BIOFUMIGATION IN AUSTRALIAN HORTICULTURE: AN INTEGRATED APPROACH TO MB REPLACEMENT.

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Biofumigation is the agronomic practice of using volatile chemicals (allelochemicals), released from decomposing *Brassica* tissues, to suppress soil-borne pests and pathogens. The use of Brassicas such as canola (*Brassica napus*) as break crops to help control take-all (*Gaeumannomyces graminis*) in cereal rotations in Australia, is an example of biofumigation. The most common volatiles produced during the breakdown of Brassicas are the isothiocyanates (ITCs). ITCs are related to the active ingredient in the commercial fumigants metham sodium and dazomet and are highly toxic to pests and pathogens. They are released following tissue damage, when myrosinase enzymes, at neutral pH, hydrolyse glucosinolates (GCs). GCs are sulfur-containing chemicals (thioglucosides) that are produced as secondary metabolites by Brassicas and most researchers believe their role is to provide resistance against pests and pathogens. The aim of this paper is to describe current strategies to include biofumigation as part of an integrated approach to MB replacement in Australian horticulture.

Identity and Production of ITCs varies between Brassicas

GC profiles and the subsequent ITCs produced varies between *Brassica* species. Researchers have identified over 100 ITCs, 20 of which are commonly produced by Brassicas and known to have a biocidal effect. The difference in structure of individual GCs and ITCs depends on their organic side-chain (aliphatic, aromatic or indole), which also influences their biocidal activity.

A preliminary trial aimed to identify and quantify ITCs produced by the freeze-dried roots of Indian mustard (*Brassica juncea*) and a turnip/canola mix (Bc/Bn) (*Brassica campestris/Brassica napus*), before and after their incorporation into a light clay loam. ITCs were extracted in ethyl acetate and water and quantified using gas chromatography mass spectrometry (GC/MS). Three main ITCs were identified from *B. juncea* roots (3-butenyl, 4-pentenyl, 2-phenyethyl) and five from the Bc/Bn mix (3-butenyl, 4-pentenyl, 2-phenyethyl, 5-methylthiopentyl, benzyl). In total, the Bc/Bn mix produced 8 times more ITCs ((7.5 µmol ITC/g root tissue) than *B. juncea* (0.9 µmol ITC/g root tissue) (Fig 1). These results positively demonstrate that different *Brassica* species produce different types and concentrations of ITCs. Similar trends were evident when roots were incorporated into soil, although ITCs were recovered at lower concentrations (Fig 2). These levels were at least 15 times below those required to control most pests and pathogens (based on commercial fumigant levels). Clearly, further research needed to investigate methods of elevating the concentration of ITCs released into soils by Brassicas, particularly under field conditions.

Increasing ITC Production using Plant Stress

Brassicas increase production of GCs in response to plant stress (eg nutrient stress, mechanical damage, disease, etc). For this reason, a field trial was conducted to determine if various stress treatments applied to *B. juncea* could increase its production and release of ITCs into soil. These stresses were applied prior to the incorporation of the crop into soil (12 weeks after sowing) and included low levels of contact herbicide (bipyridyl at 5mL/100 Lwater), mechanical damage (puncturing of roots and shoots) and a defense elicitor. Controls consisted of a non-stressed treatment and a fallow. Following incorporation, muslin bags containing a vermiculite/vegetable juice (V8) medium bearing the soil-borne pathogen *Phytophthora cactorum* were buried into soil within the different treatments. After one week, these bags were retrieved and individual pieces of vermiculite plated onto a selective medium (PARP). Viability and growth of the fungus was determined after 4 days incubation. Additionally, soil samples were taken daily over the first week following *Brassica* incorporation for ITC analysis. Six weeks after incorporation, weed biomass was estimated.

Although biofumigant treatments did not kill *P. cactorum*, they suppressed its growth by 10% compared to fallowed plots. Similarly, biofumigant treatments reduced weed biomass by an average of 30% (Fig 3). These results show a clear potential for biofumigation to suppress pathogen and weed populations in the field. There was no evidence that stress treatments increased the efficacy of biofumigation against *P. cactorum* or weeds, although ITC concentrations are yet to be fully analyzed.

Increasing ITC Production by Manipulating Plant Density

A current trial is aiming to determine if high plant densities of Brassicas increase the subsequent release of ITCs into soil. Two commercially available biofumigant lines were sown at a range of densities from very low (4 kg/ha) to very high (32 kg/ha). This trial will determine the GC differences between roots and shoots at different sowing rates and correlate it with total ITCs released into the soil after incorporation. *In vitro* toxicity of macerated biofumigants against important soil-borne pathogens will be determined as will weed biomass and changes in the composition of soil microflora.

Adoption

The trials outlined in this paper target aspects of biofumigation that need addressing before an integrated pest management (IPM) system can be successfully applied on-farm. Research needs to identify methods of increasing concentrations of ITCs released into the soil by Brassicas. Our work strives to achieve this through agronomic practices, while concurrent research around Australia is aiming to breed higher levels of GCs into *Brassica* lines and increase release efficiencies of ITCs.

Despite these obstacles, adoption of biofumigation by growers in horticultural industries is relatively high due to its successful use in cereal production. For example, 10% of the strawberry fruit industry in Victoria, 10% of growers in the strawberry nursery industry and some flower bulb growers are experimenting with ways of combining biofumigation into their cropping rotations. In this way, researchers and growers are collaborating to identify practical and effective systems for integrating biofumigation into Australian horticulture.

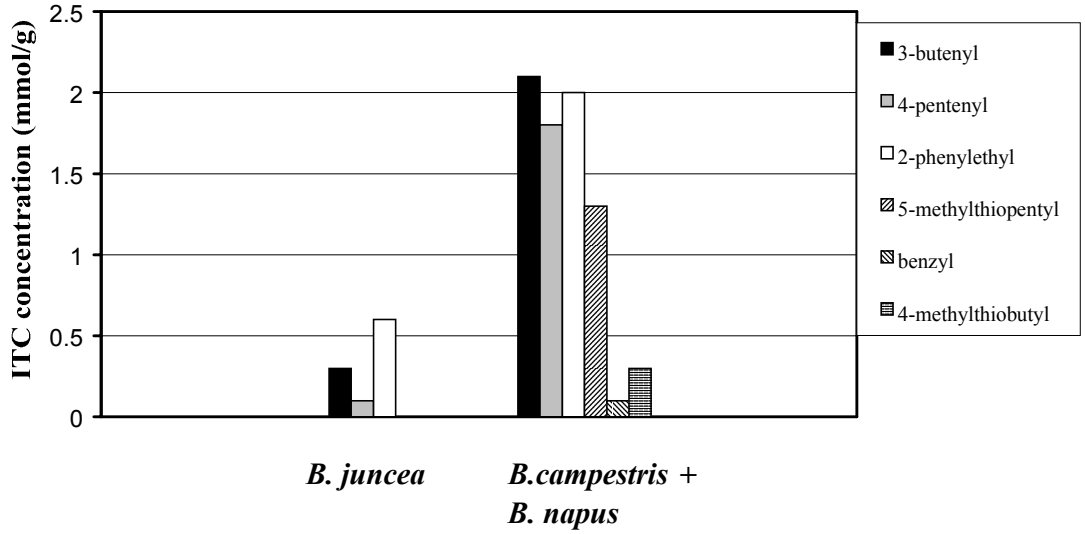

Figure 1. Level of ITCs present in Brassica root tissue

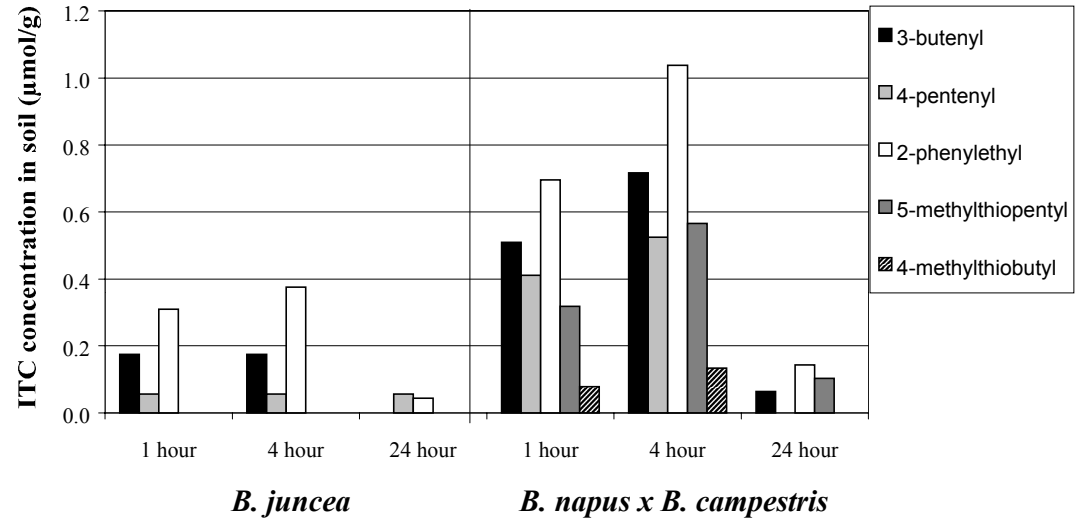

Figure 2. Level of ITCs present following incorporation of Brassica root tissues into soil

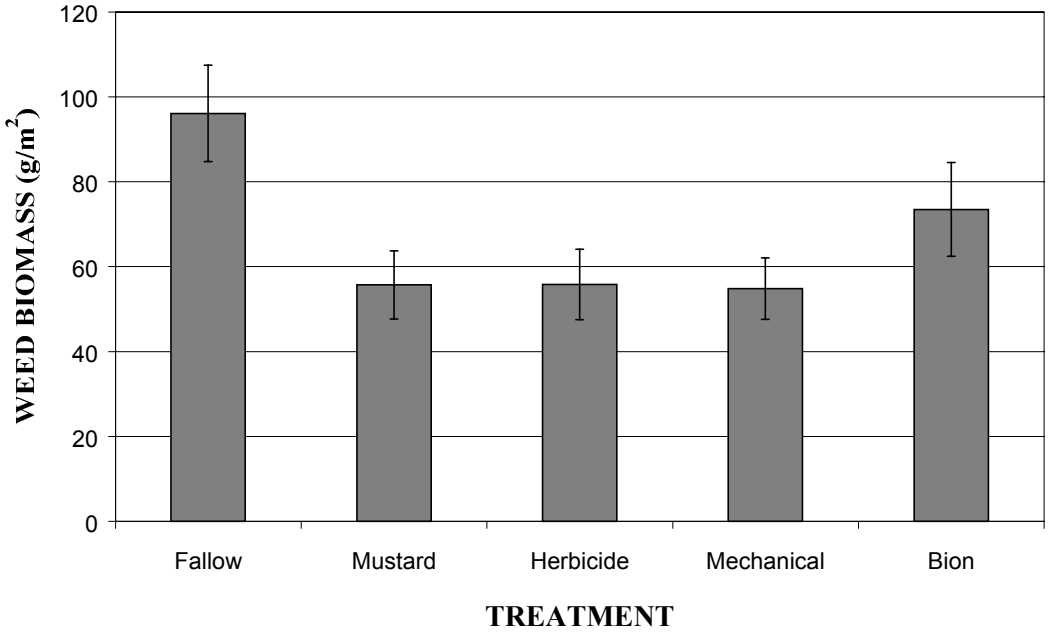

Figure 3. Average weed biomass six weeks after incorporation of *B. juncea*