BIOFUMIGATION BY BRASSICAS REDUCES TAKE-ALL INFECTION

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Abstract

Biofumigation refers to the suppression of soil-borne pests and pathogens by biocidal compounds, principally isothiocyanates (ITCs) released when glucosinolates (GSLs) in the tissues of *Brassica* plants are hydrolysed in soil. We investigated the biofumigation potential of brassicas in cereal farming systems by considering the profile of GSL in the roots, and the suppression of the Take-all fungus (*Gaeumannomyces graminis*) by the *Brassica* roots in soil. The results showed that canola roots contain predominantly the aromatic GSL, 2-phenylethyl GSL, which yields an ITC upon hydrolysis which is highly toxic to cereal fungal pathogens. Levels of this compound within current Australian canola cultivars varied 6 -fold. In pot and field experiments, *Brassica* root residues suppressed Take-all more than those of linseed, and suppression increased with higher levels of GSLs in the roots.? The results suggest there is significant potential to develop brassicas with increased biofumigation potential for suppression of soil-borne cereal pathogens in the traditional wheatbelt. The high levels of GSLs found in winter fodder brassicas suggest they may provide an effective disease break in new areas of winter wheat production in the higher rainfall areas.

Key Words: Brassica, take-all, disease, glucosinolate, suppression.

Brassica species and other members of Cruciferae contain significant quantities of the thioglucoside compounds known as glucosinolates (GSLs) in their tissues. GSLs are hydrolysed by the myrosinase enzyme (present endogenously in *Brassica* tissues) to release a range of hydrolysis products including oxazolidinethiones, nitriles, thiocyanates and various forms of volatile isothiocyanates (ITCs).These hydrolysis products, in particular the ITCs, are known to have broad biocidal activity (2).

In *Brassica* oilseed rotation crops such as canola (*B. napus*) or Indian mustard (*B. juncea*), crops are grown to maturity for seed production and shoot biomass, if incorporated, is generally mature and low in GSL concentration (4). Under these circumstances, the roots would provide the principal source of GSLs for biofumigation in the soil, either in-crop or during post-harvest decomposition of the root tissues. Accordingly, suppression of intractable soil-borne fungal pathogens by ITCs released by the roots of *Brassica* rotation crops is thought to contribute to their superiority as break crops for cereals (1, 4). The roots of most brassicas contain predominately 2-phenylethyl GSL (5), which has been shown to be highly toxic to cereal fungal pathogens *in-vitro* (10).

There have been few studies to measure the GSL profiles of *Brassica* roots in the field, and most studies of fungal suppression have been carried out in-vitro.?? We measured the GSL profiles in the roots of a diverse set of field-grown brassicas, in particular those relevant to the Australian grains industry. *Brassicas* with different root GSL profiles were then selected for pot and field studies. The aim was to determine if greater suppression of soil-borne fungi by brassicas than non-brassica break crops could be demonstrated *in-vivo*, and if the level of suppression was related to the concentration of GSL in the roots.

Materials and methods

Glucosinolate profiles

Root GSL profiles were measured in a diverse range of *Brassica* species including Australian spring canola varieties (*Brassica napus olifera annua*), winter oilseed and fodder rapes (*B. napus olifera biennis*) and Indian mustard (*B. juncea*) in 1995 (5). In 1996, a larger range of current cultivars and advanced

breeding lines of Australian spring canola were assessed. In both years, seeds were sown directly into the field during May at CSIRO Ginninderra Experiment Station near Canberra, Australia in plots 0.5 m x 1 m with 0.1 m inter-row and intra-row spacing (100 plants/m^2) and individual plots 1 m apart. Three replicate plots of each of the entries were arranged in a randomised complete block design.? The plots were topdressed with fertiliser on 26 July to supply 20 kg/ha N, 20 kg/ha P and 18 kg/ha S. The area was hand-weeded and irrigated occasionally to prevent water stress.

At flowering, six bordered plants from the inner three rows were dug from each plot to a depth of 0.15 m and taken to the laboratory with the soil surrounding the intact roots. The soil was washed from the roots which were separated and frozen at -20°C. The samples were freeze-dried, ground using a Wiley mill with 1 mm screen, and stored in sealed bottles at -20°C. GSLs from 300 mg of freeze-dried root tissues were extracted, identified and quantified according to an HPLC method described previously (6), with some modifications (5).

Pot Experiment

Field soil collected from a cultivated site near Harden NSW (0-10 cm) was sieved, fumigated with MeBr, and stored dry prior to the experiment. The soil was packed into pots (15 cm deep and 15 cm in diameter) in two layers - an initial 5 cm uninoculated soil layer was overlain by a 10 cm layer of soil inoculated with 0.5% w/w of take-all (*Gaeumannomyces graminis*) inoculum prepared on sterile ryegrass seed. The pots were then wet up to field capacity using full strength Hoaglands solution. Four replicate pots of the following species were sown: (1) Wheat (Jantz), (2) Linola (Argyle), (3) Mustard (Siromo), (4) Canola (low root GSL - Oscar), (5) Canola (high root GSL - Tamara). Four plants per pot were established (10 for Linola) and grown in a growth cabinet at 8/12°C with regular applications of nutrient solution. At 60 days after sowing the shoots of all plants were cut and removed, and the soil in the pots allowed to dry for 5 days. The soil was then removed from the pot, thoroughly mixed to incorporate the root material, replaced in pot, rewet to field capacity and then left to air dry. After 8 weeks, Jantz wheat was sown and established in the pots and grown for 5 weeks (5 leaf stage). The plants were then harvested and the roots assessed for take-all infection using a method described previously (7).

Field experiment

Six replicate plots of the same five crops used in the pot experiment were sown in field plots (15 m x 2 m) at Ginninderra Experiment Station on May 20, 1996. The wheat, linseed and brassicas were sown at 80, 40 and 5 kg/ha respectively. At sowing, the plots were inoculated with Take-all by adding 20 kg/ha of the ryegrass inoculum used in the pot experiment to the seed, and drilling it with the crop. The crops were grown to maturity and following harvest, soil was collected from within the crop rows using a soil coring tube (0-10 cm) to determine the level of Take-all inoculum remaining. The soil from 30 sites in each plot was bulked, and Take-all assessed using the bioassay technique described previously. The bioassay was conducted using Jantz wheat seedlings grown in small pots (4 replicates from each field plot) in a growth cabinet at 15°C and the level of Take-all infection was assessed at the 4 leaf stage as previously described.

Results

Root GSL profiles

The major GSL found in the roots was 2-phenylethyl GSL which comprised between 65 - 95 % of total root GSL concentration (Fig. 1). In the 1995 screening, the winter *B. napus* lines (*eg.*Tamara, Hobson, Rangi) generally had higher GSL levels than the spring canola lines tested (eg. Oscar). Indian mustard (Siromo) generally had lower levels of 2 phenylethyl GSL than *B napus*, but also contained 2-propenyl GSL, not present in *B. napus*.? Tamara, Oscar and Siromo were selected for use in the pot and field experiments on the basis of these differences in their root GSL profiles. A more comprehensive screening of current Australian spring canola lines in 1996 revealed a 6 fold variation in GSL concentration and some lines had levels as high as those measured in the 1995 winter types (highest and lowest only shown in Fig 1).

Pot and field experiment.

In both pot and field experiments, the level of take-all infection was highest following wheat, was higher after linseed than any of the brassicas, and among the brassicas was lower as the level of GSL in the roots increased (Fig. 2).

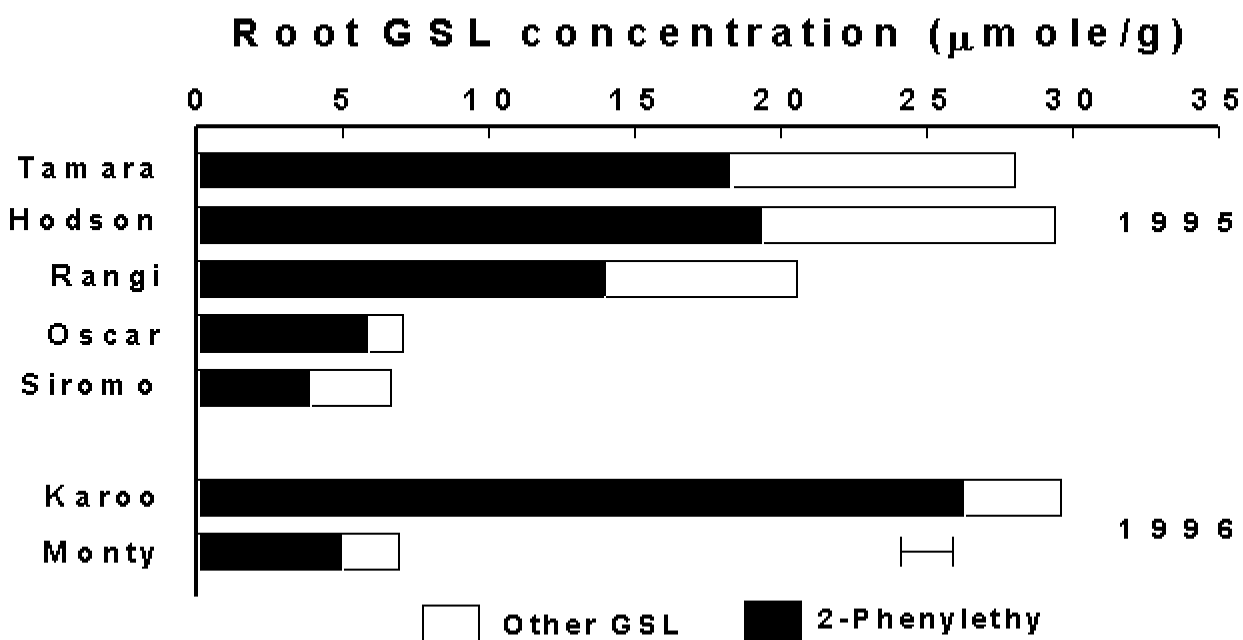

Figure 1

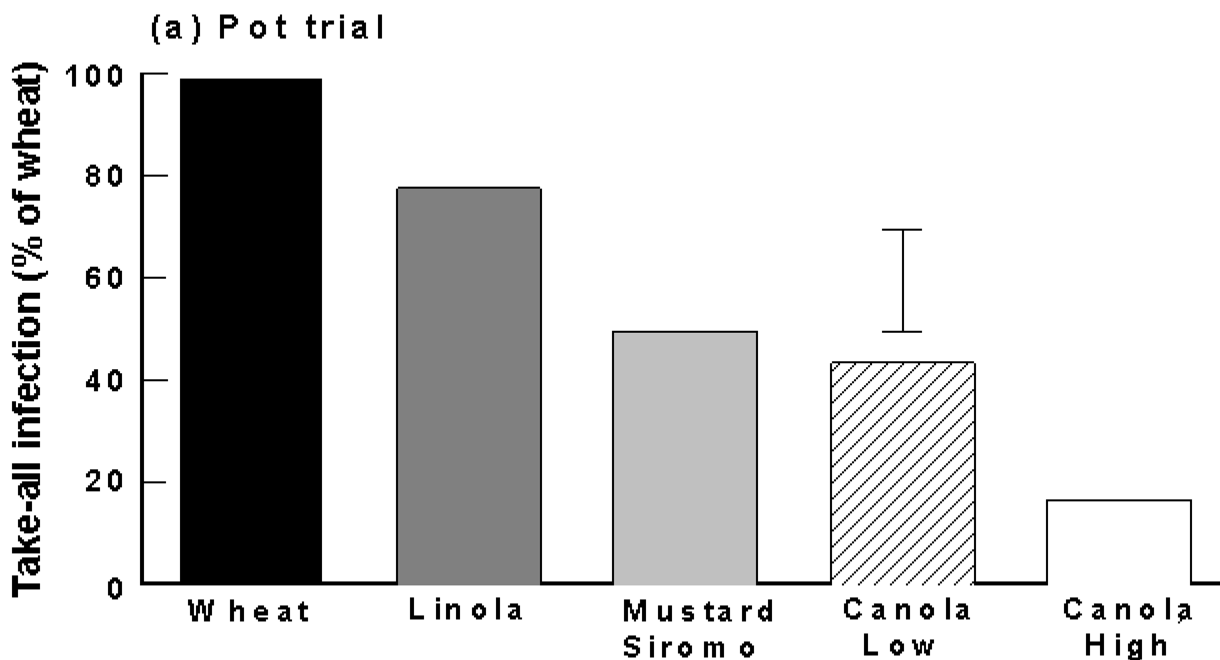

Figure 2

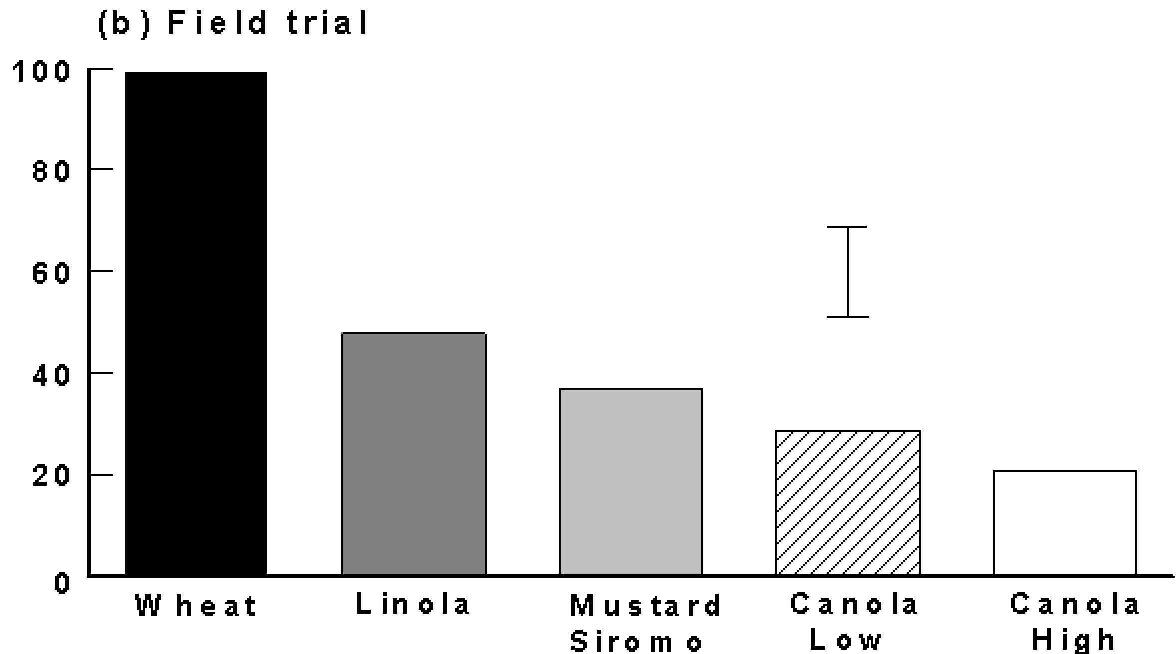

Figure 3

Discussion

The results demonstrate the suppressive potential of *Brassica* root tissues in soil and provide evidence that suppression is related to the level of GSL in the root tissues. The principal GSL in the roots, 2-phenylethyl GSL, is the precursor to 2 phenylethyl ITC which has been shown to be the most toxic of several ITCs released by the hydrolysis of different Brassica tissues against soil-borne fungal pathogens (3, 10). Take-all was selected as the test fungus as it had previously been shown to be the most sensitive of several soil-borne fungi to ITCs and is easily assessed on the roots (10). The level of suppression by the brassicas observed in both pot and field experiments (Fig. 2) relate well to the levels of GSL measured in the roots (Fig. 1). Similar results for the key role of 2-phenylethyl GSL in canola roots, in the suppression of *Pratylenchus* nematodes have also been recently reported (8). Although these results indicate the potential for enhanced fungal suppression by *Brassica* crops, a reduction in the infection of subsequent wheat crops in the field depends upon other factors associated with the persistence of the inoculum in soil. In a normal crop sequence, Take-all inoculum is likely to be reduced to low levels by all break crops except where it survives on grass weed hosts or is preserved due to dry conditions. Further studies to determine the magnitude of the benefits of biofumigation to subsequent cereal crops and the conditions under which they occur are in progress.

The variation in the root GSL levels of Australian spring canola varieties (independent of seed GSLs) provides scope to select or develop lines with increased biofumigation potential. In addition, the high GSL levels in the winter fodder brassicas may provide further opportunities to exploit biofumigation by incorporating? spring-sown fodder brassicas into the rotation in higher rainfall areas to precede winter or spring wheats. These winter fodder brassicas accumulate and maintain high concentrations of GSLs in their root tissues as the usual decline in GSL associated with flowering does not occur (8). The enhanced suppresssion of Take-all by these crops may reduce the need to remove grass weeds from pastures at an early stage, and the brassicas would provide high quality feed in summer.

Conclusions

These results show that Brassica species relevant to the Australian Grains Industry contain high levels of the aromatic GSL 2-phenylethyl GSL, and that high levels in the roots are associated with greater fungal suppression in the soil. Variation in current oilseed and fodder brassicas indicates potential to select or develop varieties with greater biofumigation potential. Further studies to determine the efficacy of brassica residues under commercial field conditions against Take-all and other pathogens are in progress.

Acknowledgments

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