PATHOGENICITY AND CONTROL USING COMPOSTS OF *APHANOMYCES EUTEICHES* PEA ROOT ROT

MONIKA WALTER¹, CHRIS M.A. FRAMPTON²,
PHILIP A.G. ELMER¹ and ROBERT A. HILL³

¹HortResearch, P.O. Box 51, Lincoln

²Lincoln University, P.O. Box 85, Lincoln

³HortResearch, Private Bag 3123, Hamilton

ABSTRACT

Since 1978, the fungus *Aphanomyces euteiches* Drechsler has been recognised as an important pea disease in New Zealand. A bioassay was developed to screen a range of bark and animal manure composts for their ability to suppress *Aphanomyces* root rot in peas in 1994/95. Disease severity was significantly affected by isolate type and inoculum concentration. Significant differences in the degree of *Aphanomyces* disease suppression were detected between different composts and compost concentrations. The effect of compost concentration was less important than compost type. Composts containing manure were more effective in disease suppression than pure bark-based composts. Physical, chemical and microbial properties of the composts and their effect on disease suppression are currently being investigated.

Keywords: *Aphanomyces euteiches*, *Pisum sativum*, root rot, disease suppression, composts

INTRODUCTION

During the summer of 1978-79 severe losses of pea crops in Canterbury and Nelson occurred, caused by the root rotting fungus *Aphanomyces euteiches* Drechsler (Manning and Menzies 1980). The fungus is considered to be the most important root rot pathogen of pea in the USA (Holub *et al.* 1991) and is also recognised as one of the most important pea diseases in Canada (Tu 1992) and Scandinavia (Bødker and Leroul 1993). Through the lack of effective fungicides, resistant cultivars and/or biological control measures (Cerenius *et al.* 1992; Rao *et al.* 1995) growers usually have to rely on disease avoidance resulting in the recommended 6-year pea rotation to delay build-up of root rot pathogens and to keep yield losses within economically acceptable limits (Oyarzun *et al.* 1993). In New Zealand, disease is measured by Disease Severity Indexing (DSI) to judge whether peas may be planted the following season (Sherwood and Hagedorn 1958). A small difference in the DSI estimate can result in a considerable difference in yield (Bødker and Leroul 1993).

In the 1960s the composting of tree bark as peat substitutes was investigated. During the utilization of composts, improved plant growth and suppression of root rot diseases were observed (Hoitink and Fahy 1986). A detailed literature review on disease suppressive properties of composts has been published elsewhere (Tränkner 1993). In New Zealand, the increasing amount of waste pine bark disposed of in land fills (Aglink 1985) presses for new alternative and industrial usages of bark. The objective of our study was to examine disease suppressive properties of pine bark and other composts against *A. euteiches*.

MATERIALS AND METHODS

A. euteiches cultures were isolated from diseased pea plants provided by Plant Protection Centre, MAFQuality, Lincoln from Disease Severity Index (DSI) trials for local farmers. Isolations were carried out on semi-selective medium (Pfender *et al.*

Proc. 48th N.Z. Plant Protection Conf. 1995: 308-313

1984). The fungus was identified according to the description of Jones and Drechsler (1925).

Root inoculation

We used the technique of Mitchell and Yang (1966) to produce zoospores of *A. euteiches*. Peas (*Pisum sativum* cultivar 'Tere') were surface sterilised in 0.3% sodium hypochlorite for 3 minutes, rinsed in sterile water and germinated in the glasshouse at 24°C for 7 days in sterile sand using 45 ml seed raising trays. Pea seedlings were lifted gently from the trays and washed free of adherent sand particles and lightly blotted dry with sterile paper towels. Pea roots were inoculated with a zoospore suspension (10^4 spores/ml) of *A. euteiches* by dipping the root and the epicotyl into the spore suspension for 3 seconds. Seedlings were then planted immediately into the moistened growth substrate in planter bags. Control plants were dipped in sterile water before planting. Planter bags each containing four to five seedlings were incubated for 18 days as described by Sherwood and Hagedorn (1958).

Pathogenicity

Ten isolates of *A. euteiches* (AE1 - AE10) from Canterbury soils and two isolates from the International Collection of Microorganisms from Plants (ICMP), Auckland (*A. euteiches* Drechsler ICMP 6477; *A. euteiches* Drechsler ICMP 6478) were used for pathogenicity tests. Pea seedlings were inoculated with zoospores or sterile water as a control. The seedlings then were planted into sterile river sand and incubated as described above. The sand was supplemented with 2 g osmocote/litre sand.

Effect of zoospore concentration was studied by increasing zoospore concentration ($1, 10, 10^2, 10^3, 10^4, 10^5$ spores/ml) of representative *Aphanomyces* isolate AE7. Screening for effect of isolate and zoospore concentration was carried out with four replicates of each treatment. A replicate consisted of the average measurements from five pea plants in one planter bag.

Composts

Nine different composts were screened for disease suppressive properties against *A. euteiches*. Brief descriptions of the compost compositions are given in Table 3.

Sterile sand (compost 4) was supplemented with osmocote (2 g/litre) and mixed with each compost to obtain compost concentrations of 0%, 25%, 50%, 75% and 100%, except for compost 10 for which compost concentrations were 0%, 25% and 50%. Screening for disease suppressive properties of each compost and each concentration was carried out in duplicate experiments with four replicates of plus and minus fungus treatments for each concentration. A replicate consisted of the average measurements from four pea plants in one planter bag.

Disease assessment

Assessment of disease was carried out by DSI % (Sherwood and Hagedorn 1958), and by measuring the wet and dry weight (24 h, 70°C) of the seedling leaves. DSI data were subject to logarithmic transformation before analysis of variance and the application of Fisher's Least Significant Difference Test. Wet weight and dry weight data were analysed without transformation. A highly significant correlation (correlation $t = 0.970$) between wet weight and dry weight was observed, therefore only wet weight measurements are presented.

RESULTS

Pathogenicity

A. euteiches isolates ICMP 6477 and ICMP 6478 were not able to produce zoospores using the technique described by Mitchell and Yang (1966) and hence were omitted from further investigations. All *Aphanomyces* isolates, except isolates AE1 and AE2, were able to induce severe root rot (Table 1), killing most of the pea plants during incubation. Isolate AE1 had little effect on the pea plants with the roots showing no symptoms or slight brown discolourations of the tap and lateral roots. Isolate AE2 caused discolourations of tap and lateral roots and browning of the epicotyl.

Pathogenicity was also significantly affected by zoospore concentration (Table 2). Root rot increased with increasing zoospore concentration. Seedlings started to show symptoms of infection when using a zoospore concentrations of 10^2 spores/ml.

However, the development of the seedlings was not affected at this concentration as indicated by wet weight measurements.

TABLE 1: Effect of isolate on pathogenicity of *Aphanomyces euteiches* to *Pisum sativum*.

<i>A. euteiches</i> (AE) isolate	DSI ^a (%)	Wet weight (g/plant)
Control	0a ^b	4.9a
AE1	8a	4.7a
AE2	67b	2.3b
AE3	96c	1.1de
AE4	92c	1.4d
AE5	95c	1.5d
AE6	91c	1.3d
AE7	97c	0.5cef
AE8	97c	1.1df
AE9	99c	0.4c
AE10	99c	0.6cef

^a backtransformed ln values

^b within a column, means followed by the same letter are not significantly different (P<0.05)

TABLE 2: Effect of zoospore concentration on pathogenicity of *Aphanomyces euteiches* to *Pisum sativum*.

<i>A. euteiches</i> isolate AE7 (spores/ml)	DSI ^a (%)	Wet weight (g/plant)
1	0a ^b	4.9ab
10	0a	5.3b
10 ²	13b	4.1a
10 ³	46c	3.3c
10 ⁴	97c	0.5d
10 ⁵	100c	0.4d

^a backtransformed ln values

^b within a column, means followed by the same letter are not significantly different (P<0.05)

Composts

No significant differences were observed between duplicate experiments, thus data from both trials were pooled. Table 3 lists the means of DSI and wet weight measurements for pea plants inoculated with *A. euteiches* or water growing in compost-sand-mixes. Significant differences in disease suppression were observed between composts. Compared to the control treatment (compost 4), composts 9 and 1 were able to suppress *Aphanomyces* root rot by approximately 57% and 48% respectively. Composts 8, 7 and 2 suppressed disease to a lesser extent, ie; 40%, 35%, and 30% respectively. The remaining composts 3, 5, 6 and 10 did not reduce the incidence of pea root rot. For all composts significant differences in wet weight measurements were observed between fungus and water inoculated treatments. The wet weight measurements also showed that compost composition had a significant influence on disease suppression and on plant growth.

Compost concentrations

Using DSI, composts 3, 5, 6 and 10 did not suppress *Aphanomyces* root rot of peas, regardless of compost concentration. Concentrations of the remaining composts

significantly influenced DSI rating, but only for composts 8 and 9 were significant differences between compost concentrations 25%, 50%, 75% and 100% observed (Table 4). The wet weight assessments showed that compost concentrations not only had a significant influence on disease suppression (Table 4) but also on plant growth (data not shown).

TABLE 3: Effect of composts on plant development and disease suppression of *Aphanomyces euteiches* on pea roots (*Pisum sativum*).

Compost	Composition	DSI ^a (%) inoculated with fungus	Wet weight (g/plant) inoculated with fungus	water
1	pine bark, waste from freezing works	48ab ^b	2.5a	4.3a***
2	pig manure, sawdust	65b	1.6b	3.3b***
3	ponga bark	100c	0.2c	3.5b***
4	sterile sand + osmocote (2 g/litre) (control)	93c	0.6d	5.0a***
5	pine bark	95c	0.8d	3.1bc***
6	pine bark, hardwood bark	100c	0.4cd	2.5cd***
7	garden waste, hedge clippings etc.	60b	1.1e	1.9de***
8	horse manure, straw, sawdust	56b	1.3e	2.6cd***
9	cow manure, straw	40a	1.3e	1.8e*
10	pine bark from young trees	95c	0.6d	3.4b***

^a backtransformed ln values

^b within a column, means followed by the same letter are not significantly different (P<0.05)

*, *** significantly different to corresponding fungus inoculated treatment at P<0.05 and P<0.001 respectively

TABLE 4: Effect of compost concentrations on disease suppression of *Aphanomyces euteiches* on pea roots (*Pisum sativum*).

	0%	Compost concentrations			
		25%	50%	75%	100%
DSI^a (%)					
compost 1	93ac ^b	75ab	34b	57bc	39b
compost 2	93a	88ab	80ab	48b	52ab
compost 7	93a	60b	54b	56b	74ab
compost 8	93a	82a	39b	31b	95a
compost 9	93a	46b	18c	46b	65ab
Wet weight (g/plant)					
compost 1	0.6a ^b	2.8b	2.7b	2.3b	2.2b
compost 2	0.6a	1.8bc	2.4b	1.3ab	0.9ac
compost 3	0.6a	0.2b	0.2b	0.2b	0.2b
compost 5	0.6a	0.8a	1.2b	0.7a	0.5a
compost 6	0.6a	0.4b	0.4b	0.4b	0.3b
compost 7	0.6a	1.4b	1.2bc	1.1bc	0.8ac
compost 8	0.6a	1.3b	1.6b	1.3b	0.8a
compost 9	0.6a	1.5b	1.5b	1.1bc	0.9ac
compost 10	0.6a	0.6a	0.6a	-	-

^a backtransformed ln values

^b within a row, means followed by the same letter are not significantly different (P<0.05)

DISCUSSION

Our results indicate that the pea bioassay is useful in investigating disease suppressive properties of composts against *A. euteiches*. The bioassay not only provided an environment favourable for plant infection, but also offered suitable conditions for plant development. Thus the relative effects of compost treatments (composition and concentration) on disease suppression and plant development were clearly expressed.

Composts containing animal waste (composts 1, 2, 8, 9) suppressed disease to a greater extent than pure bark composts. This is in agreement with previous reports (Hoitink and Fahy 1986; Dittmer 1991; Tränkner 1993). It is assumed that manure composts possess better disease suppressive properties than pure plant composts because their composition of nutrients and microorganisms is more diverse and therefore antagonistic microorganisms are more likely to occur (Tränkner 1993).

Disease suppression was found to be less dependent on the amount of compost than on absence or presence of composts. In general, disease suppressive properties of composts are likely to be influenced by compost sources, composting process, compost curing, compost age, composition of micro flora and fauna, microbial inhibitors, physical and chemical factors such as particle size, nitrogen content, cellulose and lignin content, electrical conductivity, pH, etc (Dittmer 1991; Tränkner 1993). At present, the physical, chemical and microbial properties of the nine composts used are being investigated.

The type of compost and compost concentration had a significant effect on plant health and development as indicated by weight measurements. When using composts for biological control of root diseases, the medium has to be adapted to suit the plant species in order to ensure and maintain plant growth, without undermining disease suppressive properties. More research is needed to develop potting mixes with reliable antagonistic properties.

The different reactions induced by *A. euteiches* isolates and zoospore concentrations on the pea cultivar 'Tere' support previous work that variation in virulence exists within *A. euteiches* (Manning and Menzies 1984; Rao *et al.* 1995). All *Aphanomyces* isolates studied, except for isolate AE1, were highly virulent. It needs to be stressed, that disease severity not only depends on the amount of zoospore inoculum and isolate used, but also on the pea genotype (Rao *et al.* 1995). No resistant pea cultivar exists yet. Disease suppression of various *A. euteiches* isolates using composts needs to be investigated in connection with various pea genotypes.

ACKNOWLEDGEMENTS

Our thanks to Stojan Ganev, MafQual, Lincoln, for supplying us with diseased pea roots; and to Richard Pender, Joanne Perry and Paul Steciurenko for technical assistance.

REFERENCES

- Aglink, 1985. Soil-less mixes bark and sawdust. For container-grown plants. *HPP 138:5/3000/8/1985 5th revision*. Information Services, MAF, Private Bag, Wellington, NZ.
- Bødker, L. and Leroul, N., 1993. Influence of pea cropping history on disease severity and yield depression. *Plant Disease* 77 (9): 896-900.
- Cerenius, L., Rufelt, R. and Söderhäll, K., 1992. Effects of Ampropylfos ((RS)-1-aminopropylphosphonic acid) on zoospore formation, repeated zoospore emergence and oospore formation in *Aphanomyces* spp. *Pesticide Science* 36: 189-194.
- Dittmer, U., 1991. Untersuchungen zu den Wirkungen des Kompostierungsprozesses und zum antiphytopathogenen Potential von Komposten gegen *Sclerotinia trifoliorum* Erikss., *Sclerotinia sclerotiorum* (Lib.) de Bary und *Pseudocercospora herpotrichoides* (Fron.) Deigh. Dissertation, Rheinischen Friedrich-Wilhelms-Universität zu Bonn, Bonn, Germany. 167 p.
- Hoitink, H. A. J. and Fahy, P. C., 1986. Basis for the control of soilborne plant pathogens with composts. *Ann. Rev. Phytopath.* 24: 93-114.

- Holub, E. B., Grau, C. R. and Parke, J. L., 1991. Evaluation of the forma specialis concept in *Aphanomyces euteiches*. *Mycol. Res.* 95: 147-157.
- Jones, F. R. and Drechsler, C., 1925. Root rot of peas in the United States caused by *Aphanomyces euteiches* (n. sp.). *J. Agric. Res.* 30: 293-325.
- Manning, M. A. and Menzies, S. A., 1980. Root rot of peas in New Zealand caused by *Aphanomyces euteiches*. *N.Z. J. Agric. Res.* 23: 263-265.
- Mitchell, J. E. and Yang, C. Y., 1966. Factors affecting growth and development of *Aphanomyces euteiches*. *Phytopath.* 56: 917-922.
- Oyarzun, P., Gerlach, M. and Hoogland, A. E., 1993. Relation between cropping frequency of peas and other legumes and foot and root rot in peas. *Neth. J. Plant Pathol.* 99: 35-44.
- Pfender, W. F., Delwiche, P. A., Grau, C. R. and Hagedorn, D. J., 1984. A medium to enhance recovery of *Aphanomyces* from infected plant tissue. *Plant Dis.* 68: 845-847.
- Rao, A., Gritton, E. T., Grau, C. R. and Peterson, L. A., 1995. Aeroponics chambers for evaluating resistance to *Aphanomyces* root rot of peas (*Pisum sativum*). *Plant Dis.* 79 (2): 128-132.
- Sherwood, R. T. and Hagedorn, D. J., 1958. Determining the common root rot potential of pea fields. *Wisconsin Agricultural Experimental Station Bulletin* 531: 1-11.
- Tränkner, A., 1993. Kompost und Pflanzengesundheit: Möglichkeiten und Auswirkungen der biologischen Beeinflussung pflanzlicher Oberflächen zur Krankheitsbekämpfung. Verlag Dr. Kovac, Hamburg, Germany. 123 p.
- Tu, J. C., 1992. Management of root diseases of peas, beans, and tomatoes. *Can. J. Plant Pathol.* 14: 92-99.