Suppression of soil-borne cereal pathogens and inhibition of wheat germination by mustard seed meal

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Summary. Seed meal of high glucosinolate Indian mustard (*B. juncea*) releases biocidal isothiocyanates (ITCs) when glucosinolates in the tissue are hydrolysed. We investigated its potential as an in-furrow treatment for wheat to suppress soil-borne fungal pathogens. The volatile ITCs released by the meal were fungicidal to five soil-borne wheat pathogens at low rates *in vitro*. Wheat seed germination and growth were reduced when more than 100 kg/ha was applied in the drill row with the seed, but up to 1000 kg/ha could be either incorporated into the top 5 cm or placed as a band 4 cm below the seed with no effect on seedling growth. At current prices and with conventional equipment, about 50 kg/ha of seed meal can be applied with the seed/fertilizer at sowing. Further studies in disease infected soil are in progress to determine the level of suppression achieved at that rate.

INTRODUCTION

Brassica species release biocidal compounds, principally isothiocyanates (ITCs) when the glucosinolates in their tissues are hydrolysed during breakdown in the soil (2). Biofumigation is a term which has been used to describe the suppression of pest or disease organisms by the ITCs released from *Brassica* green manure crops or rotation crops (1, 6).

The level of glucosinolates in the vegetative parts of *Brassica* plants generally declines with age, while pod and seed levels increase (5). The high levels of goitrogenic seed glucosinolates were bred out of oilseed rape (*B. napus*) to produce canola, which yields an edible oil and a seed meal which can be used as stock feed. Similar breeding objectives are being pursued for Indian mustard (*B. juncea*) (9) which normally has high seed glucosinolate levels. High seed glucosinolates are desirable in the seeds of condiment mustards or those from which a spicy mustard oil is produced. The seed meal which remains following the crushing of these varieties is high in glucosinolates and consequently is currently sold as a low value stock feed ($50/t). The seed meal may have potential as a soil amendment in horticultural industries or as a seed dressing in broad-acre crops if the ITCs released when the glucosinolates degrade in soil effectively suppress soil-borne disease organisms. However, the ITCs released are also known to be phytotoxic to germinating seeds (3). The rate, timing and placement of the meal in relation to seeds will be important to achieve suppression of pests and diseases without inhibiting seedling germination.

This paper reports the results of experiments designed to investigate the potential of mustard seed meal as a seed dressing to control soil-borne fungal pathogens of wheat.

MATERIALS AND METHODS

In vitro suppression of fungal pathogens

Five soil-borne fungal pathogens (*Gaeumannomyces graminis* var. *tritici, Rhizoctonia solani, Fusarium graminearum, Pythium irregulare and Bipolaris sorokiniana*) were isolated from field grown wheat roots from various locations in southern Australia (7) and stored in sterile water. Immediately prior to the experiment, plugs (5 mm in diameter) of actively growing mycelium were taken from the margins of fungal colonies subcultured onto quarter-strength potato dextrose agar (1/4 PDA) and transferred to the centre of freshly poured sterile plates (85 mm diameter). Seed meal of Indian mustard (*B. juncea*) was placed in small plastic vessels onto the upturned lid of the plates and the inverted bottom containing the fungal plug became the lid. Water was added to the meal to permit hydrolysis of ITCs and the plate was immediately

sealed with parafilm so that only volatiles released from the seed meal and water contacted the fungus. The rates of seed meal used were 0 (control), 2.5, 5, 10 and 25 mg and duplicate plates of each rate were prepared. The plates were incubated for 72 h at 25oC (*Pythium* for 28 h) after which time the colony diameter was measured. Plates in which no growth had occurred were opened, the seed meal removed and then reincubated to determine if regrowth could occur.

Effects on wheat germination and growth

Red earth soil (22% clay, Total C=1.3%) was collected from a field site at Harden NSW, sieved through a 5 mm sieve and loosely packed into pots 8 cm in diameter and 15 cm high. Mustard seed meal was applied to five replicate pots in the following treatments:

Treatment	Rates (kg/ha)
Control	0
(1) Applied in a band with seed	25, 50, 100, 200, 500, 1000
(2) Incorporated into the top 5 cm	200, 500, 1000
(3) Applied as a band at 5 cm depth	200, 500, 1000

Wheat seeds (cv Dollarbird) were selected for uniformity of size and sown in the pots (2 per pot) at a depth of 1 cm immediately following application of the mustard meal. The pots were watered up with 1/2 strength Hoagland's solution and were kept well supplied with water and nutrients using 1/2 strength Hoagland's solution throughout the experiment. The seedlings were grown in a growth cabinet at 15/10^{o}C temperature and 12 h photoperiod. Time to emergence (days) was recorded (DTE), and at the five-leaf stage the plants were harvested for measurement of shoot dry weight.

RESULTS

In vitro suppression of fungal pathogens

The effect of mustard meal on the growth of fungal pathogens is shown in Figure 1. The seed meal was fungicidal (i.e. no regrowth occurred following removal of seed meal) to *Gaeumannomyces* at 5 mg and to all other pathogens except *Pythium* at 25 mg. There were significant differences in the sensitivity of the pathogens to the seed meal at all levels although generally *Gaeumannomyces* was the most sensitive fungus, *Pythium* was least sensitive while others were intermediate.

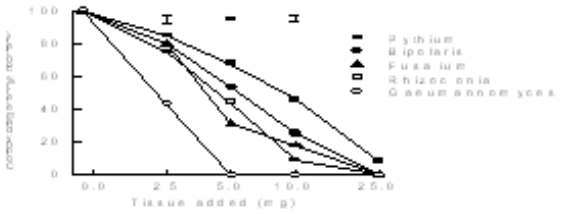

Figure 1. *In-vitro* suppression of five soil-borne cereal pathogens by mustard seed meal.

Effects on wheat germination and growth

Mustard meal banded with the seed had no effect on wheat germination or growth at rates of 100 kg/ha and below although at the lowest level there was a trend towards increased shoot growth (Table 1). At 200 kg/ha emergence time was delayed by 2 days but shoot dry weight was not reduced. At 500 kg/ha emergence was delayed by 6 days and shoot growth was reduced by 50%. The highest rate completely inhibited emergence and examination of the seed showed that germination had not occurred. The higher rates, either incorporated or banded below the seed, delayed germination by 1-2 days but had no significant effect on shoot dry weight.

Table 1. Effect of mustard seed meal on days to emergence (DTE) and shoot dry weight (DWt) of wheat seedlings at the five-leaf stage.

	Treatment[a]					
Rate (kg/ha)	Banded with seed		Incorporated 0-5 cm		Banded below seed (5 cm)	
	DTE	Dwt (mg)	DTE	Dwt (mg)	DTE	DWt (mg)
Control	10.3	604	-	-	-	-
25	10.6	668	-	-	-	-
50	10.4	664	-	-	-	-
100	11.0	635	-	-	-	-

200	12.2	620	11.4	585	11.3	648
500	16.5	395	10.6	670	11.7	640
1000	No emergence	12.4	621	12.8	613	

aLSD P=0.05 for DTE = 1.0; DWt = 72

DISCUSSION

The results of the *in-vitro* experiments indicate the potentially fungicidal effects of small amounts of mustard seed meal on soil-borne fungal pathogens. Analysis of the volatiles released by mustard seed meal in water indicated high levels of several ITCs were evolved (4), and these isothiocyanates have been shown previously to be fungicidal (1). The rates of meal required to achieve similar suppression in soil will be influenced by the sorption of ITCs onto clay and organic matter which reduces the effective concentration in the soil atmosphere (8). Further studies are underway using soil inoculated with pathogens to determine the efficacy of the ITCs in different soil types.

The results reported here indicate that up to 100 kg/ha of seed meal could be applied with the seed at the time of sowing without reducing germination or seedling growth at the five leaf stage. Higher levels of application are possible if the meal is either incorporated throughout, or placed in a band below the top 5 cm of soil. Preliminary field experiments have shown that up to 50 kg/ha of seed meal can be applied with a conventional combine before the meal causes problems with flow, or the volume becomes difficult to handle (G. Pitson Pers. Comm). Pelletising the meal may provide a way to increase the rate applied and avoid these problems although liberation of some ITCs during the pelletising process may reduce the efficacy of the meal. The ITCs are rapidly evolved when *Brassica* tissues are incorporated into soil (2) so that the timing of the biofumigation effect in relation to both the germination of the seed and the activity of the pest or pathogen is important. In horticultural enterprises large amounts of meal could be applied and incorporated prior to transplanting of vegetable crops to suppress disease organisms. In broad-acre cropping systems an application at sowing which provided protection from root rotting-fungi during the establishment phase appears to be the most promising application of the concept. Field studies are required to determine the efficacy of the meal at the rates which can be applied in the field.

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