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INVESTIGATION AND CONTROL OF SEEDBORNE *COLLETOTRICHUM* SPP. CAUSING ANTHRACNOSE OF LUPINS AND *LAVATERA*

Final Report

FINAL REPORT (June 1994)

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INVESTIGATION AND CONTROL OF SEEDBORNE*COLLETOTRICHUM* SPP. CAUSING ANTHRACNOSE OF LUPINS AND *LAVATERA*

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INVESTIGATION AND CONTROL OF SEEDBORNE *COLLETOTRICHUM* SPP. CAUSING ANTHRACNOSE OF LUPINS AND *LAVATERA*

Relevance to growers and practical application

Anthracnose of lupins and *Lavatera* is caused by seedborne fungi belonging to the genus *Colletotrichum.*

When infected seeds are sown they transmit the disease, anthracnose, to the seedlings causing leaf spots on which spores are produced. These spores are rain- or water-splashed to infect healthy plants thereby spreading anthracnose.

There have been considerable plant losses of lupins in the UK which may have resulted from the sowing of infected seeds. It is thought that failures in *Lavatera* also may have arisen from the use of infected seeds.

The purpose of this 4 month project was to a) investigate the incidence of the *Colletotrichum* species on commercial samples of Lupin and *Lavatera* seeds to obtain an indication of the dimension of the seedborne problem, b) isolate the fungi, grow them in the laboratory to find the temperatures at which they grew optimally which would indicate the plant raising temperatures best suited for their development, c) determine the location of the fungi in infected seeds which would indicate if a seed treatment with surface or penetrative action would be needed to control seedborne infection and d) select and test fungicides for the control of naturally occurring seedborne infection to obtain an effective remedial treatment.

Summary

Sixteen samples of *Lavatera* and 22 of Lupin were supplied by 5 seed companies.
Early difficulties in getting these fungi to grow from seeds were resolved when the *Lavatera Colletotrichum* was isolated and grown in culture over a range of temperatures. This test (b) showed that the fungus grew best at 25°C with little or no growth at 10°C and 35°C. All subsequent tests were done at 25_{0}C.
Therefore, temperatures of 25°C ± 5°C under moist conditions during plant raising would favour the development of this *Colletotrichum.*

Identification of this *Colletotrichum* indicates that it may be host specific and therefore confined to *Lavatera* and unlikely to infect other plant species such as lupin.

All lupin cultivars were free of infection when 150 seeds of each commercial seed type were tested on agar. Three of the 8 *Lavatera* cvs tested were infected, one severely so with almost 20% of seeds bearing the fungus internally.

Dissection followed by plating of seeds from this seed sample on to agar showed that some 20% of affected seeds bore the fungus within the seed coat and endosperm and some 3% had affected embryos. This indicated that the fungus was internally situated in seeds and

treatments which penetrated seed tissues would be necessary to control *Colletotrichum* infection.

Choice of seed treatment fungicides was based on previous screening tests made by the author against two *Colletotrichum* spp. and other selected fungi with similar morphological characteristics and on the reported efficacy of other products.
Of the 13 fungicide products applied as seed treatments prochloraz alone or prochloraz + carbendazim, or benomyl + thiram or thiabendazole + thiram were the most effective seed treatments virtually eliminating seedborne infection.

EXPERIMENTAL SECTION

INTRODUCTION

The research involved screening the 22 commercial samples of Lupin and 8 samples of the 16 samples of *Lavatera* seeds to obtain an initial indication of the dimension of the seedborne problem and in so doing to identify heavily infected samples of seeds which could be used in tests of potential seed treatment fungicides for the eradication of seedborne infection.

To detect the fungi in seed samples it was necessary to isolate the fungi from seeds, to recognise and identify them, to establish the conditions which were optimal for their growth under laboratory conditions so that an effective test method could be developed.

This was a continuing process over a series of tests at the end of which a method was developed which could be used for the testing of seed samples for the presence of the fungi and which also could be used for assessing the effect of seed treatments.

The work was divided as follows:

Detection of seedborne infection

1 Commercial samples of Lupin and *Lavatera* seeds

2 Preliminary isolation tests

3 Temperature range studies on *Colletotrichum*

4 Development of agar test for isolation of fungus from seeds

5 Recognition of *Colletotrichum* growing from seeds on agar

6 The identity of *Colletotrichum*

7 The incidence of *Colletotrichum* in *Lavatera* and lupin seeds

Seed treatment

1 The location of the fungus in seeds

2 Selection of seed treatment fungicides

3 Effects of seed treatments on disease control and seed germination

RESULTS

Detection of seedborne infection

1 Commercial samples of Lupin and *Lavatera* seeds
Varying amounts of seed were supplied by seed merchants mainly through Mr Stuart Coutts project co-ordinator. A description of the cultivars received is given in Table 1. Some merchants provided greater detail on the age of samples and their places of origin. This information is not repeated here. The samples were reported to be untreated.

TABLE 1 List of lupin and *Lavatera* seed samples provided by the seed trade

SOURCE	DESCRIPTION	NUMBER
1	Lupin - Gallery Mixed	1
	Lupin - Gallery Mixed	2
	Lupin - Gallery Mixed	3
	Lupin - Gallery Mixed	4
2	Lupin - Russell Hybrid Mixed	5
	Lupin - Lulu	6
1	Lavatera - Silver Cup	7
	Lavatera - Silver Cup	8
	Lavatera - Silver Cup	9
	Lavatera - Silver Cup	10
	Lavatera - Mont Blanc	11
3	Lavatera - Mont Blanc	12
	Lavatera - Mont Banc	13
4	Lupin - Kings Crown Strain	14
3	Lupin - Gallery Yellow	15
	Lavatera - Tanagra	16
5	Lavatera - Silver Cup	17
	Lavatera - Silver Cup	18
	Lavatera - Mont Blanc	19
	Lavatera - Silver Cup	20
	Lavatera - White	21
	Lavatera - Rosea	22
	Lavatera - Silver Cup	23
	Lavatera - Silver Cup	24
	Lupin - Lulu	25
	Lupin - Arboreus	26
	Lupin - Lulu	27
	Lupin - Lulu	28
	Lupin - Russell Mixed	29
	Lupin - Lulu	30
	Lupin - Minarette	31
	Lupin - Lulu	32
	Lupin - Russell	33
	Lupin - Noble Maiden	34
	Lupin - Russell Mixed	35
6	Lupin A	36
	Lupin Y	37
	Lupin Q	38

2 Preliminary isolation tests

In test 1 (Table 2) 100 seeds of the cvs shown were placed in 2 replicates of 50 seeds each on to moist germination pads in 120 x 80 x 20 mm polystyrene boxes. The boxes were placed in an incubator at 21°C and were examined under a low power binocular microscope for the presence of fruiting bodies of the fungus after 4, 7 and 14 days.
Colletotrichum was found on one seed only in cv 10, a *Lavatera* cultivar. None of the seeds of Lupin cvs bore the fungus.

TABLE 2 Preliminary isolation tests

TEST	CV	SEED TYPE	% COLL*
1	2	Lupin	0
	4	Lupin	0
	5	Lupin	0
	6	Lupin	0
	7	Lavatera	0
	8	Lavatera	0
	9	Lavatera	0
	10	Lavatera	1
	11	Lavatera	0

* = Colletotrichum

In Test 2 to provide a better growing medium for the fungus, seeds of cv10 of *Lavatera* were placed on Prune Lactose Yeast agar containing streptomycin and erythromycin added to inhibit bacterial development. This agar (PLYSE) was used throughout as a basic isolation medium. To reduce contaminating fungi half of the seeds were surface sterilised in a chlorine generator, a 3% solution of sodium dichloroisocyanurate dihydrate for 30 seconds, and then dried on absorbent paper in a sterile airflow for about 10 minutes. Twenty plates each bearing 5 seeds were incubated at 21°C and recorded for the presence of the fungus after 3, 5 and 7 days.
The results given in Table 3 show that 99% of untreated seeds were heavily contaminated by fungi; this was reduced to less than 60% by surface sterilisation enabling 2% of seeds infected by *Colletotrichum* to be detected. By comparison with contaminating fungi *Colletotrichum* grew very slowly from infected seeds on to the agar and was obscured by these where seeds were not surface sterilised.

TABLE 3 Preliminary isolation tests

TEST	TREATMENT	PERCENT FUNGI	COLL
3	Surface sterilised	58	2
	Not surface sterilised	99	0

3 Temperature range studies on *Colletotrichum*

A mycelial isolate (B4151) obtained on agar from infected seed was isolated into pure culture and grown for 14 days. Five mm disks cut from the periphery of this culture with a sterile cork borer were placed singly and centrally on to PLYSE agar plates. Four plates each were incubated at temperatures of 5, 10, 15, 20, 25, 30 and 35°C and growth measurements were for 2 diameters for each plate after 3, 7 and 15 days. The mean growth rates at each temperature are given in Fig. 1.

It was apparent that the *Colletotrichum* sp. isolated from *Lavatera* grew optimally at 25°C and can be regarded as a high temperature species.

4 Development of agar test for isolation of fungus from seeds

On the basis of the foregoing work all tests for the isolation of *Colletotrichum* from seeds and for the evaluation of the efficacy of seed treatments were carried out as follows:

4.1 All were done on PLYSE agar.

4.2 150-200 seeds were used per cv or treatment.

4.3 All seeds were surface sterilised except for those that had been treated with fungicides.

4.4 In most cases 25 seeds were allocated per plate.

4.5 Seedborne *Colletotrichum* was recorded after 5-7 days but where there was a doubt concerning the identity of a fungal colony the plates were left for a further 8 days and recorded again.

5 Recognition of *Colletotrichum* growing from seeds on agar

Colletotrichum from *Lavatera* seeds was recognised by its slow growth from seeds producing a star-shaped or amoeboid dark colony with a yellowish fringe 2-4 mm in depth (Plate 1). Under low power binocular examination the asexual fruiting bodies of the fungus, i.e. the acervuli, were often observed on the coats of seeds (Plate 2) and appeared on young radicles as a result of having infected them (Plates 2 and 3).

6 The identity of *Colletotrichum*

Isolates of the fungus from *Lavatera* were sent to Dr John Bailey, Department of Agricultural Science, Long Ashton Research Station, Bristol for identification to specific level. He is a specialist in this area and has identified *Colletotrichum* from *Lavatera* as being closely related to the *C. orbiculare* group. This means that this fungus is likely to be host specific and therefore unlikely to attack other plants. However, this would need to be confirmed by host testing. It may also be that the *Colletotrichum* from lupin, not yet isolated by the writer, may be a completely different species.

7 The incidence of *Colletotrichum* in *Lavatera* and lupin seeds

Lavatera

Colletotrichum was recovered from surface sterilised seeds of three cultivars of *Lavatera* at comparatively high incidences the highest being 18.7% (Table 4). This test was based on 150 seeds per cultivar in 6 replicates of 25 seeds per agar plate. To examine the effect of reducing the number of seeds per plate on the incidence of the fungus 10 plates x 20 surface sterilised seeds per plate of CV 12 were incubated at 25°C. Thirty three seeds (16.5%) bore the fungus and it was concluded that the first test had given a valid result.

TABLE 4 Incidence of Colletotrichum in surface sterilised Lavatera seeds

TEST	CV	NAME	%COLL
4	7	Silver Cup	0
	8	Silver Cup	0
	9	Silver Cup	0
	10	Silver Cup	3.3
	11	Mont Blanc	0
	12	Mont blanc	18.7
	13	Mont Blanc	6
	16	Tanagra	0

Lupin

Similar tests were made on seeds of 22 cvs of commercial lupin seed. The results given in Table 5 indicate that *Colletotrichum* was not recovered from any of the samples each of 150 seeds.

TABLE 5 The incidence of *Colletotrichum* in lupin seeds - composite table based on the results of several tests

NUMBER	DESCRIPTION	%COLL
1	Lupin - Gallery Mixed	0
2	Lupin - Gallery Mixed	0
3	Lupin - Gallery Mixed	0
4	Lupin - Gallery Mixed	0
5	Lupin - Russell Hybrid Mixed	0
6	Lupin - Lulu	0
14	Lupin - Kings Crown Strain	0
15	Lupin - Gallery Yellow	0
25	Lupin - Lulu	0
26	Lupin - Arboreus	0
27	Lupin - Lulu	0
28	Lupin - Lulu	0
29	Lupin - Russell Mixed	0
30	Lupin - Lulu	0
31	Lupin - Minarette	0
32	Lupin - Lulu	0
33	Lupin - Russell	0
34	Lupin - Noble Maiden	0
35	Lupin - Russell Mixed	0
36	Lupin A	0
37	Lupin Y	0
38	Lupin Q	0

Seed treatment

1 The location of the fungus in seeds

Seed of CV 12 were surface sterilised and soaked in sterile distilled water overnight at room temperature. Using a sterile scalpel the seed coats, including the endosperm which was strongly attached to them, were removed and plated separately on to PLYSE. The embryos were also plated out separate from but identifiable with their respective seed coats. A total of 123 seed were dissected and plated out (Table 6).

TABLE 6 The location of *Colletotrichum* in *Lavatera* seeds.

TOTAL SEEDS	% WITH FUNGUS AFFECTING SEED COAT + ENDOSPERM	EMBRYO
123	16.2	3.3

The results show that about 16% of *Lavatera* seeds bore the fungus within the tissues of the seed coat confirming the previous isolation result from surface sterilised seeds. It was impossible in this experiment to separate the endosperm from the seed coats so it is difficult to state the rate of penetration of the endosperm tissues. However, as 3% or slightly more of embryos were infected it is probable that the fungus was capable of relatively deep penetration of the seed tissues. It is presumed that the embryos were not contaminated in the course of dissection.
This result indicated that to achieve eradication of seedborne infection seed treatment fungicides with penetrant properties would be needed.

2 Selection of seed treatment fungicides

An in-house data base was used to identify fungicides which were active at a low concentration (4 ug/ml) against two *Colletotrichum* pathogens *C. spinacea* (anthracnose of red beet) and *C. lindemuthianum* (anthracnose of French bean) and *A. pisi* and *M. pinodes* two other seedborne pathogens with similar reactions to fungicides (Table 7).

TABLE 7 Fungicides selected from agar screen for testing as seed treatments

Fungicide	% fungal growth at 4 um/ml (ppm) C.lind	C.spin	A.pisi	M.pin
Thiram	78	89	21-76	18-100
Benomyl	0	53	0	6
Thiabendazole	0	27	2	57
Tridemorph	19	85	21	100
Triadimefon	100	100	100	100
Carboxin	-	-	66	100
Propiconazole	-	-	18	37
Prochloraz	-	-	7	10
Guazatine	-	-	20	-
Fenpropimorph	-	-	5	4

This provided the initial selection of chemicals for testing as seed treatments.
These included benomyl, thiabendazole, propiconazole, prochloraz, guazatine and fenpropimorph to which thiram was added as a standard.
In addition, fungicides and fungicide mixtures likely to have effect against these fungi were also tested. They included guazatine (27.6% a.i.) plus imazalil (2.3% a.i.) (Panoctine plus), triticonazole (20% a.i.), fenpiclonil (5% a.i.) (Beret), and fenpiclonil (5% a.i.) plus imazalil (1% a.i.)(Beret extra). These fungicides were applied at product rates of 1-2.2 ml/kg.
Carbendazim plus prochloraz, thiabendazole plus thiram (Hy-Tl), and benomyl plus thiram (Benlate T).

Fungicides were applied to 2.5 or 5 g weights of seeds using micro-pipettes for accurate delivery of liquid formulations and a 4-place balance for powders. After their application the 100 ml conicals which contained the seeds were rolled by hand to achieve uniform dispersion of chemical over the seeds.
Fungicides applied to seeds were tested on agar for their eradicant effects on seedborne infection in *Lavatera* (CV 12) and for their effects on seed germination. Germination tests were done in natural light conditions in the laboratory at approximately 22°C. One hundred seeds per treatment in 4 replicates of 25 seeds were placed on moistened germination pads in lidded clear polystyrene boxes (120 x 80 x 20 mm). Germination counts were made after 7 and 14 days.

3 Effects of seed treatments on disease control and seed germination

TEST 1 Twenty five percent of untreated and 18% of surface sterilised seeds were infected (Table 8). At an application rate of 5 g or ml a.i./kg all the fungicides except benomyl controlled infection. However, propiconazole, prochloraz and benomyl significantly ($P< 0.05$) reduced germination when applied at the 5 g rate (Table 8).

TABLE 8 Test 1 - Effects of seed treatments applied at 5 g a.i./kg seed

TREATMENT	%INFECTION	GERMINATION	
		%	>
Nil	24.7	77	58
Nil-SS#	18.0	81	64
Propiconazole	0	53	47
Prochloraz	0	51	46
Benomyl	0.7	46	43
Thiram	0	69	56
Fenpropimorph	0	75	61
		LSD ($P<0.05$)	9.9

= Nil-SS i.e. untreated surface sterilised seeds

> = angularly transformed data

TEST 2 In a virtual repeat of the first test all fungicides were applied at a 1 g or ml a.i./kg rate. Benomyl was omitted from this test because it failed to eradicate infection. In this test (Table 9) against similar levels of total and more deeply seated infection only prochloraz again eliminated the fungus from seeds on agar. Propiconazole significantly ($P< 0.05$) reduced the germination of seeds (Table 9).

TABLE 9 Test 2 - Effects of seed treatments applied at 1 g a.i./kg seed

TREATMENT	%INFECTION	GERMINATION %	GERMINATION >
Nil	24.7	85	67
Nil-SS#	18.0	85	68
Propiconazole	2	59	51
Prochloraz	0	75	60
Thiram	0.7	82	65
Fenpropimorph	2	83	66
		LSD(P<0.05)	10.4

= Nil-SS i.e. untreated surface sterilised seeds: > = angularly transformed data

TEST 3 Germination was unaffected by seed treatment but none of the fungicides tested at cereal rates of application eliminated seedborne infection.

TABLE 10 Test 3 - Effects of seed treatments applied at commercial rates for cereals

TREATMENT	%INFECTION	GERMINATION %	GERMINATION >
Nil	18	66	55
Nil-SS#	16	74	60
Guazatine	10.7	71	58
Guazatine + imazalil	4	78	63
Triticonazole	6.7	66	55
Fenpiclonil	5.3	74	60
Fenpiclonil + imazalil	4.7	69	57
		LSD(P<0.05)	10.8

= Nil-SS i.e. untreated surface sterilised seeds: > = angularly transformed data

TEST 4 In this test benzimidazole combined formulations with prochloraz or thiram were compared for their effectiveness in eradication. Fungicide mixtures were applied at the combined ingredient rate of 1 g or ml a.i./kg seed. Prochloraz which was completely effective in other tests was used at 0.5 ml a.i./kg seed.
All treatments eliminated seedborne infection. Benomyl plus thiram significantly ($P<0.05$) improved germination. Prochloraz reduced germination, but not significantly.

TABLE 11 Test 4 - Effects of fungicide mixtures applied at 1 g a.i./kg seed

TREATMENT	%INFECTION	GERMINATION	
		%	>
Nil	19.3	59	54
Nil-SS#	14.0	73	61
Carbendazim + prochloraz	0	62	61
Prochloraz*	0	55	49
Benomyl + thiram	0	79	65
Thiabendazole + thiram	0	67	56
		LSD(P<0.05)	6.9

= Nil-SS i.e. untreated surface sterilised seeds
> = angularly transformed data
* = prochloraz applied at 0.5 g a.i./kg

CONCLUSIONS AND FUTURE WORK

1 In the course of this study *Colletotrichum* was not islated from the 22 samples of lupin seeds supplied by the seed trade. It was isolated from 3 of the 8 *Lavatera* samples tested to date.

If the lupin seed samples were representative of those available to growers then *Colletotrichum* was not present at a detectable level in the 150-200 seeds which were tested for each cultivar.
If however, the fungus transmits at lower incidences of seedborne infection, i.e. 1 in a 1,000

or 1 in 10,000 then this would not have been detected in these tests.
If anthracnose is a current problem in lupins then it is possible that it is being transmitted from a lower incidence of seedborne infection or that it is being introduced from a source other than that of the seeds.
If the fungus is less of a problem than it was then the tests reported here may support the conclusion that the "health" of commercial seed lots has improved; however, the author has no information on the previous status of seedborne inoculum.

It is concluded that seedborne inoculum is likely to be an important source of infection in *Lavatera.*

2 The technique developed for the isolation of *Colletotrichum* was as follows:
2.1 Seeds were surface sterilised in a solution containing 3% free chlorine.
2.2 They were dried in a sterile airflow.
2.3 Seeds were then placed, 25 per plate, on to Prune Lactose Yeast agar containing streptomycin and erythromycin (PLYSE).
2.4 Plates were incubated at 25°C for 5-7 days in the dark and then assessed for the presence of colonies typical of the fungus growing from seeds.

3 Growth studies on *Colletotrichum* from *Lavatera* in agar culture indicated that it grew at an optimum rate at 25°C in the dark. Plant raising conditions having temperatures between 20-30°C would be ideal for the transmission of this fungus.

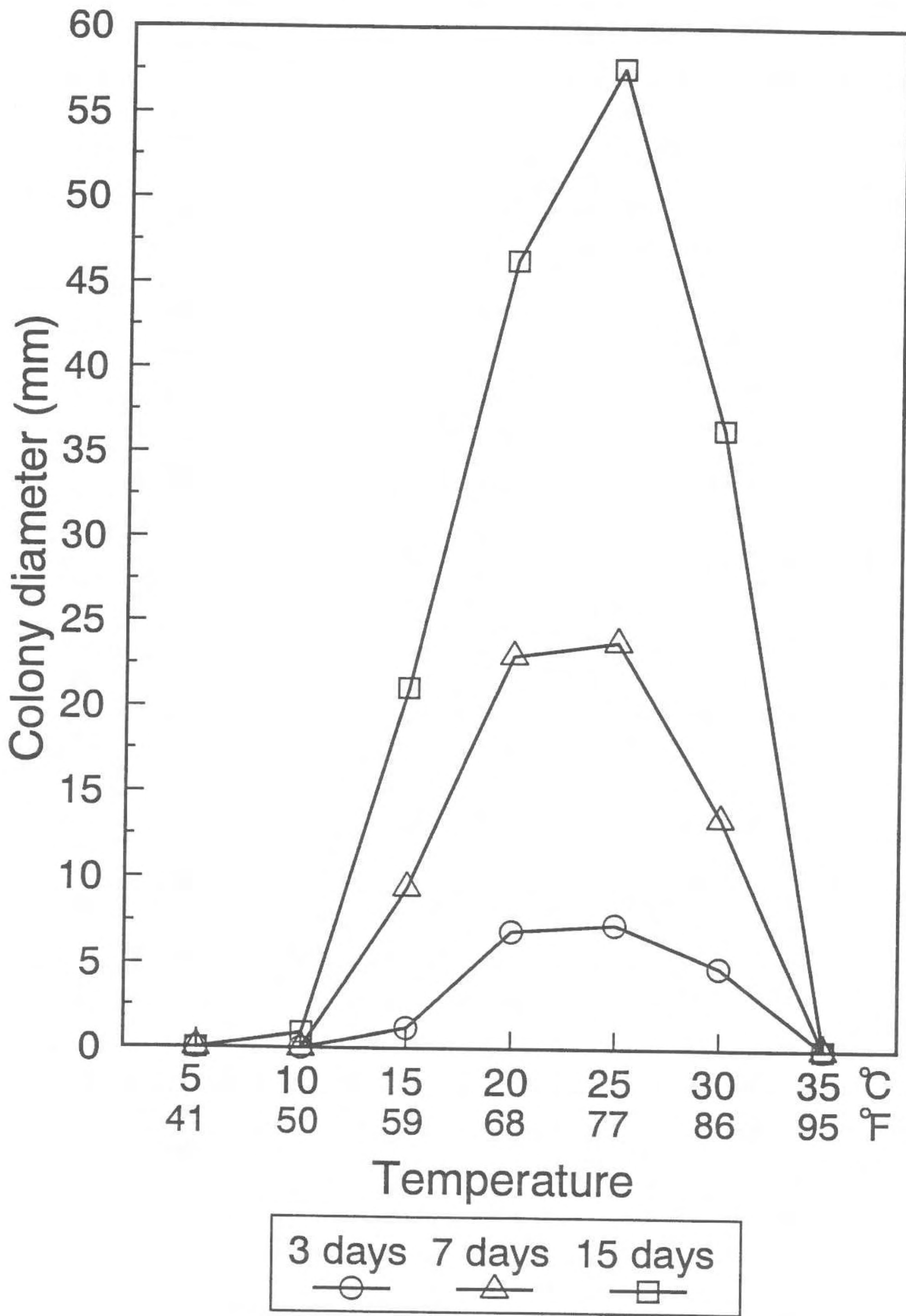
4 The fungus was observed on the seed coats and radicles of seedlings. It caused a decay of radicles on which it produced fruiting bodies (acervuli) containing spores which are the dispersal agents of the fungus. It is concluded from these observations that the fungus was a pathogen.

5 The fungus was identified as belonging to the *C. orbiculare* group which means that it is probably host specific but host range tests need to be made to confirm this. Cross inoculation studies between *Lavatera* and lupin using the *Colletotrichum* spp. which affect both types would be of considerable interest and relevance to the raisers of these crops.

6 Based on limited laboratory tests of fungicides selected by different methods it was found prochloraz alone or combined with carbendazim and thiram combined with benomyl or thiabendazole eradicated seedborne infection without harming the germination of seeds.

7 To further validate these findings it is necessary that seed treatments should be applied to larger amounts of seeds larger samples of which should be tested on agar, moist germination pads, and under plant raising conditions. The tests which have been done to date were completed within a limited time span and must be considered to be preliminary.

Fig.1 Temperature range study



THE FUNGUS

Plate 1. Growth of *Colletotrichum* from infected *Lavatera* seed onto agar.

Plate 2. Fruiting bodies (acervuli) of the fungus developing on a young radicle which has become infected from the diseased seed.

Plate 3. Close-up of the spiny fruiting bodies (acervuli) containing the spores of the fungus. The spores are splash-distributed by rain or overhead irrigation systems.