Gentians:
Integrated Control of Root Rot

HNS47

Final Report

April 1995

Typical symptoms of gentian root rot caused by
Phoma gentianae-sino-ornatae

Diseased plant **Healthy plant**

Project Number:	HNS47
Title:	Gentian - Integrated control of root rot by the use of fungicides and growing medium amendments.
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APPLICATION

The objective of the project was to determine the cause of gentian root rot and to investigate integrated control measures using a combination of compost amendments and fungicides. *Phoma gentianae* was consistently isolated from rotting roots and caused root rotting when inoculated onto healthy roots. Although other fungal pathogens including *Pythium, Rhizoctonia and Cylindrocarpon* were isolated from roots these tended to invade dead or dying root tissue and are not believed to be implicated in the disease.

Of the amendments investigated, bark gave good disease suppression, particularly in low to moderate disease situations. Fungicide drenches with Octave also gave good disease suppression. The combination treatment of bark-amended compost and Octave drenches gave good control of the disease. Dipping young 'thongs' in Octave pre-planting improved disease control dramatically and when combined with the bark compost amendment and post-planting drenches with Octave almost complete control of the disease was achieved, even under very high disease pressure.

SUMMARY OF RESULTS

The objectives of the work were to determine the cause of gentian root rot and to investigate possible control measures.

1993 results:

The incidence of plants grown in peat with no fungicide treatment which showed root rot increased from 72% one month after potting to 100% at early bud. Over the same period the proportion of root length affected increased from 2% to 29%. A *Phoma* species was consistently isolated from affected roots and identified as *Phoma gentianae*. Inoculation of gentian roots with mycelial plugs of *P. gentianae* resulted in root rot; both intact and wounded roots became affected.

The development of root rot was significantly reduced when plants were grown in peat amended with 40% bark or woodfibre; amendment with perlite was ineffective. The development of root rot was also reduced by drenching plants at monthly intervals with Octave; drenches of Bavistin and Fongarid were ineffective. When combined growing-medium amendments and fungicide drenches were evaluated bark amendment with monthly Octave drenches gave very good control, better than either treatment alone. At an assessment on 28 August, the incidence of plants wilting or dead was reduced from 36% (peat) to 15% by bark amendment, to 16% by Octave drenches and to 1% by a combined bark amendment/Octave drench treatment. None of the treatments adversely affected plant quality.

1994 results:

The disease suppression achieved by bark compost amendment and Octave drenches was confirmed in a season with much higher disease pressure than in 1993. Chitin amendments to compost gave no additional disease suppression. Pre-planting dipping of young 'thong' plants gave very significant reductions in disease levels. In combination with bark compost amendment and Octave drenches almost complete control of the disease was achieved. Under the very high disease pressure of 1994 the conventional peat-based compost resulted in 64% plant death by the end of August with no surviving plants considered saleable. The combined treatment of pre-planting dip in Octave, bark compost amendment and post-planting Octave drenches resulted in no plant losses, with 90% of plants saleable.

INTRODUCTION

During the last few years considerable losses of gentians have occurred on nurseries and garden centre outlets in the UK. The symptoms of die-back are associated with a root rot which, in its later stages, is very aggressive and may ultimately result in plant death. The problem has occurred at many outlets, particularly in England, and several ADAS plant pathologists have been involved in attempts to control the disease on commercial nurseries.

The root rot syndrome has presented considerable problems in the isolation and identification of the causal agent(s). Although several fungal organisms have been isolated these have not been shown to be the causal agent. Relatively unsuccessful attempts at fungicidal control in ADAS experiments suggest that the disease may not be due to a simple fungal infection. There may be plant physiological or stress factors which influence the disease progress. There must be continued efforts to clearly identify the causal agent(s) involved. Work by ADAS and the Scottish Agricultural College, Edinburgh has given some indications of fungal involvement and *Phoma gentianae* has been confirmed as a possible causal organism. The use of fungicidal drenches in trials has given some reduction of the disease but not commercially acceptable levels of control.

HDC - funded work on cyclamen fusarium wilt in 1991 (Project PC50) has demonstrated a significant reduction in development of this disease when bark (Cambark Fine) or woodfibre are incorporated into the growing medium. Drenching plants with Octave at monthly intervals also reduced the disease, and the combined effect of bark and Octave appeared to reduce disease more than either treatment on its own. The use of such amendments may be a valuable tool in attempting to control root rotting of gentians.

OBJECTIVES

To determine the causal agent of root rot of gentian and to define possible control measures based on growing medium amendments and the best of currently available fungicides. Specific objectives are:-

- To isolate fungal organisms present in affected roots and thongs.
- To identify such organisms.
- To determine pathogenicity of organisms isolated.
- To assess the use of growing medium amendments in conjunction with post-planting fungicide drenches.
- To investigate different types of growing medium amendment including bark, perlite and woodfibre.
- To investigate the use of chitin as an additional compost amendment.
- To assess the value of a pre-planting dip treatment with Octave.

MATERIALS AND METHODS

Source of plants

Potted plants (cv. *Sino ornata*) were provided by Angus Heathers Nursery, Letham, Forfar, Angus.

Fungal isolation 1993

At the planning stage of the experiment it was hoped to begin isolations from symptomless roots at the beginning of the season and follow the progression of fungal invasion into roots as the season progressed. However, when the plants arrived from Scotland they were already showing root rotting symptoms. The experiment was thus modified and instead of making isolations from untreated plants greater numbers of isolations were made from the compost amendment and fungicide treatments in order to try and understand the interaction of the various fungal species which may be found.

Roots from untreated plants were examined at regular periods through the season and isolations made from 'typical' root lesions. At the end of the season (25 October) 5 plants were removed from each of the 16 treatments. Sixteen isolations were made from each plant in each treatment. Thus, a total of 1,280 isolations were made from the experimental treatments. Root pieces were surface-sterilised in sodium hypochlorite, rinsed 3 times in sterile, distilled water and plated onto potato dextrose agar (PDA) amended with streptomycin. Isolation plates were incubated under near-UV light at 21°C. Colonies were identified after 7 days or sub-cultured for later identification. The main fungi isolated are very variable in their morphology and several cultures were sent to CSL Harpenden for confirmation of identification. isolates of *Phoma* which were suspected as being *Phoma gentianae-sino-ornatae* were sent to IMI, Kew for confirmation of identification.

Pathogenicity tests

Roots of young gentian plants were washed and any plants with root rot were discarded. Mycelial plugs (6 mm diameter) of *P. gentianae* on PDA were inoculated on roots and plants were incubated in a humid chamber at 21°C. Wounding of roots was by removal of a small piece of root tissue. There were 10 inoculation sites/test. The length of root rotting was measured after 4 and 10 days.

Growing media:

Ingredient	Source	Grade	Proportion used
Peat	Irish sphagnum	Medium	60%
Bark	Cambark	100	40%
Woodfibre	J. McLauchlan, Horticulture	Fine	40%
Perlite	Silva Perle	Standard	40%

Fungicides:

Product	Active ingredient	Rate used
Bavistin FL	carbendazim (50% ai)	1 ml/l
Fongarid	furalaxyl (50% ai)	1 g/l
Octave	prochloraz Mn (50% ai)	1 g/l

All fungicides were applied as a drench at 50 ml/plant.

Nutrition

Osmocote Plus (8-9 month; 16-8-12) was incorporated in all media at 2.5 kg/m^3. Magnesium (Dolomite) limestone was incorporated at 1.2 kg/m^3. Ammonium nitrate was added to the peat/bark mixture at 0.4 kg/m^3 and to the peat/woodfibre mixture at 0.2 kg/m^3. Nutrient levels and air-filled porosity (AFP) of all mixes were determined immediately before potting.

Treatments 1993:

	Growing medium	Fungicide drenches
1.	Peat	nil
2.	Peat	Bavistin
3.	Peat	Fongarid
4.	Peat	Octave
5.	Peat/bark	nil
6.	Peat/bark	Bavistin
7.	Peat/bark	Fongarid
8.	Peat/bark	Octave
9	Peat/woodfibre	nil
10.	Peat/woodfibre	Bavistin
11.	Peat/woodfibre	Fongarid
12.	Peat/woodfibre	Octave
13.	Peat/perlite	nil
14.	Peat/perlite	Bavistin
15.	Peat/perlite	Fongarid
16.	Peat/perlite	Octave

Fungicide drenches were applied at monthly intervals from May to August (5 in total). Water was applied where no fungicide was used.

Experiment design 1993

The experiment was a factorial design with two factors (growing medium and fungicide) each at four levels. Treatments were arranged in four randomised blocks with 20 plants/plot arranged pot-tight. Results were analysed by analysis of variance.

Disease Assessments

50 plants were removed at random every two weeks from a batch of 400 untreated plants grown in peat and the occurrence and extent (proportion of root affected length) of root rotting and crown rot were determined. The incidence of plants dead or severely wilting in the fungicide and growing medium experiment was assessed on 28 July, 11 August and 28 August. At termination of the experiment on 5 November, final plant quality and root development were assessed using the following keys:

Regrowth of shoots from plant crown

0 - nil
1 - slight regrowth
2 - significant regrowth

Root extent (% cover of exposed compost surface)

0 - nil
1 - <10%
2 - 11-25%
3 - 26-50%
4 - 51-75%
5 - 76-100%

Root browning (% exposed roots showing browning)

0 - Healthy
1 - <10%
2 - 11-25%
3 - 26-50%
4 - 51-75%
5 - 76-100%

Crop diary 1993

Experiment established - 5 May

Fungicide drenches -	5 May	Assessments -	3 June
	3 June		28 June (stem extension)
	28 June		28 July (early blue bud)
	28 July		11 August
	25 August		25 August (full bloom)
			1 October (late bloom)
			5 November

RESULTS 1993

Nutrition

Initial nutrient levels were satisfactory (Table 1). Amendment of peat with 40% bark, or perlite did not increase the AFP according to the test method; amendment with woodfibre resulted in a small increase.

Development of root rot

Root rot was visible at trace levels on a small proportion of plants. When they were received one month after potting 72% of plants were affected, the average extent of root rot being 2.3% of root length (Table 2). Root rot developed steadily over the next 3 months and by the end of August all plants were affected, the average extent of root rot being 37.8%, with over 75% of roots affected to a greater or lesser extent.

Effect of growing media and fungicides

The incidence of plants showing severe wilting or death of all shoots in peat growing medium and without fungicide treatment increased from 10% on 28 July to 36% on 28 August (Table 3). At an assessment on 11 August the mean incidence of dead or wilting plants was significantly reduced by two growing medium amendments (bark and woodfibre) and one fungicide treatment (Octave) (Table 3). The incidence of affected plants grown in peat/bark and drenched monthly with Octave remained at 1% throughout the experiment.

Plant quality

At termination of the experiment on 5 November none of the growing media or fungicide treatments significantly affected the mean number of green shoots/plant or regrowth from the plant crown (Table 4).

Pathogenicity tests

Inoculation of both intact and wounded gentian roots with mycelial plugs of *P. gentianae* resulted in root lesions after 4 days. No lesions developed on control plants inoculated with agar. The mean lesion length after 10 days was 14.8 mm on intact roots and 12.0 mm on wounded roots. *P. gentianae* was re-isolated from rot lesions.

Table 1. Initial nutrient levels and AFP of growing media.

Treatment	pH	Phosphorous mg/l (index)	Potassium mg/l (index)	Magnesium mg/l (index	Conduct µs (index)	Nitrate mg/l N (index)	Ammonium mg/l N (index)	AFP (mean of 2 determinations
Peat	5.1	26 (4)	70 (2)	23 (3)	73 (0)	13 (0)	14.0 (0)	16.0
Peat/bark	5.0	22 (4)	44 (1)	18 (3)	209 (1)	80 (3)	54.0 (2)	11.9
Peat/woodfibre	5.2	4 (0)	7 (0)	6 (1)	153 (1)	61 (3)	43.0 (1)	18.2
Peat/perlite	5.3	3 (0)	11 (0)	6 (1)	76 (0)	19 (1)	17.0 (0)	14.0

Table 2. Development of root rot in untreated plants

Growth Stage	Date	% plants affected	% roots affected	% root length affected	% plants with crown rot
One month after potting	3 Jun	72	21.1	2.3	1.0
Stem extension	28 Jun	96	35.3	4.5	22.0
Early blue bud	28 Jul	100	68.0	29.1	64.0
Full bloom	25 Aug	100	75.3	37.8	78.0
Late bloom	1 Oct	100	80.5	39.2	86.0
End of season	26 Oct	100	100	40.3	100 *

18% of plants were dead at the end of the season (26 October)

Table 3. Effect of treatments on incidence of dead or wilting plants

Treatment	Mean % of plants dead or wilting		
	28 July	11 August	28 August
Growing medium			
Peat	3.5	12.0	23.7
Bark	1.5	6.8	9.5
Woodfibre	2.5	8.5	14.2
Perlite	4.7	17.2	30.7
Significance	NS	**	***
SED	1.84	3.13	4.17
Fungicides			
Water	4.5	15.0	26.0
Bavistin	3.2	12.0	21.5
Fongarid	3.2	13.5	20.5
Octave	1.2	3.2	10.2
Significance	NS	**	**
SED	1.84	3.13	4.17
Growing medium x Fungicide			
P - Water	10	21	36
Bavistin	3	18	31
Fongarid	0	6	14
Octave	1	3	16
P/B - Water	3	10	15
Bavistin	2	9	12
Fongarid	0	4	10
Octave	1	1	1
P/W - Water	2	6	22
Bavistin	1	5	12
Fongarid	4	17	16
Octave	3	6	7
P/Pe - Water	3	23	33
Bavistin	7	16	31
Fongarid	9	27	42
Octave	0	3	17
Significance	NS	*	NS
SED	3.68	6.26	8.35

P - peat; B-bark; W - woodfibre; Pe - perlite

NS - not significant; * - significant at p=0.05; ** - significant at p=0.01

These data are represented graphically in Appendix 1

Table 4. Effect of treatments on final plant quality - 5 November 1993

Treatment		No. green stems/plant	No. dead stems/plant	Mean total stems/plant	Regrowth from crown (0-2)	Root extent (0-5)	Root browning (0-5)
Growing medium							
Peat		6.8	0.7	7.5	1.2	3.2	2.5
Peat/Bark		6.9	0.3	7.2	1.2	3.8	1.8
Peat/Woodfibre		6.1	0.3	6.4	1.3	3.3	1.8
Peat/Perlite		6.9	0.6	7.5	1.2	3.4	2.0
Significance		NS	*	***	NS	***	***
SED		0.31	0.17	0.27	0.06	0.15	0.15
Fungicides							
Water		6.4	0.7	7.0	1.2	3.4	2.1
Bavistin		7.0	0.5	7.4	1.3	3.6	2.6
Fongarid		6.5	0.6	7.1	1.2	3.1	1.7
Octave		6.9	0.2	7.1	1.3	3.5	1.7
Significance		NS	NS	NS	NS	*	***
SED		0.31	0.17	0.27	0.06	0.15	0.15
Growing medium x Fungicides							
Peat -	Water	6.2	1.0	7.2	1.2	3.1	2.4
	Bavistin	6.7	0.7	7.4	1.2	3.4	3.2
	Fongarid	7.2	0.6	7.8	1.2	3.0	2.2
	Octave	7.2	0.6	7.7	1.2	3.1	1.9
Peat/Bark -	Water	6.2	0.6	6.8	1.2	3.6	2.2
	Bavistin	7.2	0.4	7.6	1.2	3.8	2.5
	Fongarid	6.8	0.2	7.0	1.2	3.5	1.3
	Octave	7.4	0	7.4	1.2	4.5	1.4
Peat/ Woodfibre -	Water	5.7	0.3	6.0	1.1	3.2	2.0
	Bavistin	6.8	0.1	6.9	1.4	3.7	2.0
	Fongarid	5.8	0.5	6.3	1.4	3.1	1.6
	Octave	6.2	0.1	6.4	1.4	3.1	1.8
Peat/Perlite-	Water	7.4	0.6	8.0	1.1	3.8	1.9
	Bavistin	7.0	0.6	7.6	1.4	3.4	2.8
	Fongarid	6.1	1.2	7.3	1.1	2.9	1.8
	Octave	6.9	0.2	7.0	1.1	3.6	1.6
Significance		NS	*	NS	NS	NS	NS
SED		3.68	6.26	0.54	0.12	0.29	8.35

NS - not significant; * - significant at p=0.05; ** - significant at p=0.01; *** - significant at p=0.001

Table 5 Effects of treatment on Fungal Species Isolated

Treatment		No. of isolates of each fungal species (from 80 isolations) 25/10/93				
		P.gentianae	*T.viride*	*C.destructans*	*Penicillium*	*Others*
Peat -	Water	45	26	0	9	
	Bavistin	16	19	5	10	
	Fongarid	17	13	12	4	
	Octave	4	50	11	15	
Peat/Bark -	Water	30	10	7	3	1 *Fusarium*
	Bavistin	32	30	1	4	
	Fongarid	41	28	1	1	
	Octave	24	34	4	8	
Peat/ Woodfibre -	Water	36	20	6	1	
	Bavistin	42	20	0	0	2 *Mucorales*
	Fongarid	22	28	2	6	
	Octave	41	3	0	3	
Peat/Perlite-	Water	20	48	3	2	
	Bavistin	4	60	9	4	
	Fongarid	8	54	6	1	
	Octave	13	43	7	3	2 *Mucorales*

Key - *P.gentianae: Phoma gentianae-sino-ornatae* (See Appendix 2)
T. viride: Trichoderma viride
C. destructans: Cylindrocarpon destructans

The main fungi isolated from the gentian roots are given in the key of Table 5. Relatively few fungal species were found on the gentian roots, even late in the season. *P. gentianae* was consistently isolated from roots in all treatments and although some fungicide treatments reduced the number of isolations of each fungal species this did not correlate well with the vigour of the plant from which the isolations were made. This may reflect the situation which is frequently observed when fungicides are applied to control root diseases i.e. the fungicides are actually fungistatic. This can allow fungi to regrow as the fungistatic effect of the fungicide declines following cessation of treatment. *Trichoderma viride* was frequently isolated from roots in all treatments and it is possible that this fungus may play a part in the disease complex. It is known that *Trichoderma spp.* can be antagonistic to other root rotting fungi but its role in this root rotting complex is not understood.

Laboratory Examinations 1994:

It was planned to destructively examine 10 'control' plants grown in peat-based compost every 2 weeks throughout the season and to make 20 isolations per plant, giving 200 fungal isolations every 2 weeks. However, the disease severity was so great that as the season progressed control plants died very quickly and by the end of June few plants remained for laboratory examination.

Treatments 1994:

Treatment	Growing Medium	Fungicide Drench	Pre-planting dip
1.	Peat	Nil	Nil
2.	Peat/Bark	Nil	Nil
3.	Peat/Bark/Chitin	Nil	Nil
4.	Peat/Bark	Octave	Nil
5.	Peat/Bark/Chitin	Octave	Nil
6.	Peat	Nil	Octave
7.	Peat/Bark	Nil	Octave
8.	Peat/Bark/Chitin	Nil	Octave
9.	Peat/Bark	Octave	Octave
10.	Peat/Bark/Chitin	Octave	Octave
11.	Peat/Chitin	Nil	Nil
12.	Peat	Octave	Nil
13.	Peat/Chitin	Octave	Nil

Fungicide drenches were applied at 4 week intervals at a concentration of 1g/litre product. Water was applied where no fungicide was applied. The pre-planting dip concentration was 1g/litre product.

Compost amendments:

Bark (Cambark 100) @ 40% amendment.
Chitin (Ocean Supermix) @ 1.5 kg/M^3

Additional compost requirements for peat/bark mix:

Peat/Bark (600 litres peat/400 litres bark)

Osmocote Plus (4) 8/9 month 16-8-12 2.5 kg

Mg limestone (Dolomite) 1.2 kg

Ammonium nitrate (Nitram) 0.4 kg

Crop Diary 1994

April 1994: Thong plants potted into 75 mm square pots. Pre-planting Octave dip treatment applied to specific treatments.

19 May: Plants arrive from Scotland, experiment established.

Assessment Dates:

19 May First samples taken for root rot assessment.

7 June: Assessment of % plant death in each treatment made.

16 June: Plants taken for fungal isolation.

29 June: Plants taken for fungal isolation. Assessment of % plant death.

20 July: Plants taken for fungal isolation.

17 August: Assessment of % plant death in each treatment made.

31 August: Assessment of % plant death in each treatment made.

Treatment dates:

19 May First drench treatment applied.

16 June Second drench treatment applied.

20 July Third drench treatment applied.

17 August Fourth drench treatment applied.

Experiment design 1994

The experiment was a randomized block design with 4 replicates. Each plot comprised 20 plants.

RESULTS 1994

Development of root rot

Root rotting was apparent on plants in May on arrival from Scotland. Laboratory tests confirmed *Phoma gentianae* in 63 out of 200 isolations. The rate of disease progression and disease severity was much higher in 1994 than in the previous year. Plant death was rapid and by early June the untreated control treatments had an average of 30% plants dead.

Effects of growing media and fungicides

The effect of the pre-planting Octave dip was very clear from the first assessments made on 7 June. Untreated controls showed a mean of 30% plants dead. The mean of all Octave pre-planting dip treatments was 0.5% plants dead. Four out of the five treatments which included a pre-planting dip treatment with Octave had no plant losses at this stage. The bark amendment alone gave a reduction in plant losses from 30% to 11.3% at this stage.

By 17 August the untreated control plants showed 57.5% plants dead with only 2.5% of remaining plants considered saleable. (Saleable plants were defined as those with strong, well grown plants with several stems with flower buds visible). Treatments which included a pre-planting Octave dip ranged from 0-13.8% plants dead with saleable plants ranging from 58% to 97%. The treatment of bark amended compost plus Octave drenches which had been so successful in 1993 was less successful under very high disease pressure, resulting in 44% plants saleable in mid August. The best treatments at this stage were those which included bark amendment, Octave drenches and a pre-planting Octave dip. In such treatments plant losses were minimal (0-1.3%) with 94-97% saleable plants.

By the end of August the untreated control plots had 64% plants dead with none of the remaining plants saleable. In contrast, the treatments which included a pre-planting Octave dip, bark amendment and Octave drenches had 87-90% saleable plants with only 0-1.3% plants dead.

Results from the above assessment dates are given in full in Tables 6, 7 and 8.

Table 6. Percentage of plants dead 7 June 1994

	Treatment	% Plants Dead 7/6/94
1.	Untreated	30.0
2.	+ Bark	11.3
3.	+ Bark/Chitin	32.5
4.	+ Bark + Octave drenches	13.8
5.	+ Bark/Chitin + Octave drenches	21.3
6.	Octave Dip Peat	0
7.	Octave Dip + Bark	0
8.	Octave Dip + Bark/Chitin	2.5
9.	Octave Dip + Bark + Octave drenches	0
10.	Octave Dip + Bark/Chitin + Octave drenches	0
11.	Peat/Chitin	30.0
12.	Peat + Octave drenches	12.5
13.	Peat/Chitin + Octave drenches	18.8

Table 7. Percentage of plants dead and % plants saleable 17 August 1994

	Treatment	% plants dead	% plants saleable
1.	Untreated	57.5	2.5
2.	+ Bark	43.8	8.8
3.	+ Bark/Chitin	48.8	3.8
4.	+ Bark + Octave drenches	15.0	43.8
5.	+ Bark/Chitin + Octave drenches	20.0	38.8
6.	Octave Dip Peat	1.3	61.3
7.	Octave Dip + Bark	1.3	78.8
8.	Octave Dip + Bark/Chitin	13.8	58.8
9.	Octave Dip + Bark + Octave drenches	1.3	93.8
10.	Octave Dip + Bark/Chitin + Octave drenches	0	97.5
11.	Peat/Chitin	33.8	11.3
12.	Peat + Octave drenches	13.8	45.0
13.	Peat/Chitin + Octave drenches	15.0	11.3

Table 8. Percentage of plants dead and % plants saleable 31 August 1994

	Treatment	% plants dead	% plants saleable
1.	Untreated	63.8	0
2.	+ Bark	50.0	2.5
3.	+ Bark/Chitin	58.8	0
4.	+ Bark + Octave drenches	12.5	37.5
5.	+ Bark/Chitin + Octave drenches	18.8	31.3
6.	Octave Dip Peat	0	27.5
7.	Octave Dip + Bark	5	43.8
8.	Octave Dip + Bark/Chitin	13.8	21.3
9.	Octave Dip + Bark + Octave drenches	1.3	87.5
10.	Octave Dip + Bark/Chitin + Octave drenches	0	90.0
11.	Peat/Chitin	46.3	3.8
12.	Peat + Octave drenches	15.0	23.8
13.	Peat/Chitin + Octave drenches	13.8	3.8

The results from tables 6,7 and 8 are shown graphically in Appendix 3.

Laboratory Isolations

Root pieces were surface-sterilised in sodium hypochlorite, rinsed 3 times in sterile, distilled water and plated onto potato dextrose agar (PDA) amended with streptomycin. Isolation plates were incubated under near-UV light at 21°C. Colonies were identified after 7 days or sub-cultured for later identification. The main fungi isolated are very variable in their morphology and several cultures were sent to CSL Harpenden for confirmation of identification.

Isolations 25/5/94:

200 isolations made from 10 plants:-

Fungal Organism	No. isolations
Trichoderma viride	112
Phoma gentianae	63
Penicillium spp.	1
Mucorales spp.	4
Others (Nil, bacteria, Yeasts)	20

Isolations 6/6/94:

72 isolations made from 6 plants:-

Fungal Organism	No. isolations
Trichoderma viride	50
Phoma gentianae	13
Penicillium spp.	9

Because roots at this stage were very rotten, serological test kits (Alert Kits) were used to test for other possible pathogens. *Pythium* and *Rhizoctonia* were confirmed by serological testing and confirmed by laboratory float testing.

Isolations 20/6/94:

72 isolations made from 6 plants:-

Fungal Organism	No. isolations
Trichoderma viride	58
Phoma gentianae	14

Once again, because roots at this stage were very rotten, serological test kits ('Alert' Kits) were used to test for other possible pathogens. *Pythium* and *Rhizoctonia* were confirmed by serological testing and confirmed by laboratory float testing.

Isolations made on 12/7/94, 21/7/94, 9/8/94, 24/8/94 and 14/9/94 gave predominantly *Trichoderma, Pythium and Penicillium*. As roots became very rotten isolation of *Phoma* became more and more difficult. During this July/August period, the period during which advisory samples would be likely to arise, it would be very rare to isolate *Phoma* from the samples. This phenomenon partly explains why, during the early advisory investigations into the cause of gentian root rotting, little progress was made in identifying the pathogen involved. Interestingly, if plants survived into August (largely due to rooting of thongs from the mother plant), *Phoma* could once again be isolated from young roots. This is evidence that thongs become infected very early in their life and are almost certainly carrying infection when they are taken from mother plants which are affected by *Phoma*.

DISCUSSION

The work reported indicates that *Phoma gentianae* is a significant factor in the cause of root rot of gentians. Punithalingham & Harling (1993) have also recently reported *Phoma gentianae* associated with root rot of gentians. These are the first reports to describe the cause of a root rot on gentians. This HDC-funded experiment has confirmed the association of *Phoma gentianae* with a severe root rot of gentian and has provided information to allow a strategy for disease control.

The effect of prochloraz (Octave) drenches in providing partial control of the disease is in agreement with reports of the activity of this fungicide in controlling *Phoma* diseases of other crops (Evans *et al,* 1984).

The effect of bark and woodfibre amendments in providing partial control of the disease appears to be the first report of a growing medium amendment in controlling a disease caused by a species of *Phoma.* Growing medium amendments have been reported to control diseases caused by species of *Fusarium, Phytophthora* and *Pythium* (Hoitink *et al.*, 1991). Perlite amendment appeared to increase the occurrence of phoma root rot; this effect has previously been reported with fusarium wilts of carnation and cyclamen. The use of chitin as a further compost amendment had no significant effect on disease suppression over and above that achieved by bark alone.

The 1993 results indicated that a combined bark amendment and Octave drench treatment gave good disease control; the effect of the two control components appeared to be additive. Further work in 1994 confirmed these results. The further addition of a pre-planting Octave dip, in addition to the compost amendment and Octave drenches, achieved almost complete control of the disease. The pre-planting dip treatment was highly effective across the range of other treatments and was they key addition to the 1993 treatment list. This addition allowed almost complete disease control in a season where the 'normal' treatment i.e. untreated plants, resulted in no saleable plants.

The mechanism of root disease control using bark amendments is little understood. A biological mechanism has been suggested for several host/pathogen/amendment combinations. Further work is required to investigate the mechanism in this instance lest the effect is inadvertently obviated by a pesticide treatment or growing practice.

REFERENCES

PUNITHALINGAM, E. & HARLING, R. (1993). *Phoma gentianae-sino-ornatae* sp. nov. from *Gentiana sino-ornata* with root rot. *Mycological Research* **97**, 1299-1304. (See Appendix 2)

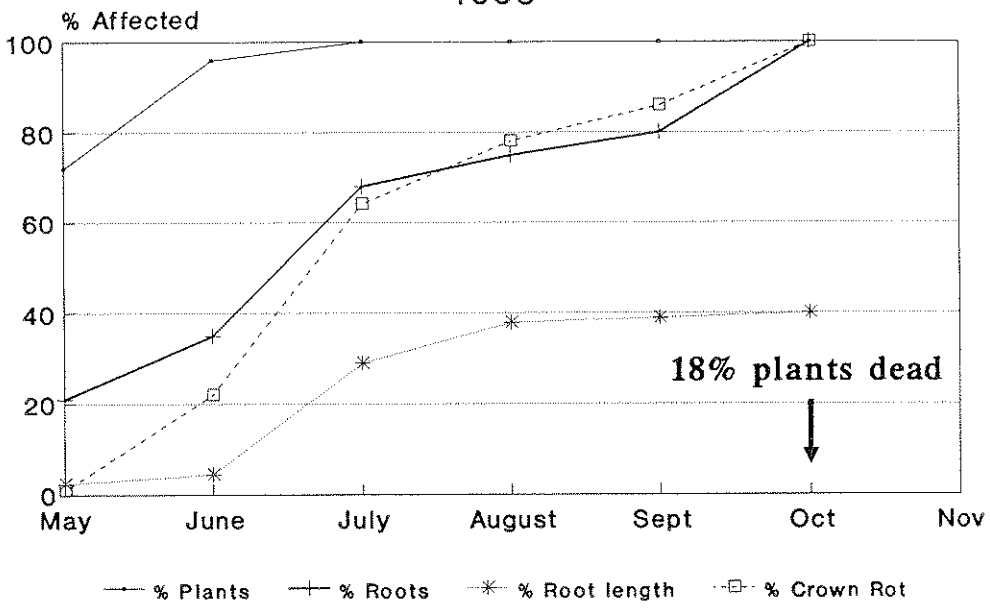
HOITINK, H. A. J., INBAR, Y. & BOEHM, M. J. (1991). Status of compost-amended potting mixes naturally surpressive to soil-borne diseases of floricultural crops. *Plant Disease* **75**, 869-870.

EVANS, E.J., GLADDERS, P., DAVIES, J.M.L., ELLERTON, D.R., HARWICK, N.V., HAWKINS, J.H., JONES, D.R. & SIMKIN, M.B. (1984). Current status of diseases and disease control of winter oilseed rape in England. Aspects of Applied Biology 6, 232-334.

Disease Development in Untreated Plants

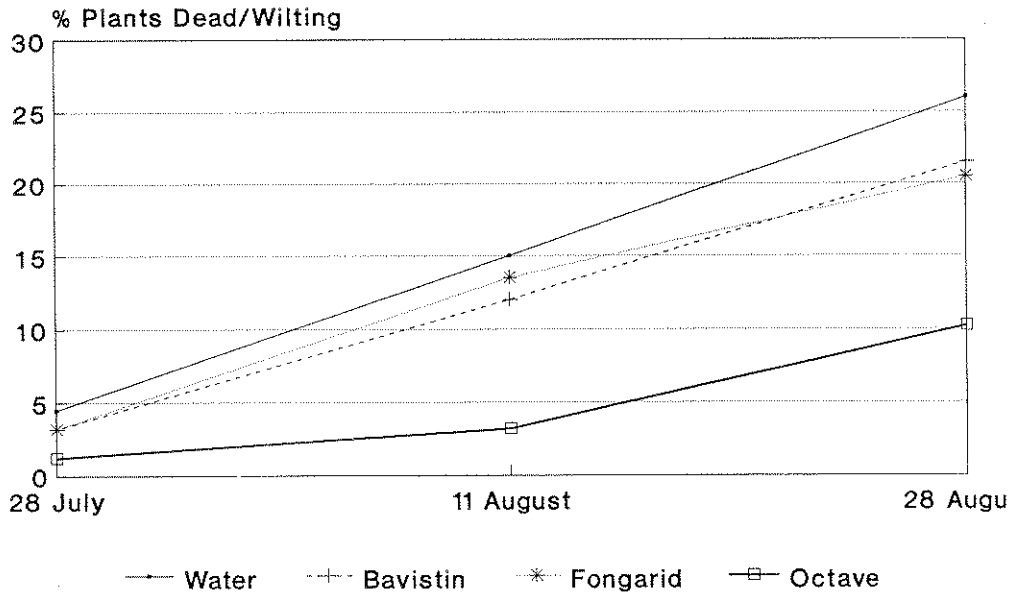
Gentian Root Rot - Phoma gentianae

1993



Gentian Root Rot Control 1993

Effects of Fungicides in Peat/Bark



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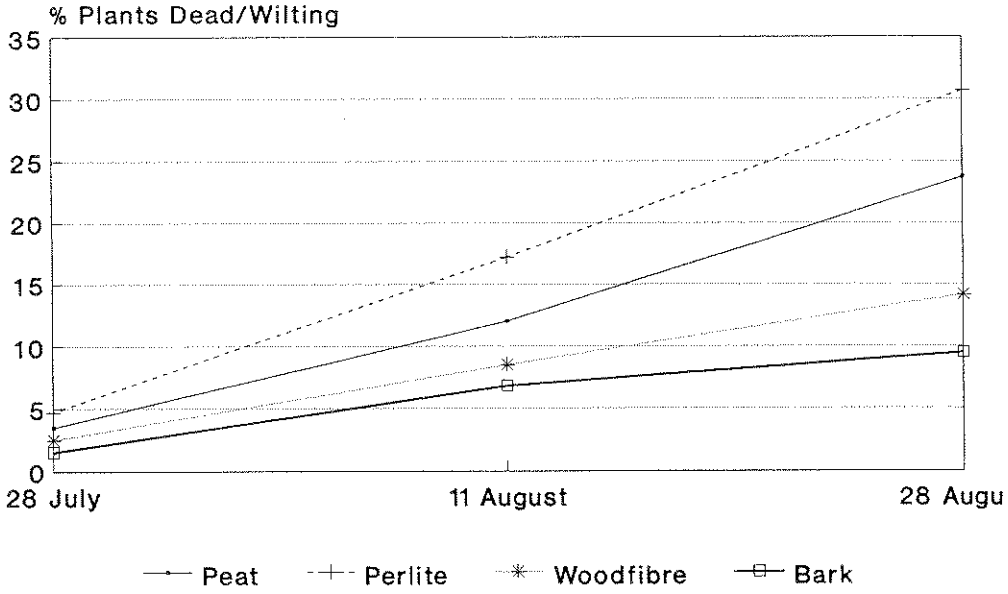

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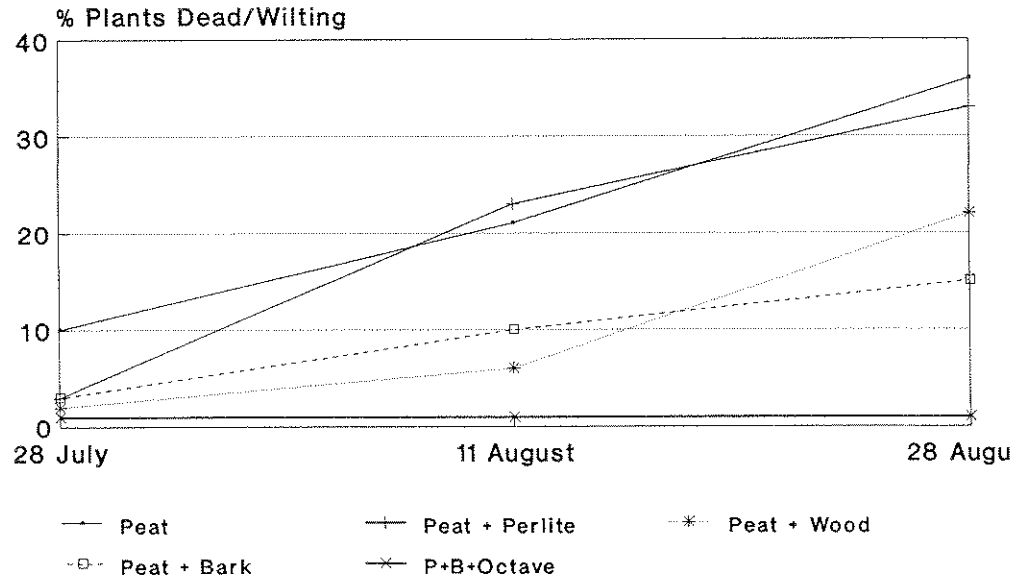

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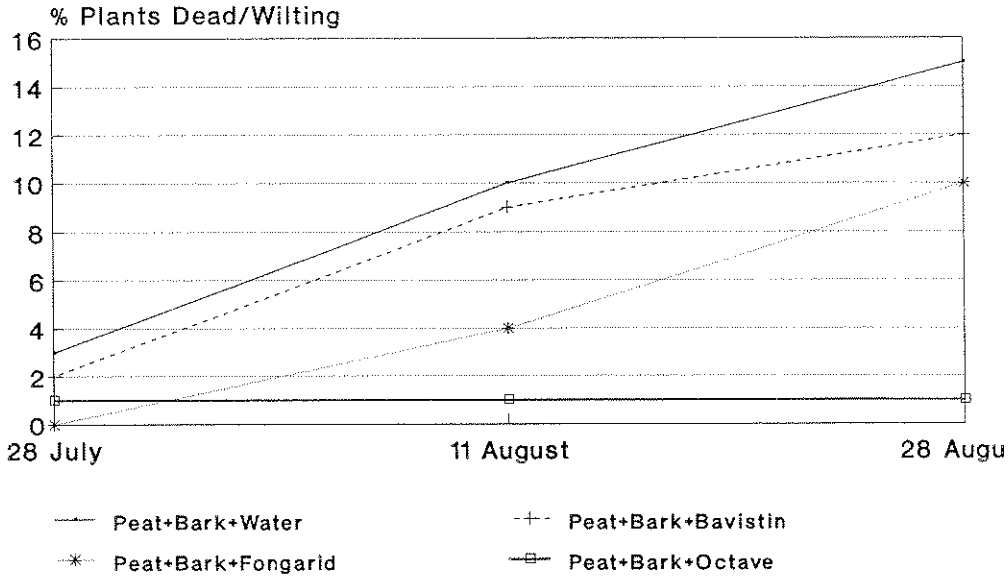

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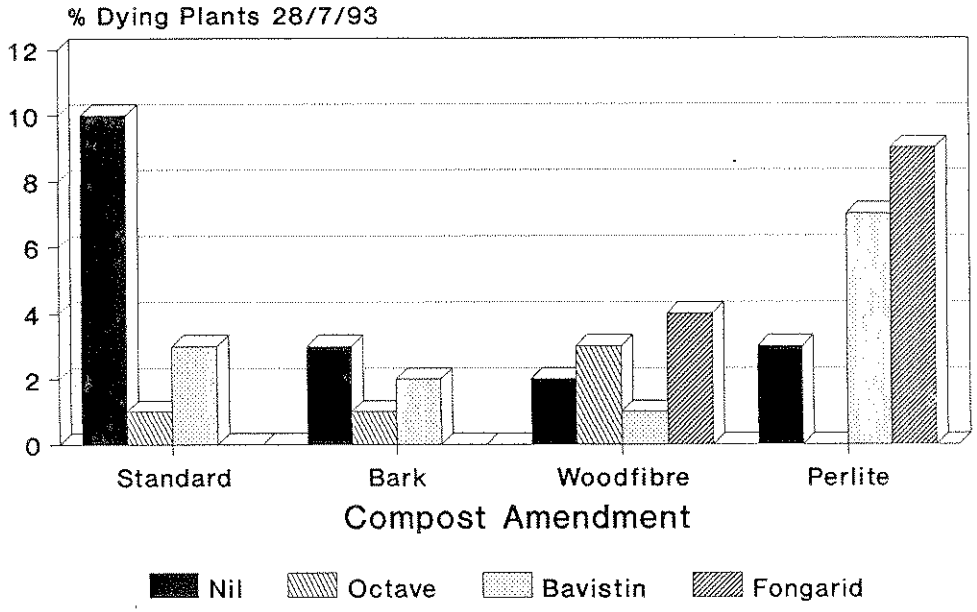

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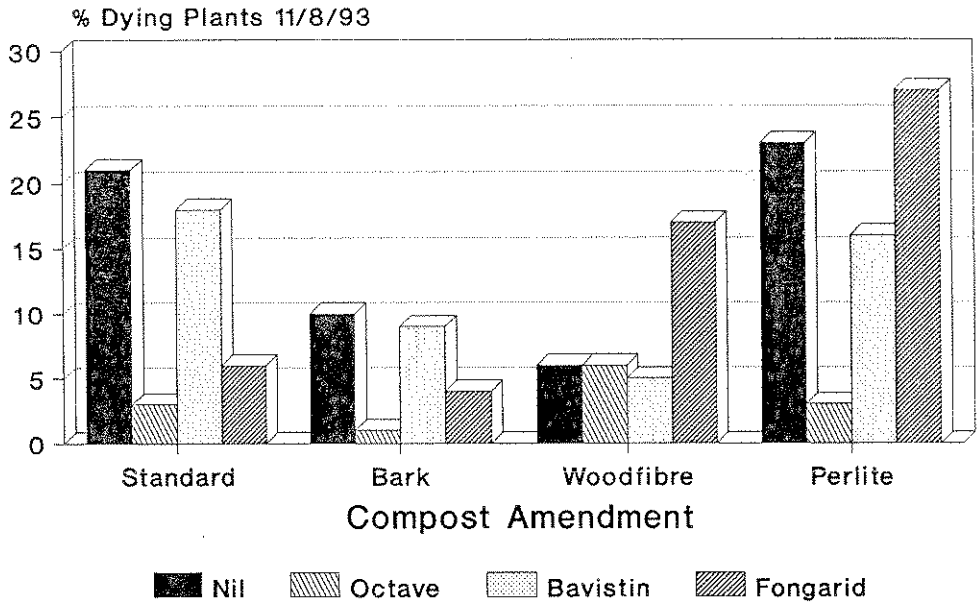
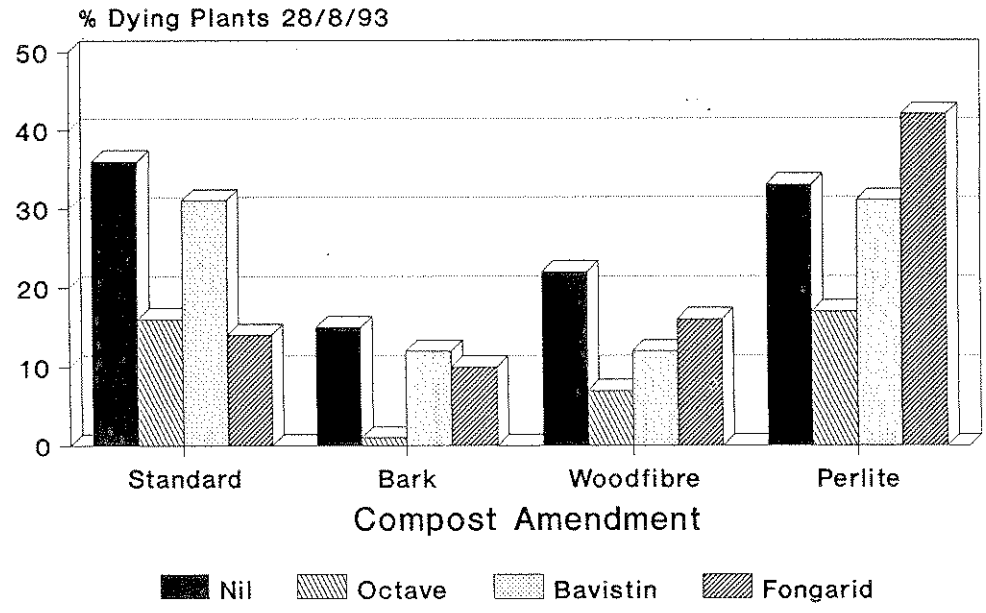


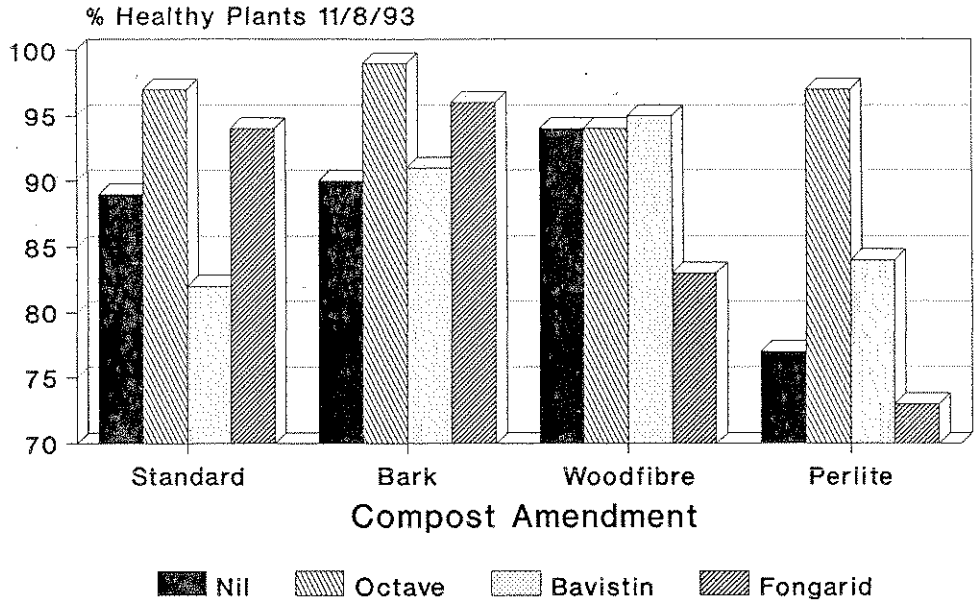
Gentian Root Rot Control

Compost Amendments and Fungicides



Gentian Root Rot Control

Compost Amendments and Fungicides



Mycol. Res. 97 (11): 1299–1304 (1993) *Printed in Great Britain*

Phoma gentianae-sino-ornatae sp. nov. from *Gentiana sino-ornata* with root rot

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Phoma gentianae-sino-ornatae sp. nov. isolated from rotting roots of living *Gentiana sino-ornata* plants in the U.K. showing root rot symptoms is described, illustrated and compared with those *Phoma* species known from gentian, and other *Phoma* species causing root rot of various plants.

Since 1989 severe root rot and blackening of tissue revealing sterile hyphal aggregations or sclerotium-like bodies have been observed in gentian, *Gentiana sino-ornata* I. B. Balf., but the fungus associated with the symptoms was only conclusively identified as a *Phoma* species in 1991. When the disease was first noticed, establishing the identity of the fungus based on morphology proved difficult, because only sterile hyphal aggregations resembling chlamydospores or sclerotium-like structures were seen in abundance on and within the infected host and in 5-wk-old cultures of the fungus on oat agar. Pycnidial conidiomata were sparse on the host and in cultures derived from rotting roots of *G. sino-ornata*. In culture, hyphal connexions between sclerotium-like bodies and pycnidia were a common feature, suggesting that the two states were linked. The behaviour of the fungus on the host plant and in culture, the pycnidia, conidia and anatomical characters of the sclerotium-like bodies indicated that the fungus belongs in *Phoma*. A comparison with those *Phoma* species already described from gentian showed differences in morphology, as did a comparison of the *Phoma* species associated with root rot of gentian with those *Phoma* species known to cause root rot of other plants. In view of the existence of sufficient recognizable differences the *Phoma* isolated from rotting roots of *G. sino-ornata* is described as a new taxon.

The *Phoma* species described here was isolated from rotting roots of *Gentiana sino-ornata* collected in a nursery by one of us (R.H.) in N.E. Scotland in 1990.

Phoma gentianae-sino-ornatae Punithalingam & Harling sp. nov. (Figs 1–9)

Etym.: reference to the host, *Gentiana sino-ornata*

Coloniae in agaro 'oat meal' floccosae, vinoso-bubalinae vel hinnuleae, mycelio aerio abundanti, marginem laeves; reversum atrobrunneum vel nigrum. *Mycelium* hinnuleum, densum, ex hyphis septatis, ramosis, laevibus vel exasperatis. *Chlamydosporae* sparsae, atrobunneae, laeves vel scaberosae, primum unicellulares vel bicellulares, postremo multicellulares. *Sclerotia* abundantia, brunnea vel nigra, globosa vel irregularia, 60–120 (–170) µm lata. *Conidiomata* sparsa, pycnidialia, brunnea vel atrobrunnea, postea nigrescentia, subglobosa, unilocularia, 180–220 µm lata, 200–250 µm alta, ostiolata. Paries pycnidialis pseudoparenchymaticus, ex 3–5 stratis cellularum (textura angularis) compositus; strato extimo flavido brunneo vel atrobrunneo, stratis interioribus hyalinis. *Ostiola* fere circularia 15–25 µm lata. *Cellulae conidiogenae* hyalinae, subglobosae, doliiformes vel ampulliformes, phialidicae, discretae, determinatae, laeves ex intimis stratis cellularum cavitatis pycnidii exorientes. *Conidia* hyalina, laevia, aseptata, in medio septata vel inaequaliter 1-septata, raro 2-septata, ad septum non constricta, cylindrica vel cylindrica-oblonga, ovoidea, interdum curvata, basi rotundata vel obtusa, apice rotundata, 6–11 (–16) × 2–3 (–3·5) µm, guttulata.

E radice putrescente *Gentianae sino-ornatae*, N.E. Scotland, U.K., 11 June 1990, R. Harling, (Ri) IMI 341116 holotypus.

Colony on 'oat agar' floccose, vinaceous buff to fawn with abundant aerial mycelium; smooth at the margin, reverse dark brown to black, with abundant sclerotia and sparse conidiomata. *Mycelium* moderately brown, dense, composed of septate, branched, smooth to roughened hyphae. *Chlamydospores* sparse, dark brown, unicellular or bicellular, later becoming multicellular, smooth or roughened. *Sclerotia* abundant brown to black, globose to irregular, 60–120 (–170) µm wide. *Conidiomata* sparse, pycnidial, brown to dark brown later becoming black, subglobose, unilocular, 180–220 µm wide, 200–250 µm high, ostiolate. Pycnidial wall pseudoparenchymatous, composed of 3–5 layers of cells, the outer layers yellowish brown to dark brown, the inner layers hyaline. *Ostiole* nearly circular, 15–25 µm wide. *Conidiogenous cells* formed from the inner layer of cells lining the pycnidial cavity, hyaline, ampulliform to doliiform, phialidic (*sensu* Sutton, 1980), discrete, determinate, smooth. *Conidia* hyaline, cylindrical to oblong, ovoid, sometimes curved, base rounded or obtuse, apex rounded, aseptate to medianly or unequally uniseptate, rarely 2-septate, not constricted at the septum, 6–11 (–16) × 2–3 (–3·5) µm, guttulate.

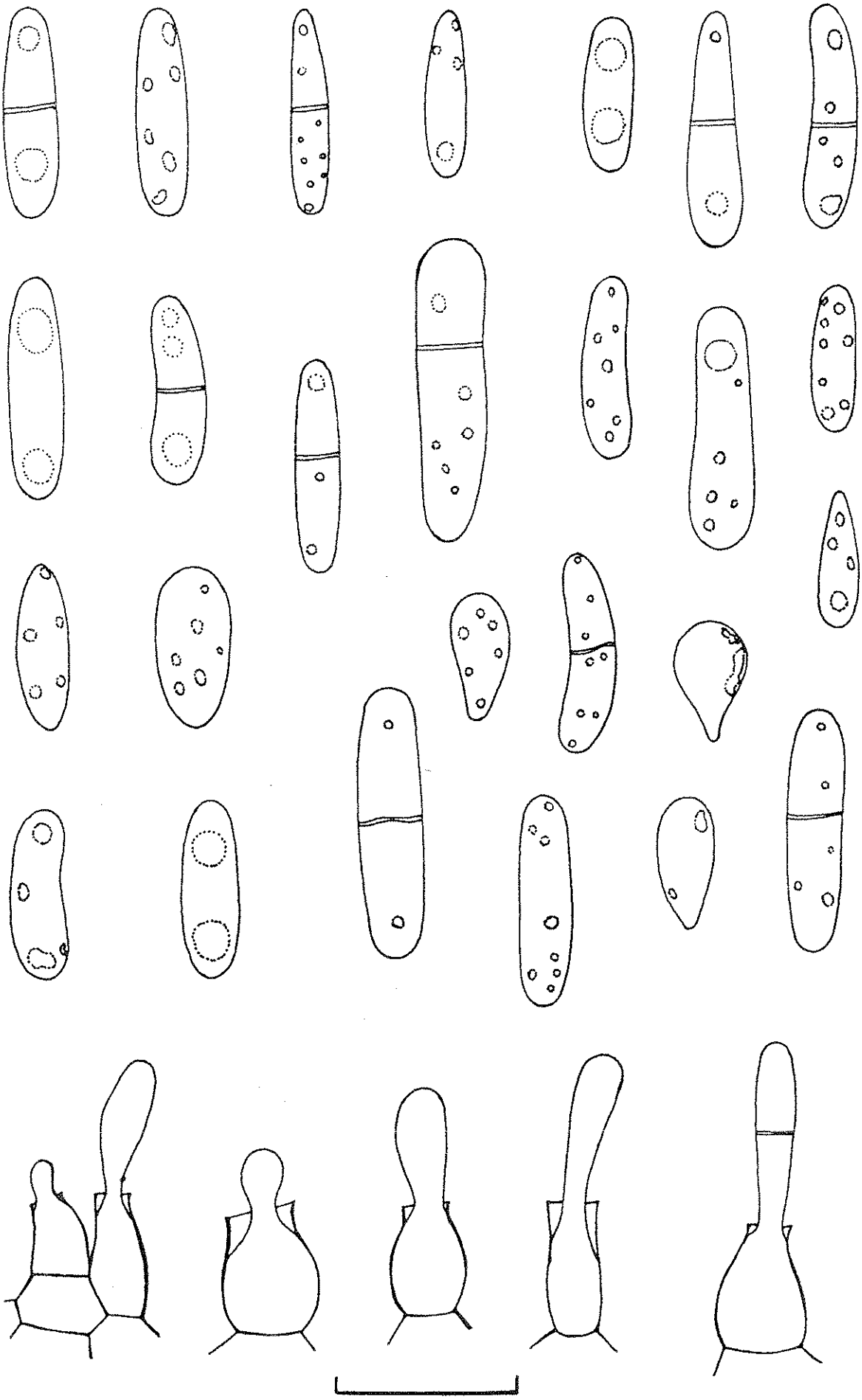
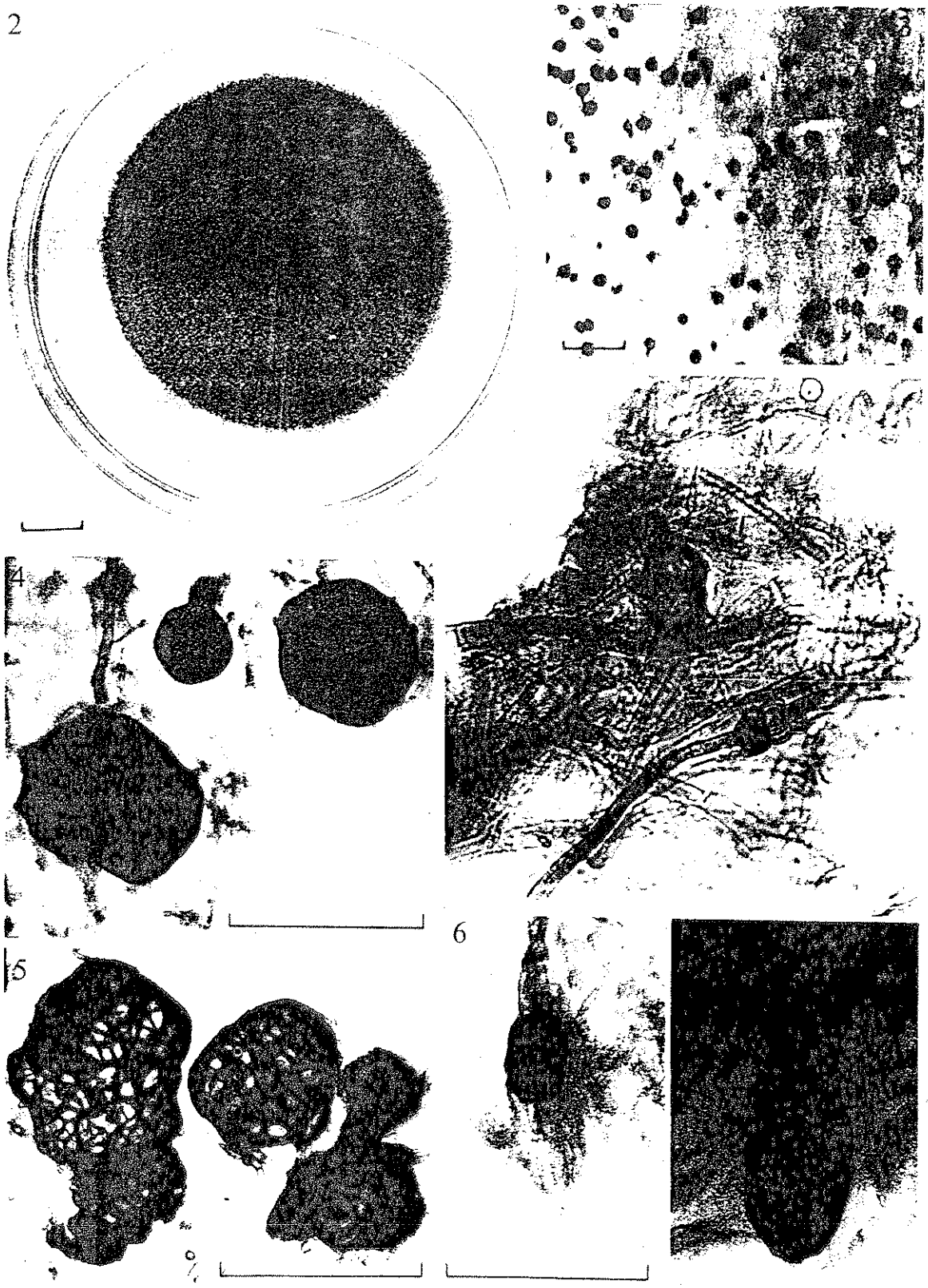


Fig. 1. *Phoma gentianae-sino-ornatae*. Conidiogenous cells and conidia produced within pycnidia developed on oat agar (bar, 10 µm).

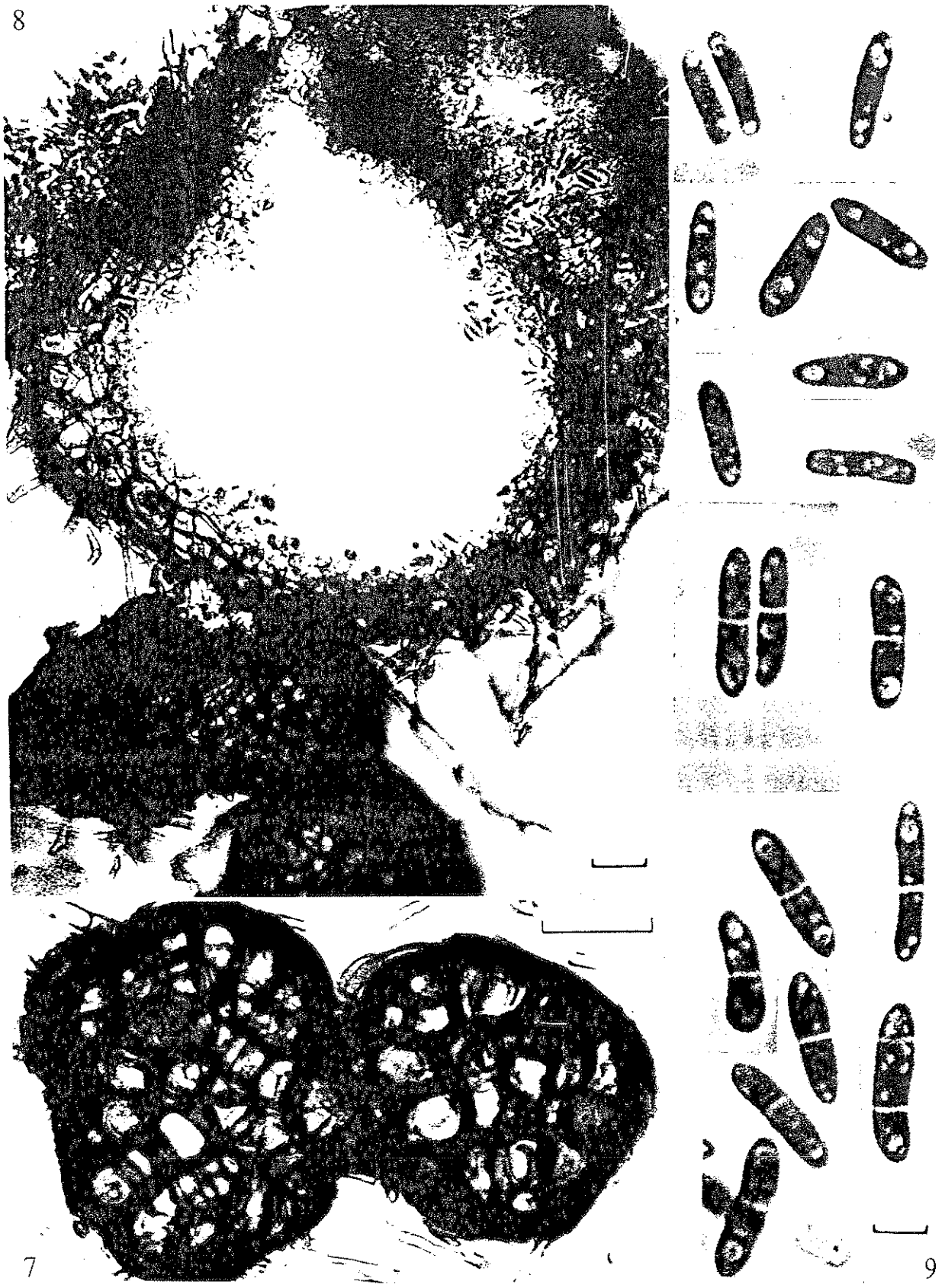


Figs 2–6. *Phoma gentianae-sino-ornatae*. **Fig. 2.** A 2-wk-old colony on oat agar with abundant sclerotia (bar, 1 cm). **Fig. 3.** Sclerotia on oat agar (bar, 300 µm). **Fig. 4.** Sclerotia at various stages of development on oat agar (bar, 100 µm). **Fig. 5.** Vertical sections of sclerotia on oat agar (bar, 100 µm). **Fig. 6.** Smooth and ornamented chlamydospores at various developmental stages (bar, 50 µm).

Isolated from *Gentiana sino-ornata* root, N.E. Scotland, U.K., 11 June 1990, R. Harling, (R); IMI 341116 holotype.

Additional material examined: on *Gentiana sino-ornata* root, Cambridge, U.K., 9 Sept. 1990, D. W. S. Clark, IMI 341834.

This species grows well at 25 °C on oat agar, colony diameter increasing at the rate of 4·5–4·7 mm in 24 h. Colonies of *P. gentianae-sino-ornatae* resemble those of *Macrophomina phaseolina* (Tassi) Goid. in the production of



Figs 7–9. *Phoma gentianae-sino-ornatae*. **Fig. 7.** Vertical section of sclerotia (bar, 25 μm). **Fig. 8.** Vertical section of pycnidial conidioma and sclerotia connected by hyphae (bar, 25 μm). **Fig. 9.** Conidia (bar, 5 μm).

Table 1. Comparison of *Phoma* species reported from *Gentiana* species

	Host	Sclerotia	Conidia
P. niesslii* Sacc. Michelia **2: 618 (1882)	*G. lutea* L.	Unknown	Aseptate, 6 × 3 µm
†*P. gentianae* Kühn *Hedwigia* **22**: 15 (1883)	*G. ciliata* L.	Unknown	Aseptate, 6–7·5 × 1·5–2 µm
P. californica* Ell. & Everh. *Bulletin Torrey Botanical Club* **24: 286 (1897)	*G. serrata* Gunn. (syn. *G. detonsa* Rottb.)	Unknown	Aseptate, 3 × 0·75 µm
P. drobnjacensis* Bubák *Botanikai Közlemények* **14: 63 (1915)	*G. asclepiadea* L.	Unknown	Aseptate, 3·5–5·5 × 0·75–1 µm
P. gentianae-punctatae* Petrak *Sydowia* **9: 581–582 (1955)	*G. cruciata* L.	Unknown	Aseptate, 3–4 × 1 µm
‡*P pedicularis* Fuckel, in M. Th. von Heuglin, *Reisen nach dem Nordpolarmeer in den jahren 1870 und 1871, III: Beiträge zur Fauna, Flora und Geologie von Spitzbergen und Novaja Semlja*: 318–319 (1874)	*G. punctata* L.	Unknown	Aseptate, 4–7 (–8) × 1·5–2 (–3) µm
§*P. gentianae-sino-ornatae* Punithalingam & Harling sp. nov.	*G. sino-ornata* I.B. Balf.	Abundant *Macrophomina*-type sclerotia produced in roots, stems and on agar media	0–, 1–, 2-septate 6–11 (–16) × 2–3 (–3·5) µm

* Measurements from original description.
† Measurements from isotype material in Herb. *K. Rabenhorst–Winter, Fungi Europaei* no. 2893, slide only in Herb. IMI (IMI 350514).
‡ Measurements from reference material (dried culture) in herb. IMI (PD 77/711 – IMI 248430), supplied for IMI by Dr G. H. Boerema.
§ Measurements from holotype material (dried culture) in Herb. IMI (Ri – IMI 341116).

Table 2. Comparison of root-infecting *Phoma* species with *P. gentianae-sino-ornatae*

	Main host and symptoms	Colony and conidial characters
P. chrysanthemicola* Hollós *Annales historico-naturales Musei nationalis hungarici* **5: 456 (1907)	*Chrysanthemum* root rot	Margin of colony almost smooth. No *Macrophomina*-type sclerotia develop in culture, but chlamydospores collect to form pseudosclerotia. Conidia mainly aseptate, rarely 1-septate
†*P. exigua* Desm. *Annales des sciences naturelles, botaniques* sér..3, **11**: 282–283 (1849)	All kinds of plants. Associated with *Solanum* tuber and *Linum* foot rot	Margin of colony often wavy or lobed. No *Macrophomina*- type sclerotia develop in culture, but sometimes abortive pycnidia form. Conidia mostly aseptate, and 5–10% are 1-septate
†*P. medicaginis* Malbr. & Roumeg. *Revue mycologique, Toulouse* **8**: 91 (1886)	*Medicago* foot rot	Margin of colony almost smooth, pycnidia in radial rows or scattered. No *Macrophomina*- type sclerotia develop in culture, but abundant chlamydospores form in sectors. Conidia mainly aseptate, and up to 10% are 1-septate
‡*P. sclerotioides* Preuss ex Sacc. *Sylloge Fungorum* **11**: 492 (1895)	Legumes root rot	Margin of colony almost smooth with abundant black 'pycnosclerotia' which are scleroplectenchymatous. Chlamydospores not known. Conidia aseptate
§*P. gentianae-sino-ornatae* Punithalingam & Harling	*Gentiana* root rot	Margin of colony smooth with abundant *Macrophomina*- type sclerotia. Chlamydospores sparse. Pycnidia not sclereoplectenchymatous. Conidia 60–70% aseptate, 30–40% septate

* Details from neotype material (dried culture) held in CBS, slide only in Herb. IMI (H. J. Wilcox, 5161 – IMI 351704).
† Details from reference cultures in Herb. IMI.
‡ Details from dried culture in Herb. IMI (PD82/1061 – IMI 284476), cited by Boerema & Loerakker (1985) as reference (or representative) material.
§ Details from holotype material (dried culture) in Herb. IMI (Ri – IMI 341116).

abundant sclerotia, but the latter species is readily distinguished by the production of large conidia having apical gelatinous appendages formed by the eversion and gelatinization of the outer sheath (Punithalingam, 1982).

During the first 4–5 weeks of growth on agar media, *P. gentianae-sino-ornatae* usually produces numerous sclerotia, which are anatomically similar to those of *M. phaseolina*. Pycnidial conidiomata are formed only sparsely intermingled amongst sclerotia, and the two structures are often seen connected by common hyphae. Of those conidia formed within pycnidia 60–70% are aseptate, 28–38% are 1-septate and < 2% are 2-septate.

In the literature several *Phoma* species have been recorded from gentian, but none causing root rot or conforming to the characters of *P. gentianae-sino-ornatae*. Amongst those *Phoma* species previously described or reported from gentian all except *P. niesslii* Sacc. and *P. gentianae* Kühn have been recorded as having distinctly smaller conidia than those of *P. gentianae-sino-ornatae* (see Table 1). The type material of *P. niesslii* was not available for study, but the conidial length

originally recorded for this species is only at the lower end of the scale for *P. gentianae-sino-ornatae*. Examination of the isotype material of *P. gentianae* held at Herb. K showed no sclerotia, but thin-walled pycnidia with aseptate conidia smaller than those of *P. gentianae-sino-ornatae*.

Phoma pedicularis Fuckel was originally described from *Pedicularis*, and *Plenodomus gentianae* (Moesz) Petrak (syn. *Sphaeronaema gentianae* Moesz) from *Gentiana punctata* L., in Europe. Boerema, Kesteren & Loerakker (1981) relegated *S. gentianae* to synonymy with *P. pedicularis* in Section *Plenodomus* (Preuss) Boerema *et al.* Examination of a reference culture of *P. pedicularis* in Herb. IMI supplied by Dr Boerema showed that it does not produce *Macrophomina*-type sclerotia but pycnidia, which produce aseptate conidia.

Although in phytopathological reports four plurivorous *Phoma* species, *P. chrysanthemicola* Hollós, *P. exigua* Desm., *P. medicaginis* Malbr. & Roumeg. var. *medicaginis*, and *P. sclerotioides* Preuss ex Sacc., have been implicated with root rot of plants other than gentian, examination and direct comparison of the neotype material or reference cultures of these species showed that they all lack the *Macrophomina*-type sclerotia produced in abundance by *P. gentianae-sino-ornatae*. Also the four plurivorous root-infecting species differ from *P. gentianae-sino-ornatae* in both colonial and morphological characters (Table 2).

We thank the Curator and Director of Herb. K for the loan of the isotype material of *Phoma gentianae* Kühn, and Dr H. A. van der Aa, CBS for the loan of the neotype material of *Phoma chrysanthemicola* Hollós. We thank Joan E. Woodhams for technical assistance.

REFERENCES

Boerema, G. H., Kesteren, H. A. van & Loerakker, W. M. (1981). Notes on *Phoma*. *Transactions of the British Mycological Society* **77**, 61–74.

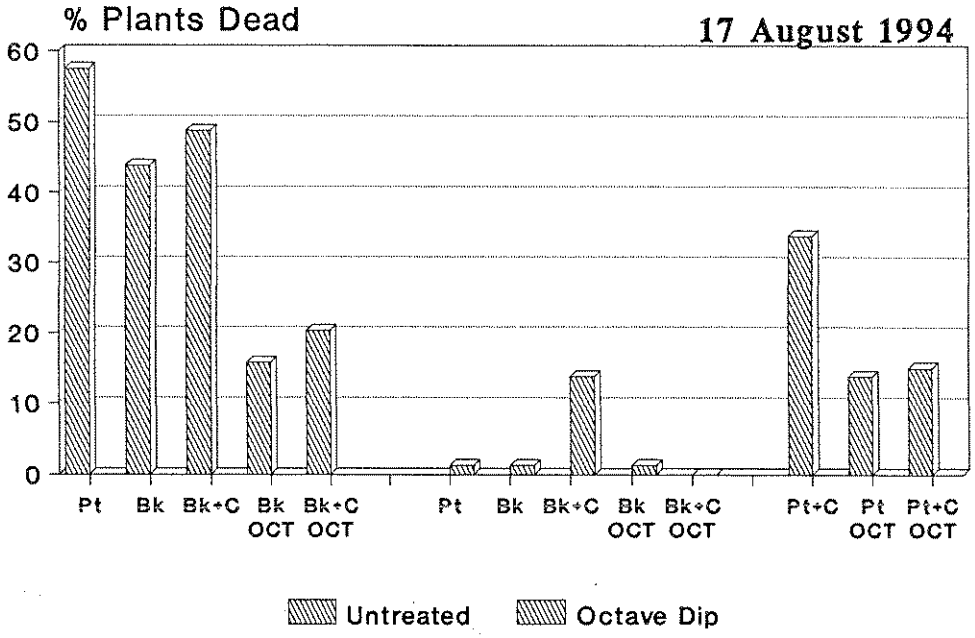
Boerema, G. H. & Loerakker, W. M. (1985). Notes on *Phoma*. 2. *Transactions of the British Mycological Society* **84**, 289–302.

Punithalingam, E. (1982). Conidiation and appendage formation in *Macrophomina phaseolina* (Tassi) Goid. *Nova Hedwigia* **36**, 249–289.

Sutton, B. C. (1980). The Coelomycetes. Commonwealth Mycological Institute: Kew.

(*Accepted 14 April 1993*)

HDC Gentian Project 1994



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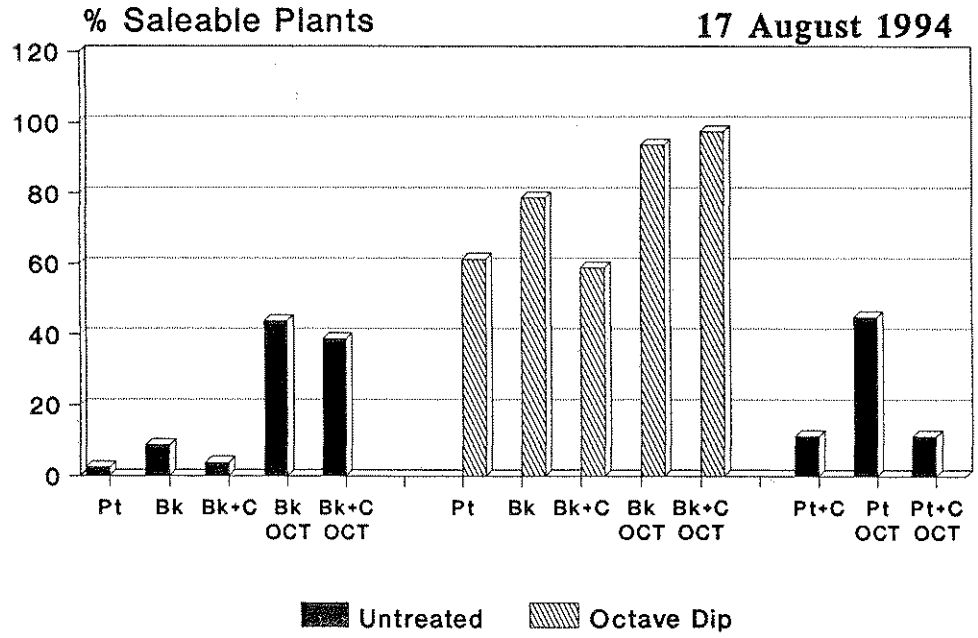

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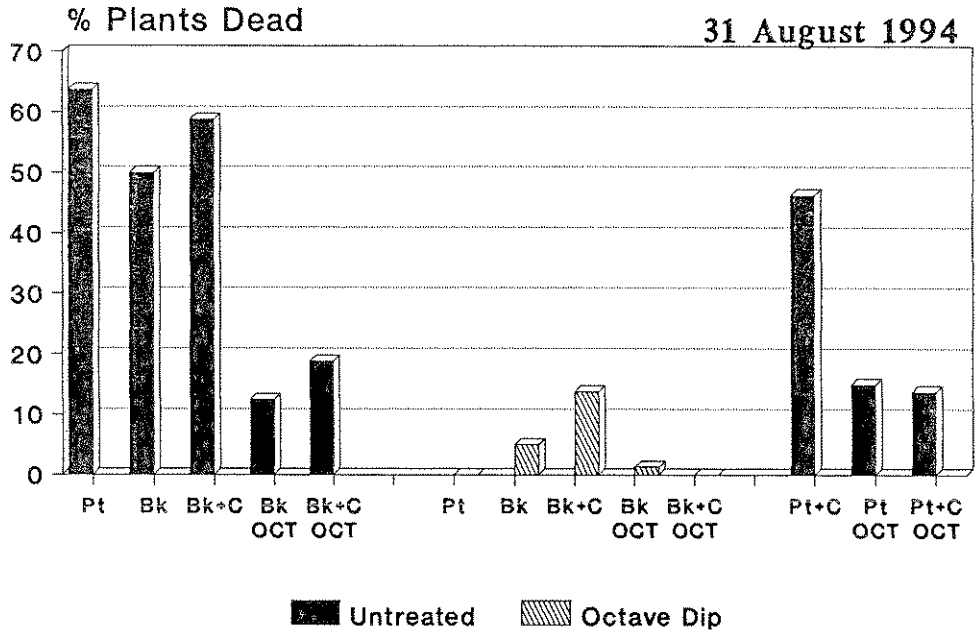

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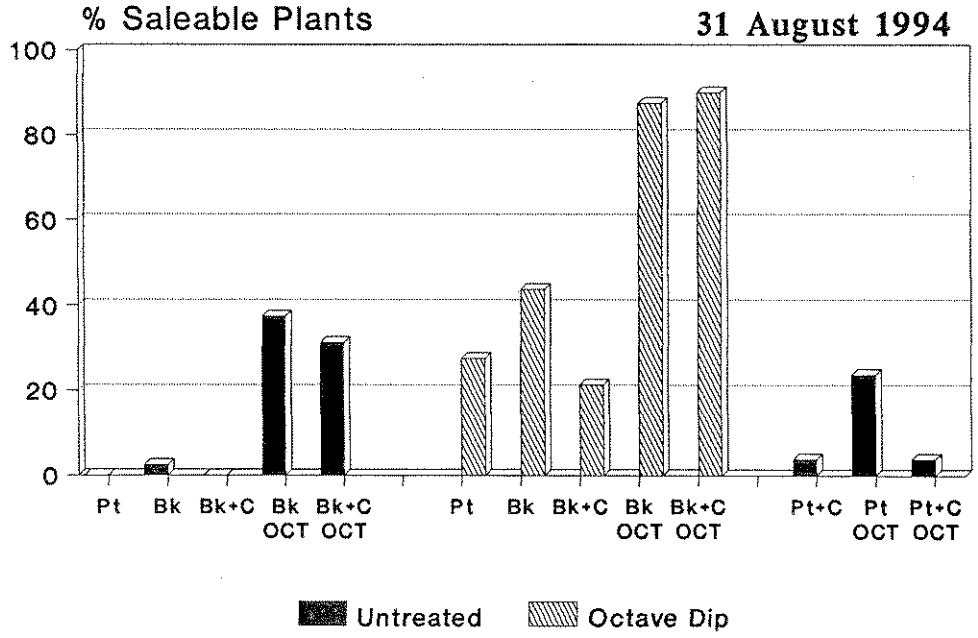
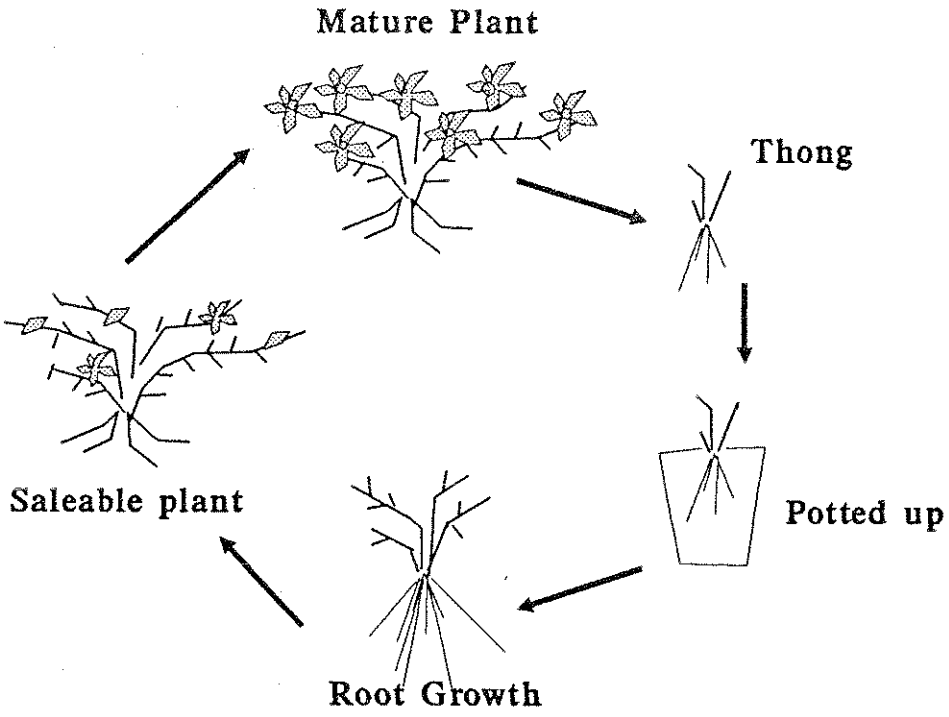


Production cycle of gentians and control measures for Phoma gentianae

Gentian Production Cycle



Gentian Root Rot - Control measures