The effect of *Brassica* crops on the level of mycorrhizal inoculum in soil

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ABSTRACT

Vesicular arbuscular mycorrhizal (VAM) fungi colonise the roots of most crop and pasture plants. Brassicas, however, are non-hosts and their roots contain glucosinolates (GSLs) that are potentially hydrolysed in soil to release isothiocyanates (ITCs), which are toxic to some soil fungi. As field experiments have indicated that VAM colonisation is reduced in crops following brassicas, the effect of brassicas on VAM inoculum was investigated. Glasshouse and field experiments indicated that the viability of VAM inoculum is not reduced by ITCs at the rates these compounds are likely to be released by canola crops grown in Australia. Low VAM colonisation of crops following canola appears to be primarily due to a natural decline in inoculum in the absence of a host. However, incorporation of larger quantities of *Brassica* tissue – as may occur in green manuring – may reduce both VAM colonisation and *Rhizobium* nodulation and this area requires further investigation.

KEYWORDS

Vesicular arbuscular mycorrhizae, isothiocyanates, glucosinolates, brassicas, biofumigation.

INTRODUCTION

Vesicular arbuscular mycorrhizal (VAM) fungi colonise the roots of most crop and pasture species grown in Australian agricultural systems. The fungi provide nutrients, particularly phosphorus, to the plant in return for photosynthate. The fungi are obligate symbionts and when no host plants are present survive in the soil as inactive spores, segments of hyphae and segments of colonised roots. The viability of this inoculum will decline with time (4). In the northern wheatbelt, low colonisation by VAM fungi after long bare fallows has been implicated in phosphorus and zinc deficiency of crops (Long Fallow Disorder) (14).

Brassicas are one of the few plant families that do not host VAM fungi (11, 15). In addition, the roots of brassicas contain glucosinolates (GSLs). These are potentially hydrolysed in soil to release isothiocyanates (ITCs) which are toxic to some soil fungi (13, 6). Wheat grown after canola (*Brassica napus annua*) generally has lower VAM colonisation than wheat grown after crops that host VAM fungi (10). This paper investigates whether compounds released from *Brassica* roots, in particular ITCs, contribute towards this lower colonisation through reducing VAM inoculum levels.

MATERIALS AND METHODS

Field experiments

At Cowra in 1997 wheat and canola (cvs. Oscar, Dunkeld and Tamara) were sown. There were eight replicates in a randomised block design. Concentrations of 2-phenylethyl glucosinolate (2pe) in roots in August were 7.5, 19.7 and 20.4 ?mol/g for Oscar, Dunkeld and Tamara, respectively (6). In 1998, wheat (cv. Whistler) was sown into the plots. In August, 25 plants per plot were removed and roots stained (3) and assessed for VAM colonisation (2).

At Longerenong in 1998, safflower (*Carthamus tinctorius*) cv. Sironaria, canola cvs. Monty (low root GSLs) and Dunkeld (medium root GSLs) and crambe (*Crambe abyssinica*)(6.4 ?mol/g 2-propenyl, 2.3 ?mol/g 2pe, 2.2 ?mol/g 1-methoxy-3indolylmethyl in September) were sown. There were three replicates in a randomised block design. In 1999, plots were sown to wheat (cv. Goldmark). Twenty plants per plot were removed in August and their roots assessed for VAM colonisation.

Glasshouse experiments

Two glasshouse experiments were conducted in which tissues of brassicas and other plants were added to soil, which was then watered and left for one or two weeks, by which time the majority of ITCs should have been released (8). Clover plants were then grown in the pots and the level of VAM colonisation in the clover roots was considered to indicate the density of VAM inoculum remaining in the soil.

Experiment 1:

Red earth soil collected from the field was air dried and passed through a 4 mm sieve. Colwell (1) available P was 10 mg/kg and mineral nitrogen 126 mg/kg. Ground, freeze-dried roots of wheat (C:N 30), canola cvs. Oscar (6.3 ?mol/g of 2pe, C:N 34) and Dunkeld (13.5 ?mol/g of 2pe, C:N 32) collected from the field at anthesis were mixed with soil at 0, 0.6 and 1.29 %w/w and 5 cm diameter tubes filled with 100 g of soil. There were 10 replicates randomly arranged in two seedling trays. The tubes were watered and after two weeks, three subterranean clover seedlings with one trifoliate leaf (cv. Goulburn) were transplanted into each pot. The trial was harvested after four weeks. Roots were weighed wet, shoots dried at 70?C for 3 days and weighed and VAM colonisation assessed. Root wet weights were converted to dry weights. Results were analysed using ANOVA.

Experiment 2

Red brown earth soil collected from the field was air dried and passed through a 4 mm sieve. Colwell (1) available P was 8 mg/kg and mineral nitrogen 38 mg/kg. Ground, freeze-dried canola shoots cv. Monty (0.5 ?mol/g of 3-indolylmethyl, C:N 23), Indian Mustard shoots (*Brassica juncea*) cv. Siromo (23 ?mol/g of 2-propenyl, C:N 15) and fodder radish roots (*Raphanus sativa*) (2 ?mol/g of 4-methylsulphinylbutyl and 23 ?mol/g of 4-methylthiobutyl, C:N 25) were mixed with soil at 0, 0.1, 0.3, 0.6, 1.2 and 2.4 %w/w and 5 cm diameter tubes filled with 100 g of soil. There were five replicates arranged in a randomised block design. The tubes were watered and placed in a glasshouse. After one week, three subterranean clover seedlings with one trifoliate leaf (cv. Goulburn) were transplanted into each pot. *Rhizobium* nodules were evident at this time. After four weeks the trial was harvested and root wet weight, shoot dry weight and VAM colonisation were assessed. *Rhizobium* nodules were counted under a dissecting microscope. Results were analysed using ANOVA.

RESULTS

Field experiments

At both Cowra and Longerenong, VAM colonisation of wheat was highest after a crop that had hosted VAM fungi and greatly reduced after a *Brassica* crop (Table 1). The three *Brassica* crops present at each site had a similar effect on VAM colonisation of the following wheat.

Table 1. Percentage of wheat root length colonised by VAM fungi after various crops (LSD for p=0.05).

	VAM hosting crop	*Brassica* crop			LSD
Cowra					
1997 crop	Wheat	Canola cv. Oscar	Canola cv. Dunkeld	Canola cv. Tamara	
1998 VAM (%)	29	7	5	6	7

Longerenong

1998 crop	Safflower	Canola cv. Monty	Canola cv. Dunkeld	Crambe	
1999 VAM (%)	30	7	8	5	5

Glasshouse experiments

In Experiment One, addition of canola and wheat roots had no effect on VAM colonisation and only slightly influenced clover shoot and root growth in some instances (Table 2).

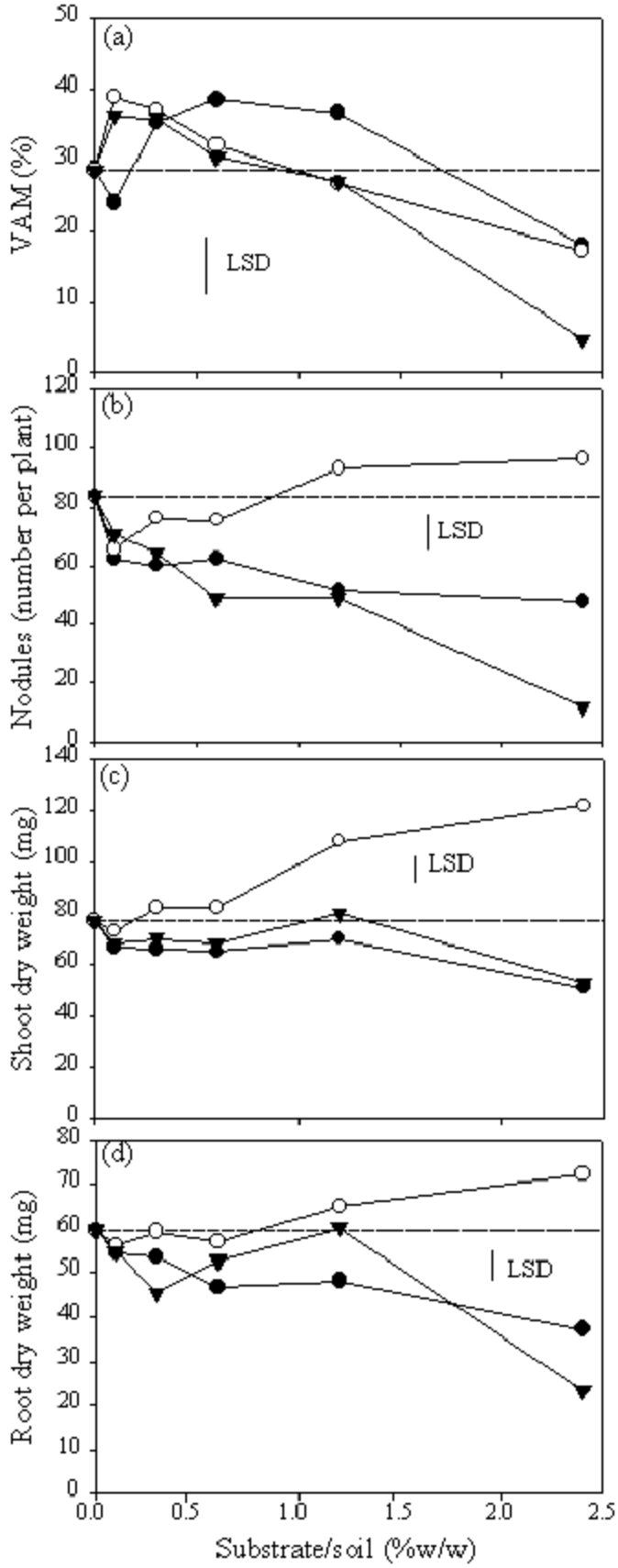

Fig. 1. The effect of adding canola shoots (—●—), radish roots (—○—) and mustard shoots (—▼—) to soil on a) the percentage of root length colonised by VAM fungi, b) *Rhizobium* nodulation, c) shoot dry weight and d) root dry weight of subterranean clover. A control with no added tissue (——) was also included. LSDs at p=0.05 are provided.

Clover shoot and root growth was little affected by the tissues, except at the higher rates of addition.

Table 2. Effect of ground wheat and canola roots on the percentage of root length colonised by VAM fungi, shoot growth and root growth of subterranean clover (LSD for p=0.05).

	Control	Wheat		Canola cv. Oscar		Canola cv. Dunkeld		LSD
Rate of addition (%w/w)		0.6	1.29	0.6	1.29	0.6	1.29	
VAM (%)	45	43	42	45	46	50	45	ns
Shoot dry weight (mg)	60	69	58	62	53	60	56	6
Root dry weight (mg)	40	48	37	42	35	37	38	0.05

In Experiment Two, there was a significant interaction ($p<0.01$) between the type of tissue applied and the level of application for all four parameters measured (Fig. 1). All tissues at 2.4 % w/w reduced VAM colonisation levels, with mustard shoots having a greater effect than the other tissues. *Rhizobium* nodulation was decreased by all rates of the canola shoots and mustard shoots, with the mustard again having a greater effect at 2.4 %w/w. The radish roots reduced nodulation only at the lowest rate.

where growth was reduced by the canola and mustard shoots, and increased by the radish roots.

DISCUSSION

In the two field experiments VAM colonisation of wheat following a *Brassica* crop was greatly reduced. While there are reports from overseas of brassicas being colonised by VAM fungi (9), a survey of *Brassica* crops across southern Australia found no instances of colonisation (11). Thus VAM inoculum levels should decline under brassicas in a manner similar to under long fallows (14). The similar levels of VAM colonisation following the three *Brassica* crops in each of the two field experiments suggests that this decline is not exacerbated by the release of ITCs by the brassicas. The laboratory experiments support this conclusion, in particular Experiment One where the addition of canola and wheat roots at 0.6 and 1.29 %w/w had no effect on VAM inoculum levels. Canola roots are commonly found in the top 15 cm of field soil at 0.1-0.5 %w/w (5).

VAM inoculum, therefore, appears less sensitive to ITCs than the inoculum of soil-borne disease fungi, which has been shown to be negatively affected by both freeze-dried *Brassica* tissues and pure ITCs (7, 12). For instance, addition of canola roots to soil at a rate of 2pe equivalent to the 0.6 %w/w Dunkeld treatment in Experiment One reduced the infection severity on wheat of *Fusarium*, *Rhizoctonia* and *Gaeumannomyces* by 40% (13). Thus, breeding canola varieties with higher root ITC concentrations to enhance the negative effects on disease-causing fungi is unlikely to further reduce VAM inoculum.

Experiment Two indicated what might occur in a situation where crops are green-manured. The highest rates of canola and mustard shoots both reduced VAM colonisation and *Rhizobium* nodulation. However, as canola shoots contain negligible amounts of GSLs it appears that compounds other than ITCs may be released from brassicas and affect soil organisms. Surprisingly, the radish roots whilst reducing VAM colonisation at the highest rate, enhanced nodulation and plant growth. The mechanism for this effect is unclear as the higher C:N ratio of the radish roots indicates that nitrogen release was unlikely to be involved. The effects of green-manuring brassicas on VAM colonisation, *Rhizobium* nodulation and growth of following crops will probably vary with the type of *Brassica* and involve compounds other than ITCs. This area requires further investigation.

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