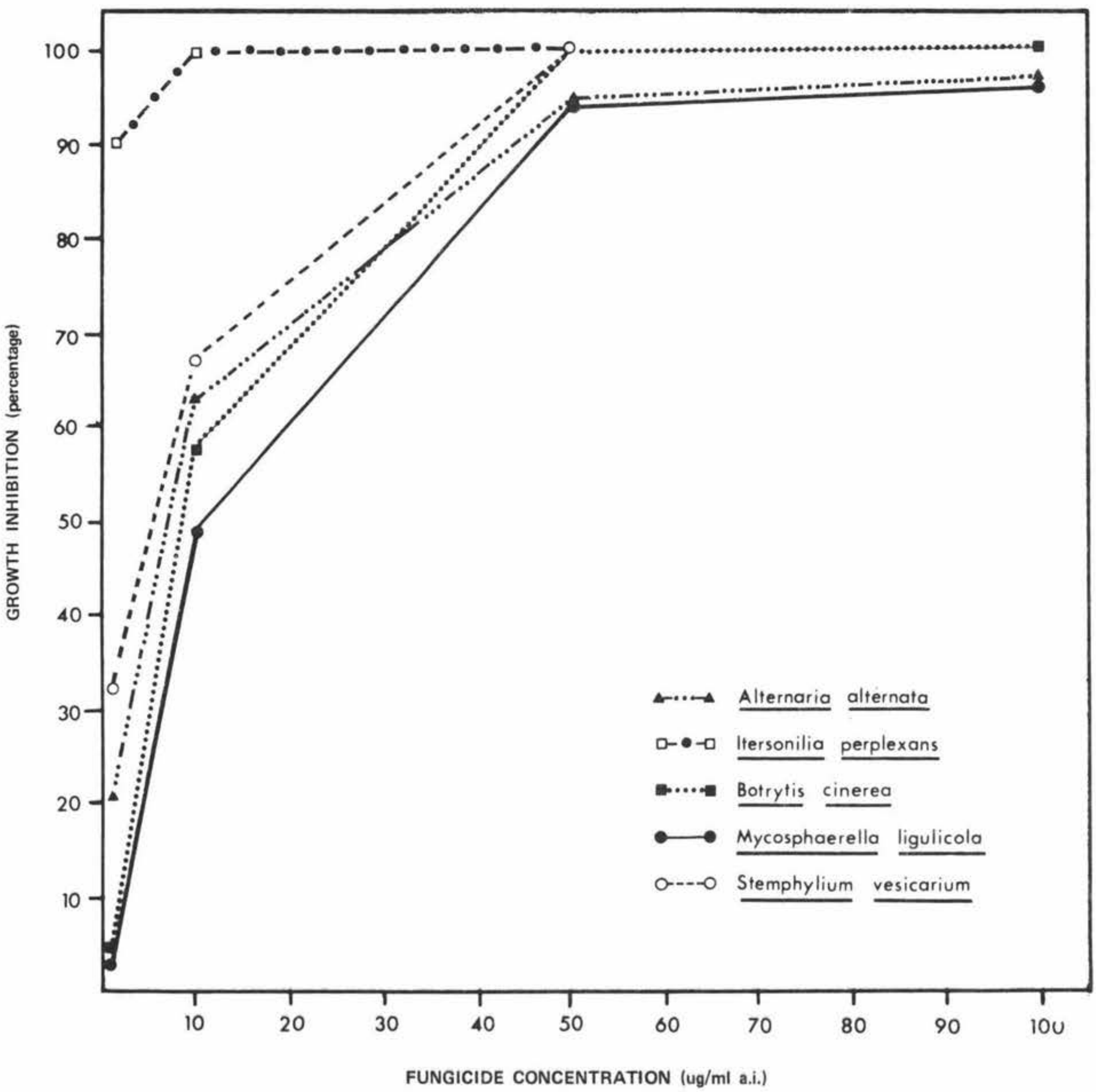

FIGURE 56 Effect of thiram, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

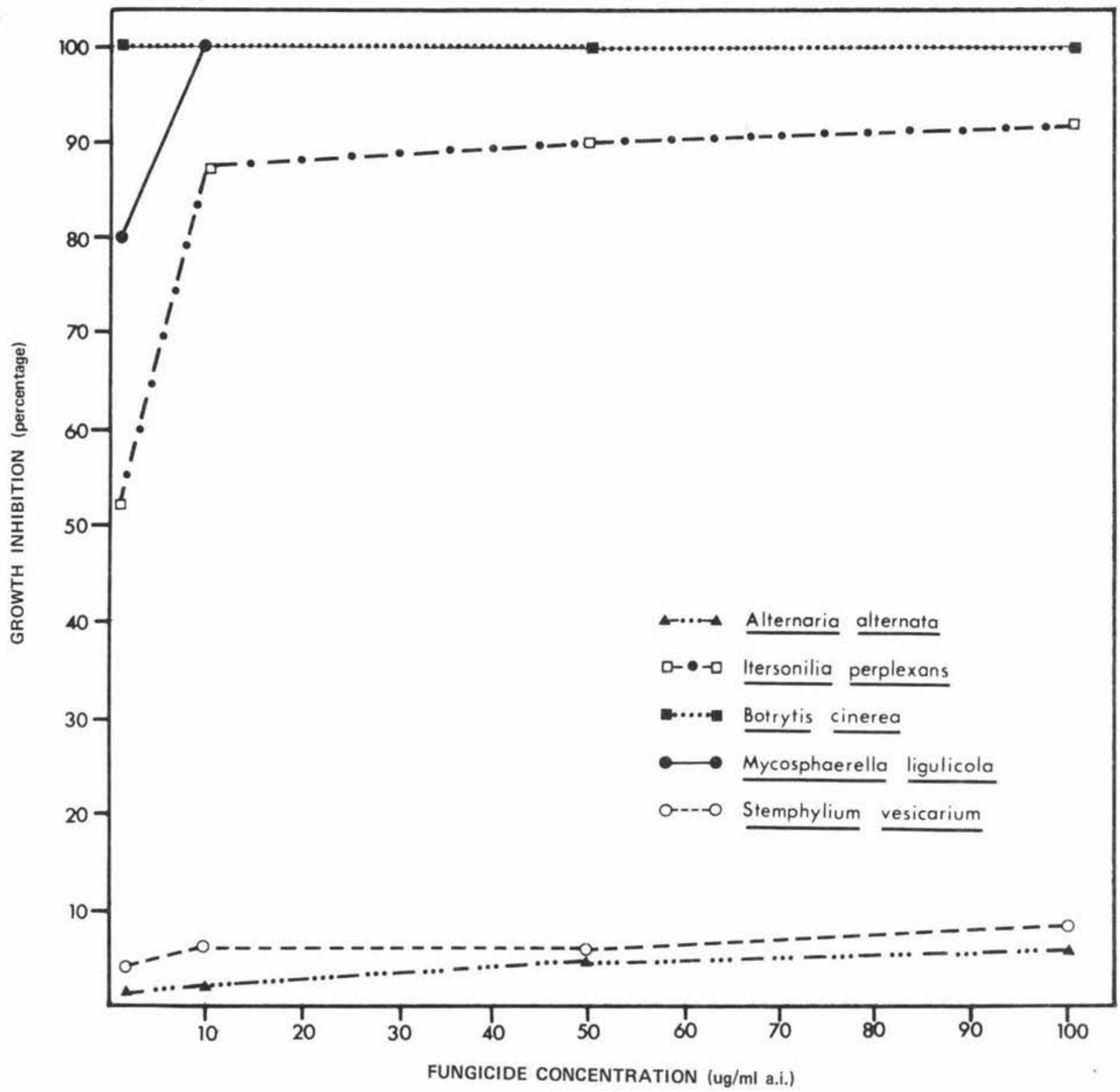

FIGURE 57 Effect of bavistin, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

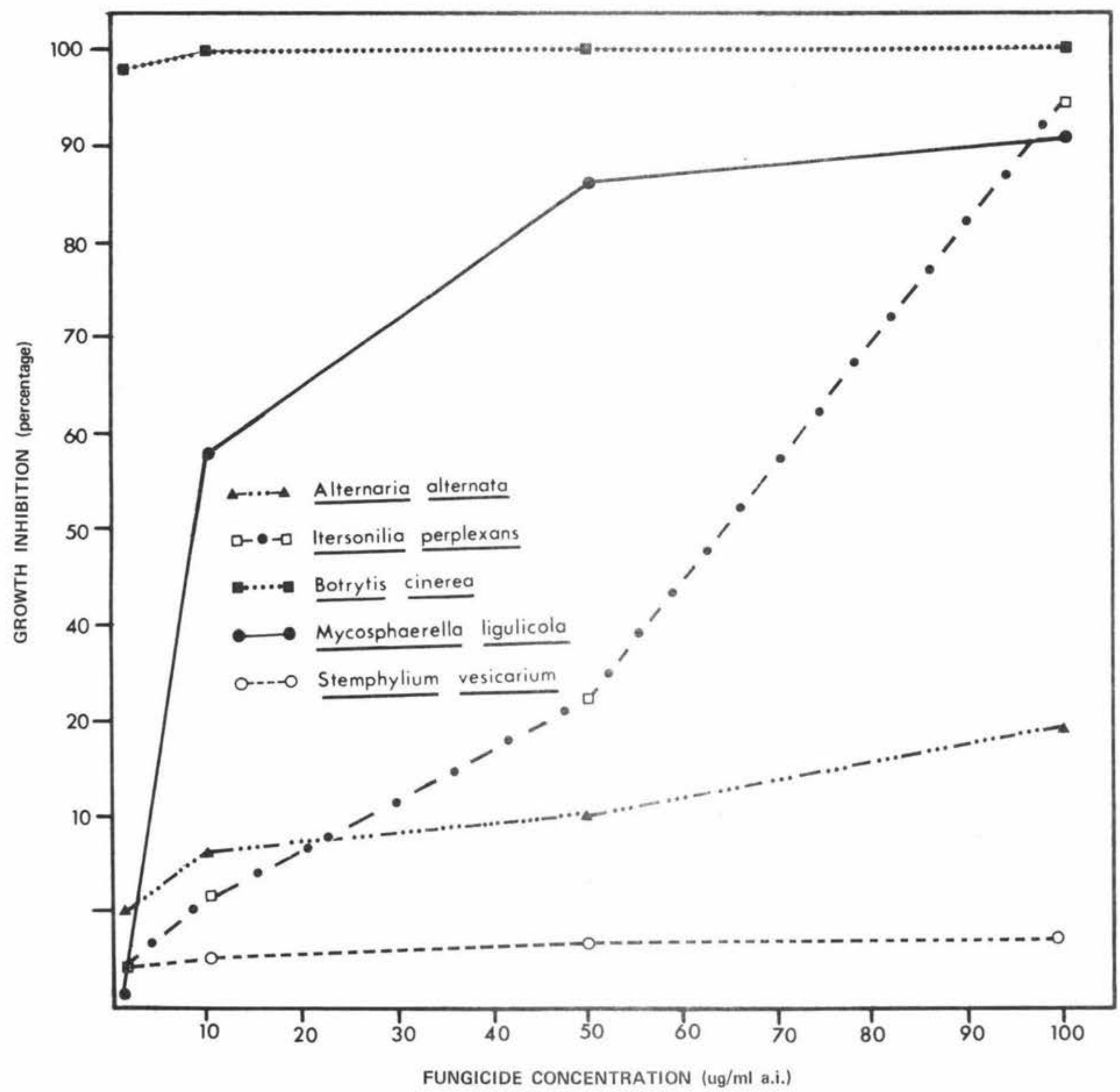

FIGURE 58 Effect of thiophanate-methyl, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

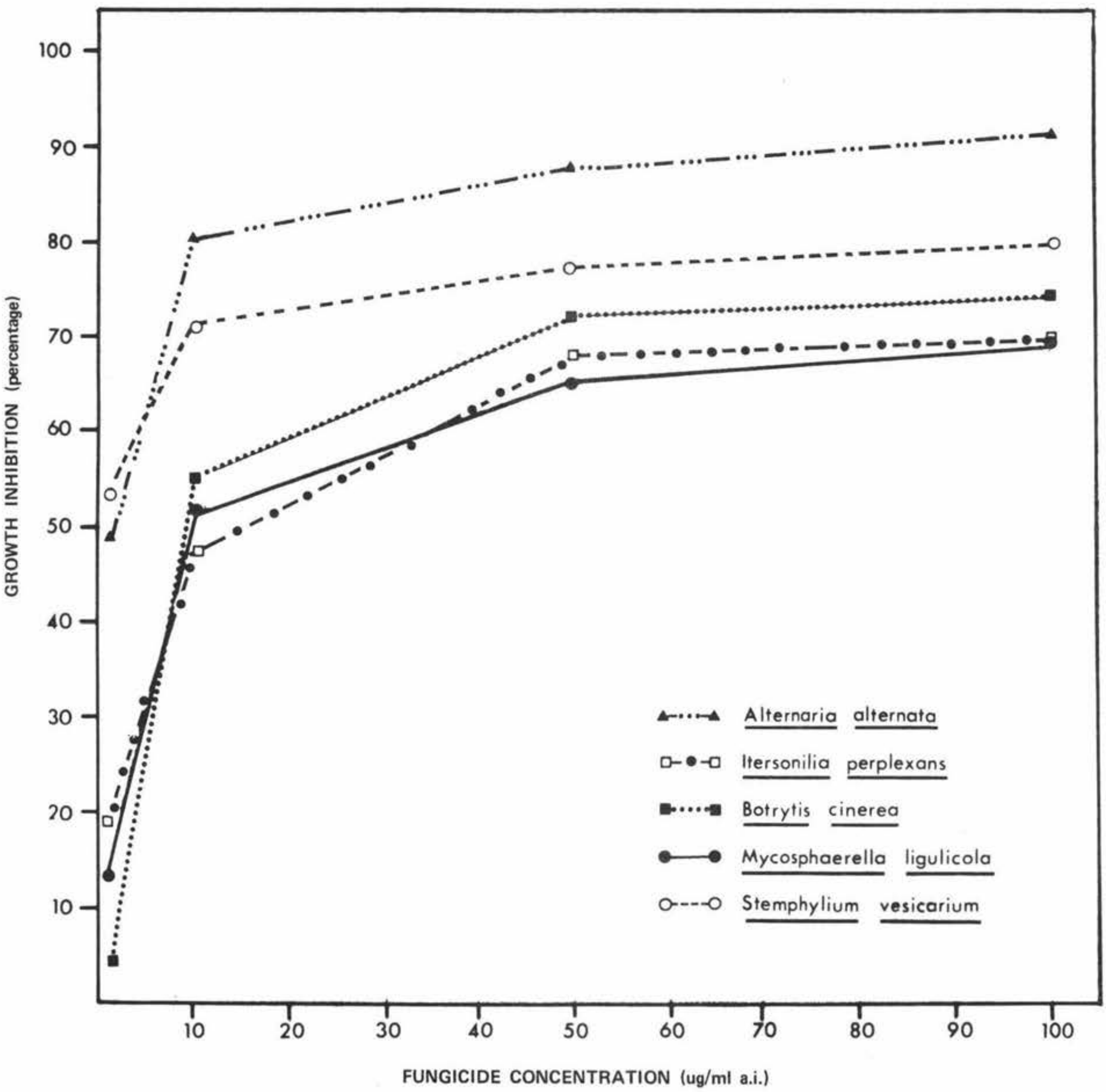

FIGURE 59 Effect of chloroneb, incorporated in laboratory potato dextrose agar, on the radial growth of five fungi causing flower blight of chrysanthemums.

MATERIALS AND METHODS

Four fungicides viz. captafol, chlorothalonil, benomyl and mancozeb were examined for their activity against three isolates each of *Pleospora* sp. (*Stemphylium vesicarium*), *Alternaria alternata*, *Botrytis cinerea* and *Mycosphaerella ligulicola*. *Itersonilia perplexans* was not included because of the difficulty of obtaining ungerminated spores for the test. The above fungicides were selected either because they display a broad spectrum of activity or have been reported as effective against the flower blight fungi under field conditions (11, 16, 17, 18).

Each fungicide was tested at concentrations of 0, 1, 10, 50, 100, 500 and 1000 µg/ml a.i., in PDA_L.

The preparation of agar-fungicide plates was very similar to that used for the poison-food technique. A stock solution of each of the fungicides at 1000 µg/ml a.i. was prepared by adding the appropriate weight of the fungicide to one litre of sterile distilled water. To test one fungicide against all the four fungi 40 ml of double strength PDA_L was poured into each of the required number of sterile 100 ml erlenmeyer flasks and maintained at 55 C in a waterbath. With the aid of a sterile pipette the required volume of the fungicidal stock solution was added to the sterile distilled water to obtain 40 ml aliquotes, each double the concentration to be tested. These were in turn added to one of the flasks of double strength PDA_L maintained at 55 C. The controls consisted of PDA_L without any fungicide. After mixing the agar and fungicide together the flasks were returned to the

55 C waterbath until required. The mixture was then poured into four plates at the rate of 20 ml/plate and allowed to cool before discs of 1 cm diameter were cut and placed on a glass slide. Four discs from each concentration were inoculated with spores of each of the three isolates of the four flower blight fungi and incubated in a moist chamber at 24 C for 6 - 12 hours (time required for the spores of the fungi to germinate).* When control discs (without fungicide) showed adequate spore germination, two discs from each concentration were mounted in lactophenol acid fuchsin and examined microscopically. The germination percentage was recorded and the average germ tube length of 20 spores measured with a micrometer eyepiece. The spores on the other two discs were allowed to grow for another 5 hours after which they, too, were mounted on lactophenol acid fuchsin and germ tube lengths determined. The result of the experiment is presented in Table 19.

RESULTS AND DISCUSSION

The results in Table 19 indicate that chlorothalonil was the most effective of the four fungicides tested, in terms of inhibition of germination of conidia of M. ligulicola, Pleospora sp. (S. vesicarium), A. alternata and B. cinerea. Mancozeb and captafol also exhibited a broad spectrum of activity against spores of all four fungi, but as anticipated benomyl was much less effective against Pleospora sp. (S. vesicarium) and A. alternata. However, benomyl was also less effective than the other three fungicides

* Criteria of germination - length of germ tube equals its width.

TABLE 19. Effect of four fungicides on germination of the conidia of four fungi causing flower blight of chrysanthemums

Fungicide	Conc. (µg/ml)	% Inhibition of germination			
		M. ligulicola	*Pleospora* sp.[a]	*A. alternata*	*B. cinerea*
chlorothalonil	1	100	100	100	93.4[b]
	10	100	100	100	100
	50	100	100	100	100
	100	100	100	100	100
	500	100	100	100	100
	1000	100	100	100	100
mancozeb	1	100	5.7[b]	24.2[b]	3.5[b]
	10	100	67.7[b]	100	100
	50	100	100	100	100
	100	100	100	100	100
	500	100	100	100	100
	1000	100	100	100	100
benomyl	1	35.9[b]	6.0[b]	7.1[b]	0.7[b]
	10	89.6[b]	7.9[b]	8.2[b]	4.1[b]
	50	96.6[b]	10.6[b]	12.0[b]	7.1[b]
	100	100	12.1[b]	18.1[b]	9.8[c]
	500	100	15.5[b]	24.6[b]	12.9[c]
	1000	100	16.8[b]	30.7[b]	16.1[c]
captafol	1	72.8[b]	34.9[b]	90.9[b]	100
	10	100	94.6[c]	100	100
	50	100	100	100	100
	100	100	100	100	100
	500	100	100	100	100
	1000	100	100	100	100

[a] with *S. vesicarium* imperfect state
[b] no inhibition of germ tube elongation
[c] inhibition of germ tube elongation.

against *B. cinerea* even at 1000 µg/ml a.i. This apparent anomally could in part be explained by the fact that benomyl does become more fungitoxic in the field when it breaks down to methyl benzimidazole carbamate (MBC). With the short time spores were exposed to fungicide this change may not have occurred.

On the basis of these results it was concluded that all four fungicides warranted testing in the field.

3.2 FIELD TRIAL

Unfortunately all the results of laboratory screening of fungicides were not available when the field trial was conducted. Fungicides tested in the field were selected on several bases, including:

(i) overseas reports of recommendations on the effectiveness against one or more of the fungi involved in the chrysanthemum flower blight e.g. dicloran (35); chlorothalonil (11, 18);

(ii) activity against one or more of these fungi on another crop e.g. benomyl and *Botrytis cinerea* on strawberries (26) or gladiolus (13);

(iii) acknowledged broad spectrum activity e.g. mancozeb;

(iv) laboratory screening results available from present study.

MATERIALS AND METHODS

Fungicides, formulations and application rates are detailed in Table 20. The trial was conducted on a growers property in the Manawatu district and because of grower's claims that 'Primrose Fred Shoesmith' was very susceptable to flower blight it was chosen as the test cultivar. Plants were grown in four ground beds divided into plots (1.5 m long by 0.75 m wide), each containing approximately 36 plants. These were stopped twice during the growth of the crop but were not disbudded, as is the normal practise, so as to produce more flowers per unit area for the purpose of the trial.

Each spray treatment was replicated three times and commenced when the flower buds began to show colour. Chemicals were applied to both flowers and leaves with a double action hand sprayer. Boards were placed on each side of the plot to control drift of the chemicals to adjacent plots. Six spray applications were made at weekly intervals commencing April 7th. The spreader Cittowet* (0.02 ml/litre) and the insecticide Lindane 50% W.P. (0.5 g/litre) were added to the spray. All spraying was carried out in the mornings. The meterological data for the experimental period is presented in Appendix II. On two occasions rainfall occurred one day after spray application. The total rainfall during the test period was 150.6 mm.

Disease assessments were made at picking, at which time flowers from the three replicates of each fungicide treatment

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* Registered trade mark of Badische Anilin and Soda-Fabrik AG

were picked and grouped into one of the following categories:

(A) marketable

(i) perfect flowers - no lesioning

(ii) slightly imperfect flowers - few lesions.

(B) nonmarketable - flowers severely blighted.

A total of three assessments were made during the trial period, the first 24 days after commencing spraying and the remaining two at 14 day intervals.

To determine which fungi were not inhibited by the spray treatment twenty tissue pieces per treatment were plated onto antibiotic agar using the tissue plating technique described in 1.1.

RESULTS AND DISCUSSION

The results in Table 21 show that the incidence of flower blight in the unsprayed plots (controls) was very high. Flowers exhibited symptoms shortly after buds opened and symptoms were readily noticeable throughout the trial period, particularly in the unsprayed plots. Almost 90% of the flowers from the latter plots were nonmarketable, indicating requirements for disease development were ideal.

Table 22 shows that irrespective of a particular fungicide treatment, and with few exceptions, each of the five pathogens involved in the flower blight complex was present. Because of the small number of isolations made per treatment and possible sampling bias (several tissue pieces may have been taken from one flower), no great significance is attached to the occasional failure to isolate a particular fungus from a treatment. The relatively poor recovery of the fungi by plating lesioned flower tissue from treated plots (Table 22) is possibly due to the inactivation of the pathogen by fungicide after a lesion is formed.

The percentage marketable flowers obtained from treated plots (Table 21) clearly shows that significant disease control was obtained with all fungicide treatments. The poorest fungicide treatment (thiram) gave 48% marketable flowers compared with only 11% from the controls.

Although perfect control was not achieved with any treatment, considering the disease pressure in the trial the degree of control obtained with the three best treatments (88-93% marketable flowers), or even the five best treatments (82-93% marketable flowers), appears promising.

Chlorothalonil was the outstanding single fungicide tested and these results would confirm the widespread use in Florida of this material for control of flower blight of chrysanthemums (11, 18). It is interesting to reflect here that chlorothalonil did not have a high total group rating on the basis of *in vitro* poison food tests (Table 17) and exhibited no fungicidal or fungistatic activity at 100 µg/ml a.i. against four of the five fungi in the complex (Figure 51). However, the spore germination tests using chlorothalonil do provide an example of an *in vitro* test perhaps more indicative of the results obtained in the field. This is in contrast to the results obtained with thiram which looked promising on the basis of laboratory tests but not in the field where only 48% of the flowers from thiram treated plots were marketable (Table 21). Cox & Winfree (10) reported that thiram was ineffective in the field in controlling B. cinerea and Mycosphaerella fragariae (Tul.) Lindau on strawberry plants.

It is debatable whether benomyl significantly improved the performance of many of the fungicides. Certainly benomyl alone performed poorly, due in part to the incidence of A. alternata and S. vesicarium in the trial. There was no evidence of phytotoxicity or unsightly residues on the foliage or flowers with any of the treatments tested.

CONCLUSION

Whilst appreciating the need for further fungicide trials of the type conducted in this study on other chrysanthemum cultivars, the broad spectrum mancozeb or captafol would appear the best individual fungicides currently registered in New Zealand for general control of flower blight of chrysanthemums. Mancozeb also has the added advantage of being recommended for control of chrysanthemum rust (Puccinia chrysanthemi Roze.) (3), Japanese rust (P. horiana) (41), and septoria foliage blight (3, 24). Applications at 3 - 4 day intervals instead of 7 days as used in the trial, may improve the effectiveness of these materials and certainly warrants investigation. Subject to registration and price suitability chlorothalonil appears a very promising alternative chemical for use in a chrysanthemum spray program.

TABLE 20. Fungicides tested in the field for control of flower blight of chrysanthemums[a]

Fungicide[b]	Application rate (lbs/100 gal or g/litre)
chlorothalonil	1.5
captafol	1.5
zineb	1.5
mancozeb	1.5
captan	1.5
dicloran	1.5
thiram	1.5
benomyl	0.5
zineb + captan	0.5 + 0.8
mancozeb + captan	0.5 + 0.8
chlorothalonil + benomyl	1.5 + 0.5
captafol + benomyl	1.5 + 0.5
zineb + benomyl	1.5 + 0.5
captan + benomyl	1.5 + 0.5
dicloran + benomyl	1.5 + 0.5
thiram + benomyl	1.5 + 0.5
mancozeb + benomyl	1.5 + 0.5

[a] fungi causing flower blight:
Pleospora sp. (*Stemphylium vesicarium*); *Alternaria alternata*; *Botrytis cinerea*; *Mycosphaerella ligulicola*; *Itersonilia perplexans*.

[b] formulations as in Table 15.

TABLE 21. Performance of seventeen fungicide treatments for control of chrysanthemum flower blight[a]

Fungicide	% Marketable		Total marketable (%)	Non-marketable (%)
	% Perfect	% Slightly imperfect		
chlorothalonil + benomyl	74	19	93	7
chlorothalonil	69	22	91	9
mancozeb + benomyl	59	29	88	12
captafol + benomyl	53	30	83	17
mancozeb	57	25	82	18
mancozeb + captan	49	31	80	20
zineb + benomyl	51	28	79	21
captafol	48	31	79	21
captan + benomyl	44	33	77	23
zineb	57	20	77	23
zineb + captan	43	31	74	26
captan	26	47	73	27
dicloran + benomyl	40	32	72	28
thiram + benomyl	41	30	71	29
benomyl	32	32	64	36
dicloran	23	26	49	51
thiram	24	24	48	52
control	2	9	11	89

[a] fungi causing flower blight:
Pleospora sp. (*Stemphylium vesicarium*); *Botrytis cinerea*; *Alternaria alternata*; *Mycosphaerella ligulicola*; *Itersonilia perplexans*.

TABLE 22. Chrysanthemum flower blight fungi present in fungicide treatments and control

Fungicide	No. of colonies out of 60 tissue pieces plated					TOTAL
	A. alternata	*B. cinerea*	*M. ligulicola*	*Pleospora* sp.[a]	*I. perplexans*	
benomyl	13	4	4	9	6	36
captafol	15	-	-	1	-	16
captan	3	2	17	-	-	22
chlorothalonil	7	6	3	4	2	22
dicloran	6	9	7	5	4	31
mancozeb	9	9	8	1	-	27
thiram	2	11	14	2	-	29
zineb	8	9	12	2	-	31
Fungicide combination						
captafol + benomyl	3	11	3	2	-	19
captan + benomyl	3	8	2	4	2	19
chlorothalonil + benomyl	7	1	2	2	4	16
dicloran + benomyl	5	4	7	1	7	24
mancozeb + benomyl	7	5	4	1	1	18
thiram + benomyl	5	7	8	1	1	22
zineb + benomyl	4	6	4	1	1	16
zineb + captan	2	5	6	4	4	31
mancozeb + captan	-	11	22	-	1	34
control	6	9	10	8	6	43

[a] with *S. vesicarium* imperfect state

TABLE 23. Fungi isolated from diseased chrysanthemum flowers in the unsprayed plots

Disease assessment No.[a]	No. of colonies out of 20 tissue pieces plated for each assessment					TOTAL
	M. ligulicola	*Pleospora* sp.[b]	*B. cinerea*	*A. alternata*	*I. perplexans*	
1	2	4	3	6	1	16
2	2	2	3	2	4	13
3	6	2	3	2	1	14
Total	10/60	8/60	9/60	10/60	6/60	43/60

[a] 3 disease assessments were made during the trial

[b] with *S. vesicarium* imperfect state

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APPENDIX I

COMPOSITION AND PREPARATION OF CULTURE MEDIA

The ingredients and method of preparation of the different media are given below. In all cases the media were sterilized by autoclaving in 250 ml flasks at 15 p.s.i. for 20 minutes.

(i) Laboratory potato dextrose agar (PDA_L)

Potatoes (peeled and sliced)	200 g
Agar (Davis)	12 g
Dextrose	10 g
Distilled water	1000 ml

The sliced potatoes were cooked gently for approximately one hour in 500 ml of distilled water, after which they were filtered through a cheesecloth. The agar and dextrose were melted in 500 ml of distilled water and the potato filterate then added to it.

(ii) Oxoid potato dextrose agar (PDA_O)

Potato dextrose agar (oxoid)	39 gm
Distilled water	1000 ml

The potato dextrose agar was soaked in the distilled water for approximately 15 minutes and then autoclaved.

(iii) Nutrient agar (NA)

Nutrient agar (BBL)	23 g
Distilled water	1000 ml

The nutrient agar was soaked in the distilled water for approximately 15 minutes, boiled for 1 minute and then autoclaved.

(iv) Malt agar (MA)

Malt extract	20 g
Agar (Davis)	25 g
Distilled water	1000 ml

The malt extract was cooked in 500 ml of distilled water for one hour and then filtered through a cheesecloth. The filterate was then added to the agar melted in 500 ml of distilled water.

(v) Cornmeal agar (CMA)

Cornmeal	20 g
Agar (Davis)	15 g
Distilled water	1000 ml

Preparation as in (iv).

(vi) Oatmeal agar (OMA)

Oatmeal	42 g
Agar (Davis)	15 g
Distilled water	1000 ml

Preparation as in (iv).

(vii) Prune agar (PrA)

Prune agar (Difco)	24 g
Distilled water	1000 ml

The prune agar was soaked in the distilled water for approximately 15 minutes and then autoclaved.

(viii) Pea agar (PA)

Pea (whole seed)	400 g
Agar (Davis)	20 g
Distilled water	1000 ml

The peas were cooked in 500 ml of distilled water for one hour and then filtered through a cheesecloth. The filterate was then added to the agar melted in 500 ml of distilled water.

(ix) Water agar (WA)

Agar (Davis)	12 g
Distilled water	1000 ml

The agar was melted in the distilled water and then autoclaved.

(x) V-8 juice agar (V-8 agar)

V-8 juice	200 ml
Calcium carbonate	3 g
Agar (Davis)	15 g
Distilled water	800 ml

The agar was melted in the V-8 juice and distilled water. To the melted agar was added the calcium carbonate and the mixture then autoclaved.

(xi) Potato marmite agar (PMA)

Potatoes (peeled and sliced)	200 g
Dextrose	20 g
Agar (Davis)	20 g
Marmite	1 g
Distilled water	1000 ml

The peeled and sliced potatoes were cooked gently for approximately 1 hour in 500 ml of distilled water and then filtered through a cheesecloth. The filterate was then added to the agar, dextrose and marmite melted in 500 ml of distilled water.

(xii) Chrysanthemum leaf agar (CLA)

Chrysanthemum leaves	30 g
Agar (Davis)	20 g
Dextrose	20 g
Distilled water	1000 ml

The sap of the leaves extracted in 200 ml of distilled water was warmed for 2 hours at 50 C and then filtered through a cheesecloth. The filterate was then added to the agar and dextrose melted in 800 ml of distilled water.

(xiii) Chrysanthemum petal agar (CPA)

Chrysanthemum petals	30 g
Agar (Davis)	20 g
Dextrose	20 g
Distilled water	1000 ml

The sap of the petals extracted in 200 ml of distilled water was warmed for 2 hours at 50 C and then filtered through a cheesecloth. The filterate was then added to the agar and dextrose melted in 800 ml of distilled water.

APPENDIX II

RAINFALL, HUMIDITY AND TEMPERATURE RECORDINGS FOR PALMERSTON NORTH, APRIL AND MAY 1972

Month and Date	Rainfall (mm)	Humidity (%)	Temperature (^{o}C)	
			Maximum	Minimum
April 1	-	83	20.1	13.5
2	-	78	20.1	8.5
3	-	79	19.4	9.4
4	-	73	21.8	12.5
5	-	91	18.9	9.3
6	1.6	88	22.2	12.2
7	6.2	87	23.7	15.3
8	8.9	85	19.8	14.5
9	-	63	16.7	11.7
10	-	79	22.1	6.1
11	-	83	21.9	8.7
12	-	94	20.1	7.6
13	-	72	14.0	11.3
14	-	67	14.9	7.1
15	-	77	16.8	4.1
16	4.6	85	17.6	5.9
17	9.0	91	16.0	8.2
18	0.1	82	17.0	8.1
19	-	79	17.7	10.0
20	-	81	16.0	6.7
21	Trace	85	17.6	10.7
22	-	82	18.5	13.0
23	2.6	46	18.8	14.5
24	6.3	90	19.5	13.0
25	27.8	89	18.0	13.4
26	-	69	16.2	12.2
27	0.8	94	17.0	4.4
28	4.7	82	18.3	8.0
29	-	92	15.1	4.6
30	1.3	89	17.6	4.5

continued over ...

Appendix II - continued

Month and Date	Rainfall (mm)	Humidity (%)	Temperature (°C)	
			Maximum	Minimum
May 1	0.5	62	14.5	9.9
2	0.2	82	16.7	4.2
3	1.5	82	15.9	5.5
4	1.4	81	15.1	10.8
5	-	64	14.2	5.4
6	0.5	76	14.8	7.5
7	-	77	14.4	11.3
8	-	80	17.0	5.0
9	1.1	97	15.7	3.3
10	-	86	16.2	7.2
11	Trace	97	15.1	2.1
12	0.3	83	15.1	6.0
13	34.1	85	17.0	9.4
14	10.5	78	11.3	11.0
15	18.6	84	15.3	7.7
16	4.9	91	17.2	7.4
17	2.9	83	14.9	11.0
18	1.8	91	14.2	9.8
19	-	91	15.1	4.8
20	2.7	97	15.5	4.9
21	1.0	92	15.6	7.8
22	Trace	82	14.0	0.4
23	1.0	84	16.1	5.1
24	8.4	72	15.2	11.0
25	1.6	70	14.0	7.2
26	-	94	10.9	0.8
27	-	76	14.4	1.6
28	-	80	14.1	6.1
29	1.5	89	13.2	2.5
30	2.5	95	13.1	4.5
31	-	87	11.0	5.3