THE EFFECT OF DAIRY-SHED EFFLUENT IRRIGATION ON THE OCCURRENCE OF PLANT PATHOGENIC *PYTHIUM* SPECIES IN PASTURE

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ABSTRACT

A preliminary survey of pastures spray-irrigated with dairy-shed effluent revealed a significant increase in the population of plant pathogenic *Pythium* species isolated from both soil and roots. *In vitro* pathogenicity tests showed the majority of these isolates to be pathogenic when inoculated onto the seedlings of white clover, subterranean clover and perennial ryegrass, although both clover species were more susceptible to *Pythium*-induced disease than ryegrass.
Keywords: *Pythium,* soil, pasture, pathogenicity, dairy-shed effluent.

INTRODUCTION

The practice of using spray irrigation for disposal of dairy-shed effluent onto pasture is a growing trend in dairying regions of New Zealand. Effluent disposal applied to pastures can affect the microbial populations in soil, for example, di Menna (1966a,b) reported changes in soil yeast populations after irrigation with dairy factory effluents. Current local research into the effects of dairy-shed effluents on soil is investigating the inoculation of potentially harmful pathogenic bacteria and protozoa in soil. However effluent effects on resident soil microfungi including potentially phytopathogenic fungi have not been investigated.

Oomycetous fungi, such as *Pythium,* are economically important soilborne pasture plant pathogens (Chakraborty *et al.* 1996) causing seed rot, damping-off of establishing pasture seedlings and root rot diseases (Hawthorne 1988) of leguminous and graminaceous pasture plants. This affects the productivity and composition of pasture swards (Chakraborty *et al.* 1996). It has also been reported previously that the phytopathogenic oomycete genera *Pythium* and *Phytophthora* can be present in, and stimulated by, irrigation effluents (MacDonald *et al.* 1994). The effect of dairy-shed effluent amendments on phytopathogenic oomycete populations in pasture was investigated in a preliminary survey of a case study trial site in the Waikato.

METHODS

Soil core samples containing white clover plants were removed from a dairy farm where dairy-shed effluent was routinely applied to pasture. Soil was taken from pastures where effluent had been applied within the past seven days. Sampling was conducted over summer (January – March). Due to the pasture grazing rotation management on the farm over this period, effluent was applied to three soil types at various times: Puketaha soil (Jan/Feb), consolidated peat soil (Feb/Mar) and Horotui sandy loam (March only).

To provide control comparisons, soil cores were also removed from the same soils where dairy-shed effluent had never been applied to pasture. Each treatment was replicated three times. Soil and root samples were collected using a 5 cm soil corer to a depth of 10 cm, samples were bulked together and initially sieved through a 1 cm sieve to separate soil, debris and roots.

Isolation from soil

Two methods were used in parallel to selectively obtain oomycetous fungi from soils.

The first was a soil dilution plating method described by Martin (1992). Ten grams of each soil sample was suspended in 100 ml sterile distilled water and shaken for 15 min. Serial dilutions of the soil suspensions (10^0, 10^{-1} and 10^{-2}) were made and 0.1 ml spread onto a selective medium, Modified Pimaricin Vancomycin with Minor elements (MPVM, Ali-Shtayeh *et al.* 1986). The second method was a simplified particle filtration procedure as outlined by Bills & Polishook (1994). Soil samples were homogenised, washed and sieved to obtain samples of plant debris and soil particles <125 µm in size which were then plated onto MPVM plates. Each treatment of both methods was replicated three times.

Isolation from roots

White clover roots were removed from soil cores, washed in running tap water to remove soil, rinsed four times in sterile distilled water and dried on sterile filter paper. Root pieces were then cut to segments of 2-5 mm and plated onto MPVM, five segments per plate. A total of 100 root pieces from each treatment were plated.

The same procedure was also used to plate pieces of decomposing roots obtained by sieving soil samples. Fifty decomposing root pieces (2 –5 mm) from each treatment were plated.

Fungi obtained from MPVM plates were subcultured onto potato carrot agar for identification. Preliminary identification of isolates was undertaken according to methods of Domsch *et al.* (1980).

Pathogenicity screen

A standard petri plate technique (Christensen *et al.* 1988) was used to assess the pathogenicity of *Pythium* spp to axenically grown seedlings of white clover (*Trifolium repens* L. cv. Grasslands Huia), subterranean clover (*Trifolium subterranuem* L. cv. Grasslands1003) and perennial ryegrass (*Lolium perenne* L. cv. Grasslands Nui). Seedlings were inoculated with 5 mm culture plugs of 52 *Pythium* isolates obtained from effluent-amended pastures and assessed after 10 days for disease symptoms using criteria outlined previously (Waipara *et al.* 1996a).

All data were analysed by analysis of variance using LSD tests for separation of means.

RESULTS AND DISCUSSION

Isolation of *Pythium*

A total of 78 *Pythium* isolates were recovered on MPVM plates (Table 1), with most being isolated from root pieces. This result was not unexpected as most species are primary or secondary attackers of plant root systems (Waterhouse 1973) and parasites of living plant tissues. To date four species have been identified: *Pythium afertile* (2 isolates), *P. debaryanum* (3 isolates), *P. irregulare* (11 isolates) and *P. ultimum* (7 isolates).

TABLE 1: Total number of *Pythium* isolated from effluent amended pastures from January to March 2000.

	Month						
	January		February		March		Total
Effluent status	(+)	(-)	(+)	(-)	(+)	(-)	
Roots	18	4	19	7	3	0	51
Soil	5	6	1	0	12	3	27
Sub totals	23[1]	10	20[1]	7	15[1]	3	78
Total	33		27		18		

[1] No. of *Pythium* significantly higher between (+) and (-) effluent treatment (P<0.05).

The number of *Pythium* isolates recovered from effluent irrigated pastures was significantly higher (P<0.05) than unamended control pastures (Table 1), clearly showing that dairy-shed effluent irrigation stimulated *Pythium* populations in pastures. A decline in the number of isolates obtained from roots was observed during the course of the summer (Table 1). However, the sub-total of *Pythium* numbers isolated from soil rose during this time, indicating propagules were still present despite the loss of their primary root substrates. Rainfall measured over the farm area surveyed also decreased over the sampling period (January 68 mm, February 43 mm and March 31 mm). The lower rainfall may have caused the drop in *Pythium* isolations from roots in February and March, since higher soil moisture promotes root infection, sporulation and growth of this genus (Ingold and Hudson 1993).

The total number of *Pythium* isolates obtained from Puketaha pastoral soil was higher (Table 2) than both consolidated peat or Horotui sandy loam soils, however the latter was only sampled once and the difference in isolation frequency between treatments was not significant. Effluent irrigation significantly increased *Pythium* numbers in both Puketaha and consolidated peat soils (Table 2).

TABLE 2: Total number of *Pythium* isolated from soil from January to March 2000.

	Puketaha soil		Consolidated Peat		Horotui sandy loam		Total
Effluent status	(+)	(-)	(+)	(-)	(+)	(-)	
Roots	25	5	12	6	3	0	51
Soil	5	6	10	2	3	1	27
Total	30[1]	11	22[1]	8	6	1	78
	41		30		7		

[1]No. of *Pythium* significantly higher between (+) and (-) effluent treatment (P< 0.05).

Although *Pythium* was the dominant genus of fungi isolated in this case study, isolates of another Oomycete genus, *Phytophthora,* were obtained from clover roots. In addition, isolates from related Zygomycete genera *Mortierella, Gongronella* and *Absidia* were also recovered from all treatments.

Pathogenicity of *Pythium* spp.

Approximately 80% of the 52 isolates of *Pythium* tested against white clover, perennial ryegrass and subterranean clover seedlings were pathogenic, ranging from being mildly pathogenic (inhibition of root growth and disease lesioning of roots) to highly pathogenic (shoot and root rot and necrosis/damping off) (Fig. 1). Ryegrass

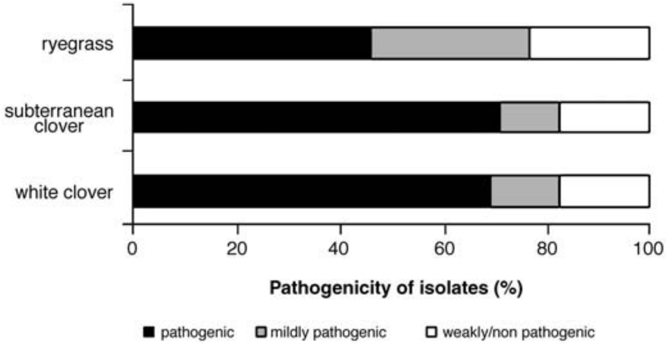

FIGURE 1: Percentage pathogenic *Pythium* isolates recovered from effluent irrigated pastures.

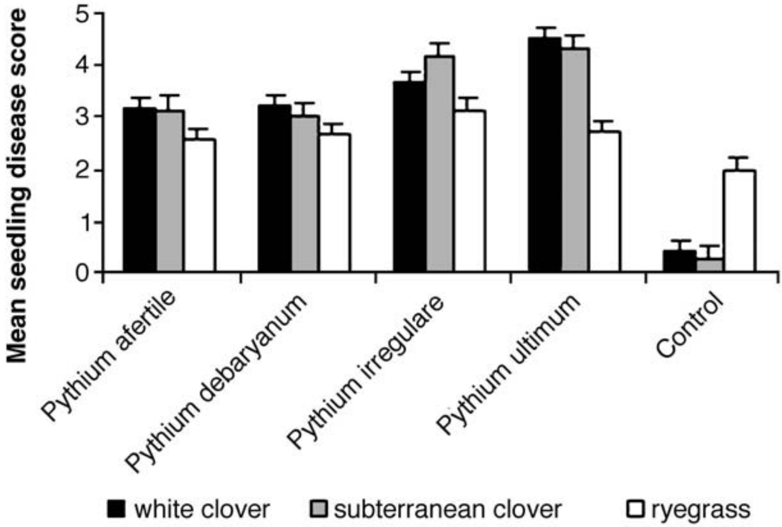

FIGURE 2: Pathogenicity of four *Pythium* species to pasture seedlings. Error bars represent LSD at P<0.05.

seedlings were less susceptible to disease than clover as slightly more *Pythium* isolates were non-pathogenic and a lower number of pathogenic isolates were also observed. A higher susceptibility of pasture legumes to fungal root diseases, compared to pasture grasses, has been frequently observed (Waipara *et al.* 1996a,b).

All seedlings inoculated with *Pythium* had more ($P<0.05$) root rot symptoms than the uninoculated controls (Fig. 2). The four species of *Pythium* differed in pathogenicity to the three hosts tested (Fig. 2) with *P. ultimum* and *P. irregulare* most pathogenic, particularly to both clover hosts, where significantly ($P<0.05$) more disease symptoms were observed. Root disease symptoms (root lesions and root browning) were observed on all inoculated ryegrass seedlings, which indicated a similar susceptibility to all four *Pythium* species.

Although an increase of pathogenic *Pythium* populations in soils could affect pasture establishment, productivity and sward composition (Chakraborty *et al.* 1996), other soil micro-organisms, such as beneficial competitive *Trichoderma* saprophytes and mycoparasites, may also be stimulated in effluent amended soils. This could limit the overall impact of pathogenic *Pythium* species. For example, long-term irrigation was reported to stimulate *Trichoderma* in pastoral soils (Ruscoe 1973). Other beneficial effects from effluent irrigation, such as increased nitrogen levels stimulating pasture growth, may also counter any losses to plant growth caused by pythiaceous pathogens. It is unclear whether the irrigation water with effluent, the effluent solids or both lead to the increase in *Pythium* numbers.

CONCLUSIONS

The application of dairy-shed effluent to pasture was found to increase the number of plant pathogenic *Pythium* strains isolated from roots and soil. Although pathogenicity varied between *Pythium* species, and isolates of species, more pathogenic than non-pathogenic strains were found to be present. Further investigation is needed to elucidate *Pythium* populations in pastoral soil ecosystems and how dairy-shed effluent irrigation affects overall microbial biodiversity in soil, in particular the economically significant deleterious plant pathogens.

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