

Differential responses by some insect pests to novel insect-resistant *Brassica napus* L.

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Introduction

Introgression of resistance from other brassicaceous species to *Brassica napus* L. has been used to develop insect-resistant canola. *Sinapis alba* L. is resistant to many insect herbivores⁷ so was chosen as a source of resistance to three important insect pests: *Ceutorhynchus obstrictus* (Marsham) (syn. *C. assimilis* (Payk.)) (Coleoptera: Cuculionidae), *Delia radicum* (L.) (Diptera: Anthomyiidae) and *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae)^{3, 6, 9, 7}.

Antixenosis resistance has been demonstrated for several lines of novel germplasm. However, three hybrid lines found to be *D. radicum*-resistant did not significantly reduce *P. cruciferae* feeding⁶. Several *D. radicum*-resistant lines did not reduce *C. obstrictus* larval infestation⁴. These results suggest distinct mechanisms of resistance to these insects.

Factors contributing to *C. obstrictus*-resistance include 2-phenylethyl- (NAS) and 1-methoxy-3-indolylmethyl-glucosinolate (NEO) contents. Previous work reported relationships of High performance Liquid Chromatography peak heights associated with these compounds and weevil oviposition and NAS peak heights and olfactory responses^{13, 17}. Concentrations of these compounds may also influence *D. radicum* and *P. cruciferae*.

Floral and foliar reflectance were found to differ among *B. napus*, *S. alba* and several *S. alba* x *B. napus* lines and floral reflectance was related to *C. obstrictus* responses¹⁸. Since *D. radicum* and *P. cruciferae* both exhibit phototactic behaviors to specific frequencies of light^{2, 19}, differences among *S. alba* x *B. napus* lines may also influence attractiveness to these insects.

Here we present results of laboratory tests to assess differences in the responses of *D. radicum* to some *C. obstrictus*-resistant and -susceptible lines. Differences in olfactory responses, oviposition preferences and foliar reflectance were tested. We also discuss factors that likely contribute to differential responses of these insects and *P. cruciferae* to some novel *S. alba* x *B. napus* germplasm.

Methods and Materials

Olfactory responses of individual female *D. radicum* to plant material were assessed with a Y-tube olfactometer¹⁷. The proportions of total flies responding and proportion captured on the treatment side were compared among hybrid lines, *B. napus* var. Q2 and *Sinapis alba* var. AC Pennant (hereinafter referred to as *B. napus* and *S. alba*). All plants were at the 5-6 leaf stage and grown in 5-cm pots. These results were compared to weevil responses to the same lines¹⁷.

Antixenosis resistance to *D. radicum* was determined using a no-choice oviposition test. Plants were grown to the 6 leaf stage. Potting medium was covered with 1 cm of washed, sterilized sand to facilitate removal of *D. radicum* eggs. Female flies were introduced to four replicate cages with four plants each at one fly per plant for 48 h. Seven *S. alba* x *B. napus* lines currently being assessed for *D. radicum* resistance (RM lines), five lines previously tested for *C. obstrictus* resistance (CSW lines)^{16, 17}, *B. napus* and *S. alba* were tested. Correlations of *D. radicum* oviposition and NAS and NEO peak heights of CSW lines¹³ were assessed.

Reflectance of 4-5 mature leaves from different plants at the 6-leaf stage was assessed using a dual-beam spectrophotometer operating between 250 and 700 nm¹⁸. The visual system of *D. radicum* has been reported to include spectral peaks of 340-350 nm and 460-546 nm and a 'pseudo-peak' at 630 nm²; phototactic responses to 366 nm, 430 nm and 540 nm light have been reported⁸. In the current study, comparisons of foliar reflectance at 350 nm, 366 nm, 430 nm, 500 nm, 540 nm and 630 nm were made and relationships of *D. radicum* oviposition and reflectance at these frequencies were assessed.

Data Analyses

All analyses were conducted with SAS version 9.2¹². Proportions of *D. radicum* females responding and proportions captured on the treatment side of the olfactometer were compared using proc GENMOD, specifying a binomial distribution. Differences among individual lines were assessed with the LSMEANS statement and DIFF option. *Delia radicum* oviposition was assessed with the LSMEANS statement DIFF option in proc MIXED. Relationships among weevil and fly olfactometer responses and oviposition and glucosinolate content were assessed using proc CORR. Relationships between oviposition and foliar reflectance were assessed using proc REG with the Selection = Stepwise option.

Results

Greater proportions of *D. radicum* responded to CSW-171S than CSW-121R or *S. alba* in olfactometer tests (Table 1). Greater proportions of *D. radicum* females were attracted to the treatment side with *B. napus* than CSW-173R or *S. alba* ($P < 0.05$ for both comparisons) (Table 1). No correlation of *D. radicum* and *C. obstrictus* olfactometer responses was apparent ($P > 0.05$ for total or proportion of respondents to the treatment side of the olfactometer).

Correlations of total *D. radicum* responding and NAS and NEO peak heights were apparent ($R = 0.830$; $P = 0.041$ and $R = -0.884$; $P = 0.019$, respectively). However, no relationships of NAS or NEO peak heights and fly proportions responding to the treatment side of the apparatus were detected ($R = 0.664$; $P = 0.150$ and $R = -0.612$; $P = 0.197$, respectively).

CSW lines: more *D. radicum* eggs were laid near CSW-171S and *B. napus* than any other line ($P < 0.05$ for all comparisons) (Table 1). RM lines: oviposition was greater for *B. napus* than RM-229, RM-308 and *S. alba* ($P < 0.05$ for all comparisons). *Delia radicum* and *C. obstrictus* oviposition on CSW lines was not correlated ($P > 0.05$). *Delia radicum* oviposition and both NAS and NEO peak heights were correlated ($R = 0.913$; $P = 0.011$ and $R = -0.892$; $P = 0.017$). Proportions *D. radicum* responding to the treatment side of the olfactometer and oviposition were correlated ($R = 0.829$; $P = 0.0212$). Functions associated with oviposition: eggs = $0.234 \times (\text{NAS peak height}) + 1.66$ for *C. obstrictus* and eggs = $0.531 \times (\text{NAS peak height}) + 3.30$ for *D. radicum*.

For CSW and RM lines tested, reflectance at all frequencies tested differed ($P < 0.05$ for all comparisons) (Figure 1). No significant relationship of *D. radicum* oviposition and foliar reflectance at these frequencies was detected (at $\alpha = 0.05$).

Discussion

Differences in *D. radicum* olfactory responses indicate differences among the odors of these plants. Differences reported among CSW-line plants that should contribute to these differences include NAS concentrations^{13, 17}. Attractiveness and susceptibility of CSW lines to *C. obstrictus* are influenced by NAS concentrations^{16, 17}; this is also apparently the case for *D. radicum*. NAS- isothiocyanate (ITC) has been shown to be a *D. radicum* attractant, particularly when odors also include allyl-ITC⁵. Differences in allyl-glucosinolate among CSW-line plants were not detected¹³. However, differences in NAS content and additive or synergistic effects with allyl-ITC have the potential to strongly influence *D. radicum* and therefore the attractiveness and susceptibility of potential host plants.

Differences in *D. radicum* oviposition were also apparent and were correlated with NAS and NEO contents of test plants. The hydrolysis products of NEO have limited volatility¹⁰ therefore should not influence olfactory responses but may influence oviposition. *Hellula undalis* (Fabricius) (Lepidoptera: Pyralidae) oviposition is reduced by indolyl glucosinolates¹⁰.

Although NAS-ITC is also attractive to *C. obstrictus*¹⁷, no correlations of *C. obstrictus* and *D. radicum* olfactory responses or oviposition were detected. Examination of the slopes of functions associated with NAS content and oviposition offers some explanation for this observation and suggests a greater affinity of *D. radicum* for this compound, perhaps owing somewhat to an interaction with allyl-ITC.

Although differences in reflectance of specific frequencies were detected among test plants, all plants tested shared a great similarity in foliar reflectance pattern: moderate reflectance at 350 nm, 366 nm, 430 nm, 500 nm and 630 nm and a peak in reflectance at ca. 550 nm. These reflectance patterns are similar to those of radish, a preferred host plant of *D. radicum*⁸ and likely do not differ enough to influence host choices. A lack of correlation between oviposition and reflectance supports this conclusion.

Some *D. radicum*-resistant lines are not *C. obstrictus*-resistant⁴; some are not *P. cruciferae*-resistant⁶. NAS and NEO contents apparently influence both *C. obstrictus* and *D. radicum*. Effects of NAS on *P. cruciferae* are negligible¹¹ and although beetle feeding induces indolyl glucosinolates in *B. napus*, these compounds apparently do not dissuade feeding¹. Physical parameters also likely affect differences in *C. obstrictus* and *D. radicum* host selection. Differences in *D. radicum* damage ratings between *D. radicum*-resistant and -susceptible lines were negated if stem diameter was considered a covariate in analyses¹⁵.

References

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Test line	P_D		P_{DT}		P_C †		P_{CT} †		Eggs _D		Eggs _C ††	NAS‡	NEO‡
<i>Brassica napus</i> var. Q2	0.65 (0.16)	ab	0.45 (0.16)	a	0.95 (0.04)	a	0.43 (0.03)	a	7.06 (0.48)	a	3.07 (0.45)	6.47 (0.61)	3.23 (0.74)
<i>Sinapis alba</i> var. AC Pennant	0.30 (0.15)	c	0.10 (0.12)	b	0.78 (0.04)	b	0.21 (0.03)	b	3.12 (0.33)	b	0.04 (0.04)	n/a	n/a
CSW 121R	0.40 (0.16)	bc	0.30 (0.15)	ab	0.74 (0.04)	b	0.19 (0.03)	b	4.06 (0.33)	b	2.21 (0.45)	1.17 (0.34)	9.99 (0.38)
CSW 154S	0.65 (0.16)	ab	0.25 (0.15)	ab	0.94 (0.04)	a	0.37 (0.03)	a	4.69 (0.44)	b	4.39 (0.71)	5.67 (0.40)	3.95 (0.72)
CSW 171S	0.80 (0.14)	a	0.30 (0.15)	ab	0.92 (0.04)	a	0.34 (0.03)	a	7.31 (0.49)	a	3.32 (0.79)	7.33 (0.61)	2.56 (0.22)
CSW 173R	0.50 (0.16)	abc	0.15 (0.14)	b	0.67 (0.04)	b	0.15 (0.03)	b	2.69 (0.23)	b	2.18 (0.52)	0.34 (0.30)	10.05 (0.54)
CSW 276R	0.60 (0.16)	abc	0.20 (0.14)	ab	0.65 (0.04)	b	0.18 (0.04)	b	4.00 (0.32)	b	1.64 (0.45)	0.98 (0.31)	8.44 (0.44)

Table 1. Mean proportions (SEM) of *Delia radicum* and *Ceutorhynchus obstrictus* responding to *Brassica napus* var. Q2, *Sinapis alba* var. AC Pennant and *S. alba* x *B. napus* lines in olfactometer experiments, mean (SEM) fly and weevil oviposition and mean (SEM) glucosinolate content. P_D : proportions of *D. radicum* responding. P_{DT} : proportion of female flies attracted to test materials. P_C : proportion of *C. obstrictus* responding. P_{CT} : proportions of weevil females attracted to test materials. Eggs_D: *D. radicum* eggs. Eggs_C: *C. obstrictus* eggs. NAS and NEO: mean peak heights of 2-phenylethyl glucosinolate and 1-methoxy-3-indolylmethyl glucosinolate, respectively. † Tansey *et al.* (2010b); †† Tansey *et al.* (2010a); ‡ Shaw *et al.* (2009).

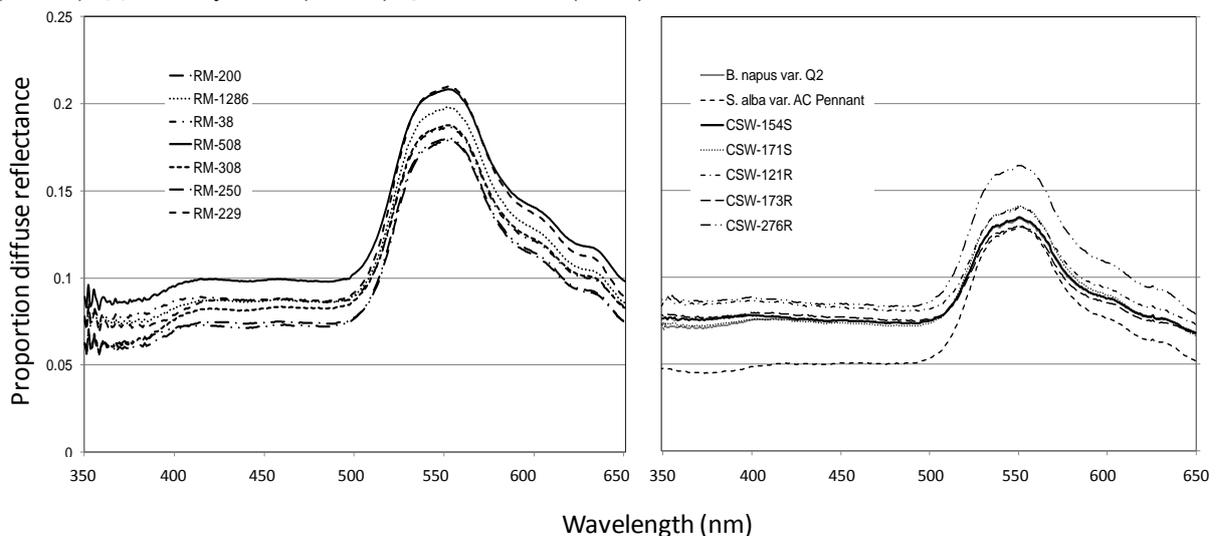


Figure 1. Reflectance properties of the test plant material. CSW indicates lines tested for *Ceutorhynchus obstrictus* resistance. RM indicates lines tested for *Delia radicum* resistance.