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Reduced Susceptibility of *Brassica napus* to *Pratylenchus neglectus* in Plants with Elevated Root Levels of 2-Phenylethyl Glucosinolate

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Abstract: The effect of canola (*Brassica napus*) as a crop suppressive to *Pratylenchus neglectus* is in part due to the release of nematicidal isothiocyanates, particularly 2-phenylethyl isothiocyanate, from degrading root tissues. However, many cultivars of canola are relatively susceptible to *P. neglectus* and will fail to reduce soil populations of the nematode. A survey of *B. napus* accessions and closely related species revealed limited scope to decrease the susceptibility of canola through conventional intercrossing. Susceptibility to *P. neglectus* was not related to the total glucosinolate levels, but there were significant, negative correlations ($r = -0.619, -0.517$; $P < 0.001$) between root levels of 2-phenylethyl glucosinolate (isothiocyanate precursor) and plant susceptibility to *P. neglectus:* plants containing more than a certain threshold level of 2-phenylethyl glucosinolate showed reduced susceptibility to the nematode. Selection for high root levels of 2-phenylethyl glucosinolate should reduce the susceptibility of the plants during the growing season while also increasing the nematicidal impact of the degrading root tissues, thereby improving the suppressive benefits of the crop when used in rotation with cereals.

Key words: 2-phenylethyl glucosinolate, *Brassica napus,* canola, disease break, isothiocyanate, nematode, *Pratylenchus neglectus.*

Canola (*Brassica napus* L.) is a potentially suppressive crop which may reduce the level of soil-borne disease within the southern Australian cereal rotation, leading to increased early vigor and final yield of following wheat crops (Angus et al., 1991; Kirkegaard et al., 1994). The negative impact of *Brassica* tissues on harmful soil organisms is well documented (Akhtar and Alam, 1991; Chan and Close, 1987; Kirkegaard et al., 1993; Owino et al., 1993) and has been reviewed by Brown and Morra (1997). The biocidal nature of the tissues is thought to be due to the presence of glucosinolates, which are amino-acid-derived products of secondary metabolism stored in the vacuoles of most *Brassica* plant cells. These glucosinolates are stable and relatively non-toxic but, when tissues are damaged, exposure to the myrosinase enzyme cleaves the glucosinolates to produce a range of biologically active products (Underhill, 1980). Under normal cellular conditions, the primary products of this enzymatic cleavage are isothiocyanates (Larsen, 1981). The biocidal activity of these isothiocyanates has led to their development as soil fumigants (Matthiesson et al., 1996; Olthof, 1987; Saeed et al., 1996). Fifteen different glucosinolates, distinguishable by variation in the side chain, are found within *B. napus* (Sang et al., 1984). As the chemical qualities of the side chain affect the biocidal activity of the different isothiocyanates (Borek et al., 1995; Drobnica et al., 1967; Horakova, 1966; Mojtahedi and Santo, 1996), the nature of the individual glucosinolates present will be as important as the total glucosinolate level in determining interactions with pests and diseases of the plant tissue (Giamoustris et al., 1994).

In vitro studies demonstrated that the biocidal effect of *Brassica* root tissues on the root-lesion nematode (*Pratylenchus neglectus* Filipjev) was correlated with the presence of a single glucosinolate, 2-phenylethyl (2-PE) (Potter et al., 1998), the dominant glucosinolate in the roots of *Brassica* spp. (Kirkegaard and Sarwar, 1998). Breeding to increase root levels of 2-PE glucosinolate should therefore increase biocidal activity

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against *P. neglectus* (Potter et al., 1999). However, the effectiveness of canola as a crop suppressive to *P. neglectus* will be a function not only of the nematicidal qualities of chemicals released from the root tissues as they degrade but also of the susceptibility of the crop to the nematode during the growing season. As *B. napus* is a host of several *Pratylenchus* spp., including *P. neglectus* (Bernard and Montgomery-Dee, 1993; Evans and Webb, 1989; Webb, 1996), the effects of the degrading root tissue on the nematodes will be counteracted, to some extent, by the susceptibility of the crop.

This paper discusses the variation in susceptibility of *B. napus* accessions and closely related species to *P. neglectus,* and explores the relationship between the levels of glucosinolate in the root and the susceptibility of the individual plant. The objective of these studies was to improve the nematicidal potential of degrading canola tissues, reported in Potter et al. (1998). Since susceptible wheat cultivars are a major host of *P. neglectus* and intolerant cultivars suffer yield loss (Vanstone et al., 1998), improved efficacy of canola in reducing soil populations of *P. neglectus* will be of benefit within the southern Australian cereal rotation.

Materials and Methods

Brassica accessions: Accessions were obtained for study from the Australian Temperate Field Crops Collection (Horsham, Victoria), representing the species *B. carinata, B. juncea, B. napus, B. nigra, B. oxyrrhina,* and *B. rapa* (Table 1). *Triticum aestivum* cv. Machete, susceptible to *P. neglectus* (Vanstone et al., 1998), was used as a control.

Pratylenchus neglectus inoculum: Cultures of *P. neglectus* were grown aseptically on carrot root pieces using the method of Nicol and Vanstone (1993) and nematodes extracted from carrot pieces using the mister technique (Southey, 1986). After extraction, nematodes were collected on a 20-µm-pore filter and washed with distilled water to remove possible bacterial contaminants. A suspension of 400 nematodes/ml distilled water was prepared for inoculation.

Table 1. Accessions assessed for variation in susceptibility of *Brassica* spp. to *Pratylenchus neglectus.*

Species	Accession	Accession number
B. carinata	054100	94036
	054108	94044
B. juncea	Cutlass	94200
	Lethbridge 22A	93437
	Mustard 1	91167
	Pusa Bold	93786
B. napus	Bienvenu	90043
	Cresus	90111
	Dunkeld	94713
	Eureka	90524
	Korina	90037
	Libravo	90059
	Lirakotta	90028
	Lirawell	90088
	Midas	90617
	Norin 16	90634
	Ridana	90038
	Start	91021
	Tamara	90039
B. nigra	Black Mustard	92971
	BNI 1	95067
	91072	91072
B. oxyrrhina	Box 1	95060
	Box 2	95061
B. rapa	Arlo	90119
	Bunyip	90139
	Duro	90164

Efficiency of mister method for nematode extraction: The time (120 hours) required to extract nematodes from *Brassica* root tissues resulted in significant reduction in the levels of glucosinolates within the roots (M. Potter, unpubl.). Because degrading glucosinolates may have released isothiocyanate during the misting process, potentially reducing the efficiency of nematode extraction, the numbers of nematodes obtained by misting were compared with numbers observed in stained roots. Eight plants each of Dunkeld canola and Machete wheat were grown singly in 300-ml pots (without drainage holes) of pasteurized University of California (UC) soil mix, and each plant infected with 400 *P. neglectus.* Soil temperature was maintained at 19 °C by placing pots in a controlled temperature water-bath. Plants were watered with distilled water as required. After 6 weeks, roots were washed free of soil under running tap-water. The root systems were severed from the tops and divided evenly

into two parts and weighed. Nematodes were extracted from one half of the roots from each plant by misting. The other half was stained following the method of Byrd et al. (1983) and the nematodes counted.

Susceptibility trials: Ten replicates of each accession were sown singly in 300-ml pots containing pasteurized UC soil mix which had been passed through a 3-mm-pore sieve. Susceptibility trials were carried out in a glasshouse under ambient conditions, with soil temperature maintained at 19 °C by placing the pots in a controlled temperature water-bath. Plants were watered with distilled water as required.

Seeds were partially surface-disinfested by immersion in 70% ethanol for 10 seconds, and then were washed in distilled water. Seeds were sown directly into each pot at a depth of 5 mm. Two weeks after germination, three vertical tunnels were made in the soil around each plant by inserting a 2-mm-diam. skewer into the soil to the depth of the pot. One milliliter of the nematode suspension was pipetted into each hole, providing ca. 1,200 nematodes to each pot. After 6 weeks, the roots were washed free of soil over a 3-mm-pore sieve. Roots were separated from the plant tops with a razor blade, and chopped into 30-mm lengths. Nematodes were extracted from the roots over a 120-hour period in the mister and were counted according to a modification of the method of Doncaster (1962). The numbers of nematodes were expressed as a proportion of the numbers extracted from the wheat control plants.

Relationship between susceptibility to P. neglectus and root glucosinolate levels: Levels of glucosinolates and degree of susceptibility to *P. neglectus* of individual plants from a range of *Brassica* spp. (Trial 1) and from a range of *B. napus* accessions (Trial 2) were compared (Table 2). Plants were grown and inoculated as described above. At harvest, roots were cut into 30-mm lengths which were weighed and divided into two equal parts. Nematodes were extracted from one part by use of the mister. The other was placed into liquid nitrogen, then lyophilized and ground into a powder for glucosinolate analysis. Glucosinolates in the *Brassica* root tissues were assessed by high-performance liquid chromatography (HPLC) in a desulphated form, following a modified protocol of Heaney et al. (1986), as described by Potter et al. (1998). The relationship between susceptibility to *P. neglectus* and root glucosinolate levels was analyzed by simple, linear correlation (Gomez and Gomez, 1984).

TABLE 2. Accessions of *Brassica* spp. tested to determine correlation between plant susceptibility to *Pratylenchus neglectus* and root glucosinolate levels.

Species	Accession	Replicates
	Trial 1: Interspecific	
B. carinata	054108	8
B. juncea	99Y11	6
B. napus	Dunkeld	10
B. nigra	BNI 1	4
B. oxyrrhina	Box 1	2
B. rapa	Bunyip	8
	Trial 2: Intraspecific	
B. napus	AGA95-01	9
	Barossa	10
	Dunkeld	7
	Hyola	12
	Karoo	6
	LL96-2	9
	Monty	9
	Narendra	6
	Oscar	10
	Yickadee	7

RESULTS

Efficiency of mister method for nematode extraction: The stained wheat roots contained 1,615 *P. neglectus*/g dry root, and misting gave an estimate of 1,396 *P. neglectus*/g dry root. From canola roots, 1,727 or 1,677 *P. neglectus*/g dry root were detected, respectively. As there was no significant difference in numbers of nematodes in canola or wheat roots using either misting or staining, the loss of glucosinolate from the tissues during misting was considered to have had no significant effect on the efficiency of nematode extraction.

Susceptibility trials: There was much variation in the numbers of nematodes extracted from the roots of the different *Brassica* spp. (Table 3). Mean numbers of nematodes were lower in roots of *B. napus* accessions

than in the Machete wheat control. The three *B. rapa* accessions supported similar numbers of *P. neglectus* to the wheat. Roots of the *B. carinata, B. juncea,* and *B. nigra* accessions all contained greater numbers of nematodes than Machete. In contrast, both accessions of *B. oxyrrhina* contained fewer *P. neglectus* than all other accessions examined. The 13 *B. napus* lines displayed little variation in susceptibility, with a mean (relative to Machete wheat) of 0.44 and a standard deviation of only 0.07 (Table 3).

Relationship between susceptibility to P. neglectus and root glucosinolate levels: In Trial 1, neither total glucosinolate nor 2-propenyl glucosinolate levels were related to host suitability of the individual plants from the six *Brassica* spp. examined (Fig. 1A,B). However, increasing levels of 2-PE glucosinolate significantly ($r = -0.619$, $P < 0.001$) reduced plant susceptibility to *P. neglectus* (Fig. 1C). Accessions with 2-PE glucosinolate levels above ~8 µmol/g tissue had significantly fewer nematodes in the roots (Fig. 1C; Table 4). In Trial 2, accessions containing 2-PE glucosinolate levels above ~12 µmol/g tissue had significantly fewer nematodes in the roots (Fig. 2; Table 4), and the negative relationship between 2-PE level and nematode susceptibility was significant ($r = -0.517$, $P < 0.001$) (Fig. 2).

Discussion

More nematodes were extracted from the roots of all 13 *B. napus* accessions than were originally added to the plants, suggesting that the species is a suitable host for *P. neglectus* and supporting the findings of Bernard and Montgomery-Dee (1993), Vanstone et al. (1993), and Webb (1996). As no significant variation was observed among the different *B. napus* accessions examined, there is limited scope to reduce the susceptibility of the crop through intraspecific hybridization. There also seems to be limited scope to reduce susceptibility of the crop to *P. neglectus* by hybridization with closely related species, since the *B. napus* lines were the least suitable hosts of *P. neglectus* of all the commercial *Brassica* spp. examined. Although the *B. oxyrrhina* lines were less susceptible than *B. napus,* use of these in the improvement of *B. napus* through hybridization is limited by the potential to introduce undesirable agronomic traits.

The high susceptibility of the *B. carinata, B. nigra,* and *B. juncea* accessions suggests a possible link between susceptibility and the B-genome, present in all three species (U, 1935). This link has implications for the use of B-genome crops in areas with established *P. neglectus* problems, particularly where 'canola quality' mustards (*B. juncea*) are being developed for use within the southern Australian cereal rotation. The widespread use of such crops could lead to increased numbers of *P. neglectus* in the field, particularly as the root tissues have been shown to have only limited nematicidal potential as they degrade in the soil (Potter et al., 1998). However, high 2-PE glucosinolate *B. juncea* lines have been identified (Kirkegaard and Sarwar, 1998) and may be more beneficial within the rotation.

There appears to be a relationship between susceptibility to *P. neglectus* (these results) and nematicidal potency (Potter et al., 1998) of canola: accessions of low susceptibility show high nematicidal potency, and highly susceptible accessions show low nematicidal potency. As the nematicidal effect of the root tissues is due to 2-PE glucosinolate (Potter et al., 1998), it was hypothesized that 2-PE glucosinolate levels may also be linked to plant susceptibility to *P. neglectus.*

Table 3. Variation in susceptibility of *Brassica* spp. to *Pratylenchus neglectus,* expressed as a proportion of the number of nematodes extracted from Machete wheat.

Species	Number of accessions	Mean[a]	Standard error
B. carinata	2	1.30	–
B. juncea	4	1.65	0.12
B. napus	13	0.44	0.07
B. nigra	3	1.83	0.22
B. oxyrrhina	2	0.10	–
B. rapa	3	0.93	0.09

[a] Number of nematodes extracted from test plant per number of nematodes extracted from Machete wheat control.

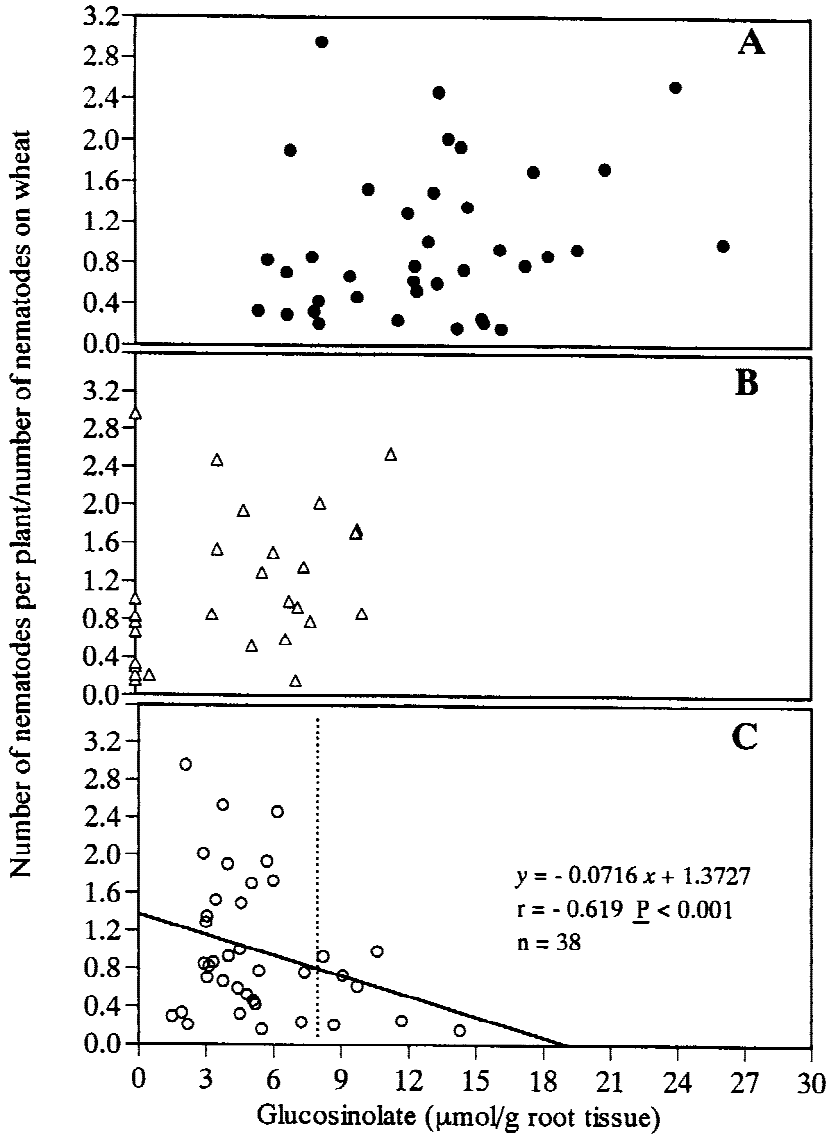

FIG. 1. Relationship between root concentrations of glucosinolates and host suitability to *Pratylenchus neglectus* for individual plants from different *Brassica* spp. (Trial 1). A) Total glucosinolates. B) 2-propenyl glucosinolate. C) 2-phenylethyl glucosinolate. (Vertical line represents threshold level of 8 µmol/g tissue.)

Both Trials 1 and 2 showed a significant, negative relationship between levels of 2-PE glucosinolate in the roots and susceptibility of the accession. Plants with roots containing more than a critical level of 2-PE glucosinolate (8 to 12 µmol/g tissue) consistently contained significantly fewer nematodes at the end of the 6-week growing period, suggesting a threshold level beyond which nematode numbers were reduced. No such relationship was observed between susceptibility and 2-propenyl or total glucosinolate. This supports the assertion that 2-propenyl glucosinolate in root tissues does not contribute significantly to the suppressive qualities of plants to *P. neglectus* (Potter et al., 1998). These findings also agree with the view that individual glucosinolates play different roles in the relationship of *Brassica* spp. to pests and pathogens (Giamoustris et

TABLE 4. Average numbers of nematodes extracted from the roots of individual *Brassica* spp. plants containing levels of 2-phenylethyl glucosinolate below or above a theoretical critical level (six *Brassica* spp. [Trial 1] and 10 *B. napus* accessions [Trial 2]).

	Range of 2-phenylethyl glucosinolate (μmol per g tissue)	Number of plants assessed	Average nematode extraction[a]	Standard error
Trial 1	0–8	31	0.54	0.07
	8–14	7	0.27	0.06
Trial 2	0–12	71	0.68	0.08
	12–20	13	0.46	0.06

[a] Number of nematodes extracted from test plant per number of nematodes extracted from Machete wheat control.

al., 1994), and support the suggestion that 2-PE isothiocyanate may be a more efficient biocide than 2-propenyl isothiocyanate within the soil environment (Sarwar et al., 1998).

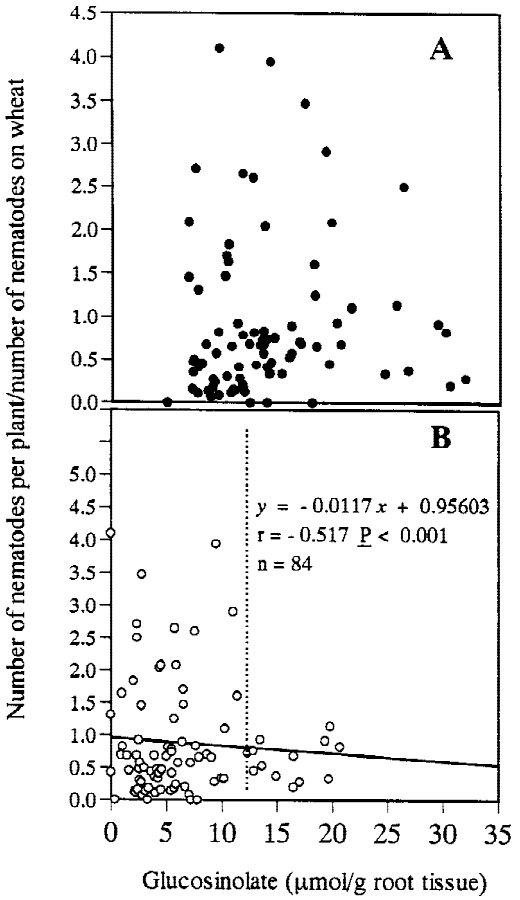

FIG. 2. Relationship between root concentrations of glucosinolates and host suitability to *Pratylenchus neglectus* for individual plants from different *Brassica napus* accessions (Trial 2). A) Total glucosinolates. B) 2-phenylethyl glucosinolate. (Vertical line represents threshold level of 12 μmol/g tissue.)

Other factors may be involved in determining the susceptibility of *Brassica* spp. to *P. neglectus*. In both trials, many plants contained low numbers of *P. neglectus* despite having negligible levels of 2-PE glucosinolate (<3 μmol/g root tissue). Some of these plants may have been misses, due to the random nature of the nematode infection, but enough plants with low glucosinolate levels contained low nematode numbers to suggest a potential for decreasing susceptibility of *B. napus* through identification and selection for alternative resistance mechanisms. However, a high level of 2-PE glucosinolate within the roots does appear to aid plant defense against *P. neglectus*. Efforts to increase mean levels of 2-PE glucosinolate within *Brassica* roots should decrease susceptibility to *P. neglectus,* concurrently increasing the nematicidal potency of the degrading tissue (Potter et al., 1998). Ample variation in the levels of 2-PE glucosinolate has been identified within commercial canola accessions (Kirkegaard and Sarwar, 1998), with strong heritability observed across three generations of breeding studies (Potter et al., 1999). Further, divergent plant populations from a single accession containing high or low root levels of 2-PE glucosinolates showed significant differences in susceptibility to *P. neglectus* (Potter et al., 1999). It seems likely that a sub-population of plants could be selected for increased root 2-PE glucosinolate levels, thus having lower susceptibility combined with greater nematicidal potency against *P. neglectus* and providing a more useful rotational crop in the cereal growing regions of southern Australia.

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