Potential use of isothiocyanates in branched broomrape eradication

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Summary Bulgarian research has shown that branched broomrape, Orobanche ramosa L., germination could be stimulated with various isothiocyanate (iTC) chemicals. These are naturally produced as breakdown products of glucosinolates in brassicas and methyl iTC is the active ingredient in metham sodium and dazomet fumigants. Such fumigants have been used in Bulgaria to cause suicidal germination of broomrape in tobacco, but there has been limited uptake of iTCs as broomrape germination stimulants elsewhere in the world.

The potential applicability of iTCs for the branched broomrape eradication program in South Australia was investigated by comparing germination responses to both iTC chemicals and brassica species/lines. Methyl iTC stimulated high levels of branched broomrape germination at 0.1mM. A hydroponic comparison of brassicas did initially show higher branched broomrape germination as the root iTC profile increased. However, this result was inconsistent in subsequent experiments.

Keywords Branched broomrape, Orobanche ramosa, isothiocyanate, dazomet, germination.

INTRODUCTION

Branched broomrape (Orobanche ramosa L.) is a parasitic weed that is the subject of an eradication program in the western Murray-Mallee region of South Australia (SA). Broomrapes (Orobanche spp.) are obligate root parasites, mainly of herbaceous, dicotyledonous plants. Broomrape seeds are microscopic and have low nutrient reserves. In order to maximise chances of attachment (and hence survival), seeds need to be in close proximity to suitable host roots. Consequently, broomrape seeds will only germinate if they detect specific chemicals from the roots of host plants.

The search for a durable chemical that can be applied to infested fields to trigger the suicidal germination of broomrape seed remains a key goal in parasitic plant research. Recent research has largely focused on strigolactone analogues, following the success of the standard laboratory stimulant chemical GR 24 (Wigchert et al. 1999, Sato et al. 2003). However, past Bulgarian research into isothiocyanates (iTCs) as germination stimulants seems to have been largely overlooked.

Zhelev (1987) observed branched broomrape germination was stimulated with low concentrations (0.1–10 mg L\(^{-1}\)) of phenyl iTC and allyl iTC (i.e. 2-propenyl iTC), whilst germination was inhibited at relatively high concentrations of these iTCs (100–1000 mg L\(^{-1}\)). In addition, ethanol extracts from seeds and roots of various brassica crops (Brassica spp.) also promoted germination of branched broomrape. Brassicas produce glucosinolates, which after tissue injury are converted to iTCs by myrosinase enzyme.

Zhelev’s (1987) research provided two possibilities for reducing soil seed banks of branched broomrape in SA. Firstly, if the local strain of branched broomrape was triggered to germinate by iTCs then it may be possible to use commercially available methyl iTC-based fumigants (e.g. Basamid\(^{®}\)) to cause suicidal germination. Such an approach has been trialled in Bulgaria (Chalakov 1998) but their results lacked detail. Secondly, brassica varieties high in root glucosinolates could be grown as host crops to maximise branched broomrape germination, with the crop being killed prior to emergence of broomrape flowering stems.

This paper reports on laboratory trials examining the germination response of the SA strain of branched broomrape to iTC concentrations and to brassica varieties differing in glucosinolate profiles.

MATERIALS AND METHODS

Branched broomrape seed used in the experiments was collected from mature plants at several infestation sites in the SA quarantine area in November-December 2000. Seeds were stored and experiments conducted in a quarantine facility in rooms kept at 20\(^{0}\)C.

Germination response to iTCs Three iTC dose response experiments were conducted in April 2002 (chemical trial 1 – CT1), December 2002 (CT2) and August 2003 (CT3). Chemicals compared were methyl
iTC, 2-phenylethyl iTC, 2-propenyl iTC (not in CT3) and GR 24. In CT1, concentrations tested were 0.1, 1, 10, 100 and 1000 mg L\(^{-1}\) with three replicates. In CT2, concentrations tested were 0.01, 0.1, 1, 10, 100 and 1000 mg L\(^{-1}\) with six replicates. In CT3, concentrations tested were 0.1, 1, 5, 10 and 100 mg L\(^{-1}\) with four replicates. Chemicals were first dissolved in acetone to make 10,000 mg L\(^{-1}\) solutions before being diluted, in deionised water for the iTCs and in 0.3mM buffer of 2-(N-morpholino) ethanesulfonic acid for GR 24 (Kroschel 2001 p. 38).

For each replicate in each trial, 50–100 branched broomrape seeds (surface sterilised in 1\% NaOCl for 10 minutes and then rinsed twice in reverse osmosis water) were placed on a 25 mm Whatman GF/A filter paper disc. Discs were moistened with RO water and sealed in Petri dishes in the dark for approximately 10 days whilst seeds conditioned (Kroschel 2001 p. 37). After conditioning, discs were placed on standard filter paper to dry, before being placed on top of a new 25 mm GF/A disc that had been saturated in a germination response chemical treatment. Discs were again placed in sealed Petri dishes (with chemical treatments kept separate) and kept in the dark. After 10–20 days seeds were assessed for germination under a microscope.

**Germination response to brassicas** Three hydroponic experiments were conducted using the polybag method (Kroschel 2001 pp. 56-9), in April-June 2001 (brassica trial 1 – BT1), December 2001–February 2002 (BT2) and December 2002–February 2003 (BT3). In BT1 22 brassica species/lines were compared with six replicate polybags each. BT2 had 10 brassicas with fifteen replicates. BT3 had 10 brassicas with twelve replicates. Species/lines are shown in Figure 2.

The polybag method of studying broomrape parasitism (Kroschel 2001) consists of a host plant with its roots growing on a glass-fibre paper sheet, which is enclosed in a suspended, clear, polyethylene bag. The base of the sheet is immersed in hydroponic solution. Broomrape seeds are sprinkled over the host’s roots and germination and attachment are observed microscopically.

Dimensions used were GF/A sheets of 115 × 285 mm in bags of 240 × 350 mm. The top of each bag was folded over a bamboo rod and stapled at each side to hold it in place. Various cuts were made in bags to allow for planting brassicas, applying branched broomrape seeds and inspecting germination, and adding hydroponic solution. Bags were suspended in black plastic tubs.

Brassica and branched broomrape seeds were surface-sterilised with NaOCl prior to use. In BT1, brassica seeds were germinated in punnets of vermiculite. Seedlings were then transplanted into polybags at the 2–4 true leaf stage, vermiculite being rinsed off in RO water. In BT2 and BT3, seeds were germinated directly in the polybags at the top of the GF/A sheets, covered by a 25 mm GF/A disc. Each trial had one brassica per bag. Hydroponic nutrient solution was applied at half-strength for the first month, then full strength thereafter, at 50 mL per application every 2–4 days depending on host size. Brassicas were grown with supplementary lighting of eight hour day length.

Conditioned branched broomrape seeds were applied in close vicinity to brassica roots once these had grown substantially down at least half the length of their GF/A sheet. Branched broomrape germination was assessed microscopically by sub-sampling across brassica root systems, at approximately six to eight weeks after brassica germination. Differences in germination rates were assessed for each trial using ANOVA, data being arcsine transformed prior to analysis.

**RESULTS**

**Germination response to iTCs** Data are presented in Figure 1 in mM (mmol L\(^{-1}\)) for clearer presentation of treatment results. There was a branched broomrape germination response to all three iTCs, with germination levels similar to or greater than GR 24 at concentrations of <0.1mM. Germination with methyl iTC peaked around 0.01mM (around 1 mg L\(^{-1}\) = 1 ppm), whereas germination levels for 2-phenylethyl iTC were maintained or improved at lower concentrations. The 2-propenyl iTC germination response was similar to methyl iTC in CT1 and lower than methyl iTC in CT2. Higher germination levels achieved in CT3 were probably due to greater viability of the seed lot used. Overall, methyl iTC gave the highest and most consistent germination response amongst the iTCs.

**Germination response to brassicas** Whilst the first trial, BT1, showed significant differences between brassica species/lines (F = 4.67, P < 0.001), there were no significant differences in BT2 (F = 0.83, P = 0.60). BT3 had significant differences (F = 6.10, P < 0.001) but there were some inconsistencies with BT1. Data are presented in Figure 2.

In BT1 there was a much higher rate of branched broomrape germination with *Brassica × napus* L. var *napus* ‘Dunkeld’ (high) compared to *B. × napus* ‘Dunkeld’ (low). However, the two lines did not give significantly different branched broomrape germination (P > 0.05) in BT2 and BT3. *Brassica barrelieri* (L.) Janka *ssp. oxyrrhina* (Coss.) Regel had a particularly
A high level of germination in BT1 whilst two *Sinapis alba* L. lines induced low to very low germination. *Brassica carinata* A.Braun 94120 and *B. × juncea* ‘Rangi’ caused high germination in both BT1 and BT3. *B. × napus* ‘Nemcon’ and *Brassica × juncea* L. Czern. ‘Nemfix’ were significantly different in both BT1 and BT3, however their positions reversed between the trials.

**DISCUSSION**

The chemical dose response trials repeated Zhelev’s (1987) results, demonstrating that iTCs can stimulate germination of branched broomrape. Methyl iTC performed the best, which is fortunate as the commercial soil fumigants metham sodium and dazomet are based on the release of this chemical. A disadvantage of methyl iTC is its high volatility, making it difficult to retain in soil for long periods. However, it is more biologically active than either 2-propenyl or 2-phenylethyl iTC over a range of soil and temperature conditions (Matthiessen and Shackleton 2005).

Methyl iTC has potential as a germination stimulant for branched broomrape in South Australia. It is applied at a rate of 30–40 kg ha⁻¹ of dazomet in Bulgaria (Chalakov 1998). However, this is an extremely low rate for applying the powder-like dazomet, which is beyond the capabilities of currently available machinery for broad acre application. The Branched Broomrape Eradication Program is instead focusing on using dazomet at higher rates (i.e. 120 kg ha⁻¹), with promising results for decline in the branched broomrape seed bank (N. Secomb pers. comm.). It may be that seed death is occurring both through methyl iTC toxicity and suicidal germination along the

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**Figure 1.** Germination response of branched broomrape to concentrations of iTCs and GR 24 in trials CT1, CT2 and CT3 (with standard error bars).

**Figure 2.** Germination response of branched broomrape to different brassica species/lines in trials BT1, BT2 and BT3 (with standard error bars).
concentration gradient in the soil. However, there are sampling challenges in detecting this effect (Williams et al. 2006). Current research is focusing on better understanding the movement and activity of dazomet in branched broomrape infested soils.

There were indications that branched broomrape germination varies in response to the root glucosinolate profiles (and hence iTCs) of various brassicas. The high and low B. × napus ‘Dunkeld’ lines were bred by Potter et al. (2000) to have high and low concentrations of 2-phenylethyl iTC in root tissue, and this aligned with branched broomrape germination levels in BT1. Similarly, Potter et al. (1998) found B. harvelieri ssp. oxyrrhina had higher root concentrations of 2-phenylethyl iTC than B. napus lines, which correlated with results in BT1.

However, the lack of species/line differences in BT2 and inconsistencies between BT1 and BT3 places some doubt on the reliability of using brassicas high in iTC-liberating root glucosinolates. There was a lack of correlation between brassica root 2-phenylethyl glucosinolate concentration and branched broomrape germination in BT1 (data not shown). There can be considerable environmental variation in the production of glucosinolates by brassicas (Sarwar and Kirkegaard 1999). Field research with brassica lines has been hampered by soil sampling difficulties for branched broomrape seed levels (Williams et al. 2006). Further field research is still warranted though, as both the Clearfield™ canola system and green manure crops of brassicas are likely to reduce branched broomrape seed banks through stimulation of germination.

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REFERENCES


