

Feeding of turnip root fly (*Delia floralis*) and cabbage root fly (*Delia radicum*) larvae on *Brassica napus* L. transgenic *MINELESS* plants – Effects on insect development

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Abstract: The dual component glucosinolate-myrosinase defence system is present in plants of the Brassicaceae. Upon insect attack, the defence system gets activated and forms glucosinolate hydrolysis products, which affect insect herbivory. The transgenic *Brassica napus* *MINELESS* plants have been produced by genetic ablation of myrosin cells, cells containing the myrosinase enzymes acting in the mustard bombs or toxic mines. In this study we analysed how the larvae of *Delia radicum* and *D. floralis* perform on wild-type and *MINELESS* *B. napus* plants and how these plants are affected. The larvae of both *D. radicum* and *D. floralis* gained more weight after feeding on *MINELESS* roots. Both aboveground and belowground plant biomass were observed to be higher for *MINELESS* plants compared to the wild-type on day 4 regardless of attack by *D. radicum* and *D. floralis*. We conclude that ablation of myrosin cells had a slight but not dramatic effect on the feeding behaviour for *D. radicum* and *D. floralis*.

Key words: Turnip fly, cabbage root fly, *Brassica*, *Delia radicum*, *Delia floralis*, myrosin cells, Myrosinase, oilseed rape, plant defence, roots

Introduction

A distinct plant defence system, well recognized as the glucosinolate-myrosinase defence system is present in plants of the Brassicaceae. Glucosinolates are amino acid derived secondary compounds that upon tissue disruption are hydrolysed by the enzyme myrosinase into a variety of degradation products, which can deter insect herbivory, termed as “the mustard oil bomb” (Bones & Rossiter, 2006, Bones & Rossiter, 1996, Kissen *et al.*, 2009). Seeds of oilseed rape *Brassica napus* cv Westar were genetically modified to remove myrosinase containing myrosin cells, also known as the “toxic mines”. The modified plants have been named *MINELESS* due to lack of these toxic mines (Ahuja *et al.*, 2011, Borgen *et al.*, 2010). A study with cabbage aphids *Brevicoryne brassicae* (a specialist) and *Myzus persicae* (a generalist) showed that ablation of myrosin cells affects the selection behavior of a specialist and a generalist (Borgen *et al.*, 2012).

The cabbage root fly (*Delia radicum*) and the turnip root fly (*D. floralis*) are considered to be economically important pests on *Brassica* crops (Ahuja *et al.*, 2010). The larvae of *Delia* flies cause damage to plants by feeding on plant roots (Klingen *et al.*, 2002). The damage caused by *Delia* flies to *Brassica* spp. roots lead to significant reductions in yield,

flowering and seed production as well as leaf, stem and root biomass (Ahuja *et al.*, 2010, Blossey & Hunt-Joshi, 2003).

Glucosinolates have been shown to affect larval and pupal weights (Björkman *et al.*, 2011, van Leur *et al.*, 2008). Higher levels of indole glucosinolates in roots may negatively affect the development of *D. radicum* larvae (Björkman *et al.*, 2011, Soler *et al.*, 2007). Knowledge on insect responses to plants with known glucosinolate-myrosinase system properties opens up the possibility of breeding for higher levels of pest resistance by manipulating the levels of glucosinolates and their breakdown products (Hopkins *et al.*, 2009).

The present study aimed at analysing the effects on survival and development of *D. floralis* and *D. radicum* larvae when feeding on *B. napus* wild-type and *MINELESS* plants. We also aimed to get insights into defence responses of *MINELESS* and wild-type plants, after being attacked by the larvae of these two root fly *species*. We expected that due to lack of myrosin cells and consequently myrosinase, the glucosinolate-myrosinase defence system has been modulated, and *MINELESS* plants would be more susceptible to attack by the larvae of specialist *Delia* species.

Material and methods

Plant material

Seeds of wild-type and transgenic *MINELESS Brassica napus* were sown in pots in climate-controlled rooms at the Norwegian University of Life Sciences (NMBU), Ås, Norway. The pots were placed in chambers that were kept at 21 °C during the day and 16 °C at night at a light intensity of 70-80 $\mu\text{mol}/\text{m}^2 \text{ s}$ and 70-80% humidity. The plants were grown for 6 weeks.

Addition of the root fly larvae and harvesting the plants

Five larvae (0-24 h after hatching) of *D. floralis* or *D. radicum* were added to the stem base of each plant (3-4 weeks after germination), when they had 5 to 6 true leaves. Four, ten and fourteen days after infestation with larvae, 5-8 samples were taken from each treatment. Larvae and pupae were retrieved and weighed. After retrieval of the larvae/pupae and scoring of damage, the roots of the plants were quickly washed with water to remove remaining soil, dried with paper tissue and severed from the shoot. The above-ground plant parts (leaves + shoots), and below-ground plant parts (roots) were harvested, photographed, weighed and flash frozen in liquid nitrogen. The samples were stored in -80 °C freezer for further analysis of glucosinolates.

Results and discussion

The insect infestation experiments showed that the larvae of both *Delia* species that have been feeding on *MINELESS* roots gained more weight by day 10 (Table 1). Larvae of *D. radicum* also showed higher weight for day 14 after feeding on *MINELESS* roots compared to the wild-type. However, for *D. floralis* an opposite trend was observed where larvae that had been feeding on *MINELESS* roots had lower weights than those feeding on wild-type roots (Table 1).

Table 1. Average larval body mass (mg) of *D. radicum* and *D. floralis* larvae feeding on roots of *B. napus* wild-type and *MINELESS* plants (n = 6-9).

Day	Insect species	Wild-type	<i>MINELESS</i>
4	<i>D. radicum</i>	0.392	0.355
4	<i>D. floralis</i>	0.671	0.674
10	<i>D. radicum</i>	5.983	6.973
10	<i>D. floralis</i>	7.057	8.137
14	<i>D. radicum</i>	9.199	12.799
14	<i>D. floralis</i>	16.199	14.639

Both above-ground and below-ground plant biomass was observed to be higher for *MINELESS* plants compared to the wild-type on day 4 irrespective of the *Delia* species (Table 2). For day 10, *MINELESS* plants also showed higher weights for above-ground biomass for both *D. radicum* and *D. floralis*, but for below-ground *MINELESS* showed slightly lower weights than the wild-type. Interestingly, for day 14, *MINELESS D. radicum* challenged roots showed almost two-fold reduction in weight compared to the wild-type *D. radicum* challenged roots (Table 2). This corresponded well with the higher weight of *D. radicum* larvae that had been feeding on *MINELESS* roots (Table 1).

Table 2. Above-ground (shoots + leaves) and below-ground (main + fine roots) fresh biomass (g) of *B. napus* wild-type and *MINELESS* plants (n = 6-9) after 4 d, 10 d and 14 d of infestation with *D. radicum* and *D. floralis* larvae.

Day	Insect	Wild-type		<i>MINELESS</i>	
		Aboveground	Belowground	Aboveground	Belowground
4	<i>D. radicum</i>	8.837	0.258	9.067	0.336
4	<i>D. floralis</i>	7.874	0.227	9.887	0.250
10	<i>D. radicum</i>	24.342	0.548	30.216	0.527
10	<i>D. floralis</i>	22.975	0.360	28.706	0.344
14	<i>D. radicum</i>	26.057	0.704	25.823	0.379
14	<i>D. floralis</i>	23.944	0.265	21.474	0.254

The higher root weights of *MINELESS* plants at day 4 shows that larvae of both *Delia* species have problems initially accepting *MINELESS* plants (Table 1). However, by day 14, larvae of both *Delia* species appear to accept *MINELESS* plants, as root weights were nearly the same for both wild-type and *MINELESS* plants.

Before this main experiment, a preliminary study was done on wild-type and *MINELESS* plants where *D. radicum* larvae had been feeding on roots of these plants for 11 days. This study showed quicker development of larvae on roots of *MINELESS* plants evidenced by retrieval of more pupae than larvae. Nevertheless, lower retrieval of larvae and lower root damage also suggested that *D. radicum* larvae have problems accepting *MINELESS* plants.

Above-ground and below-ground tissue of control (non-challenged) and *Delia* larvae challenged wild-type and *MINELESS* plants have also been sampled for glucosinolate analysis.

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References

- Ahuja, I., Borgen, B. H., Hansen, M., Honne, B. I., Müller, C., Rohloff, J., Rossiter, J. T., Bones, A. M. 2011: Oilseed rape seeds with ablated defence cells of the glucosinolate–myrosinase system. Production and characteristics of double haploid *MINELESS* plants of *Brassica napus* L. *Journal of Experimental Botany* 62(14): 4975-4993.
- Ahuja, I., Rohloff, J., Bones, A. M. 2010: Defence mechanisms of Brassicaceae: implications for plant-insect interactions and potential for integrated pest management. A review. *Agronomy for Sustainable Development* 30(2): 311-348.
- Björkman, M., Klingen, I., Birch, A. N. E., Bones, A. M., Bruce, T. J. A., Johansen, T. J., Meadow, R., Mølmann, J., Seljåsen, R., Smart, L. E., Stewart, D. 2011: Phytochemicals of Brassicaceae in plant protection and human health – Influences of climate, environment and agronomic practice. *Phytochemistry* 72(7): 538-556.
- Blossey, B., Hunt-Joshi, T. R. 2003: Below ground herbivory by insects: Influence on plants and aboveground herbivores. *Annual Review of Entomology* 48(1): 521-547.
- Bones, A. M., Rossiter, J. T. 2006: The enzymic and chemically induced decomposition of glucosinolates. *Phytochemistry* 67(11): 1053-1067.
- Bones, A. M., Rossiter, J. T. 1996: The myrosinase-glucosinolate system, its organisation and biochemistry. *Physiologia Plantarum* 97(1): 194-208.
- Borgen, B. H., Ahuja, I., Thangstad, O. P., Honne, B. I., Rohloff, J., Rossiter, J. T., Bones, A. M. 2012: ‘Myrosin cells’ are not a prerequisite for aphid feeding on oilseed rape (*Brassica napus*) but affect host plant preferences. *Plant Biology* 14(6): 894-904.
- Borgen, B. H., Thangstad, O. P., Ahuja, I., Rossiter, J. T., Bones, A. M. 2010: Removing the mustard oil bomb from seeds: transgenic ablation of myrosin cells in oilseed rape (*Brassica napus*) produces *MINELESS* seeds. *Journal of Experimental Botany* 61(6): 1683-1697.
- Hopkins, R. J., van Dam, N. M., van Loon, J. J. A. 2009: Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annual Review of Entomology* 54(1): 57-83.
- Kissen, R., Rossiter, J., Bones, A. 2009: The ‘mustard oil bomb’: not so easy to assemble?! Localization, expression and distribution of the components of the myrosinase enzyme system. *Phytochemistry Reviews* 8(1): 69-86.
- Klingen, I., Meadow, R., Aandal, T. 2002: Mortality of *Delia floralis*, *Galleria mellonella* and *Mamestra brassicae* treated with insect pathogenic hyphomycetous fungi. *Journal of Applied Entomology* 126(5): 231-237.

- Soler, R., Bezemer, T. M., Cortesero, A. M., Putten, W. H., v. d. Vet, L. E. M., Harvey, J. A. 2007: Impact of foliar herbivory on the development of a root-feeding insect and its parasitoid. *Oecologia* 152: 257-264.
- van Leur, H., Raaijmakers, C. E., van Dam, N. M. 2008: Reciprocal interactions between the cabbage root fly *Delia radicum* and two glucosinolate phenotypes of *Barbarea vulgaris*. *Entomologia Experimentalis et Applicata* 128(2): 312-322.