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Effects of herbivory by *Delia radicum* on the roots of *Brassica nigra* on the performance of the solitary parasitoid *Cotesia rubecula* and its host *Pieris rapae*



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Abstract

Performance and behaviour of aboveground trophic levels can be affected in different directions by belowground attackers of host plants, in which changes in secondary plant compounds and plant volatiles seem to play a large role. To find a link between the observed behaviour of *Cotesia rubecula* and its performance, in this paper it is studied whether root herbivory by the fly *Delia radicum* is influencing the development of the parasitoid *Cotesia rubecula* and its caterpillar host *Pieris rapae* with *Brassica nigra* as host plant. This study shows that *D. radicum* infestation does not influence larval survival, weight, or development time of *C. rubecula*. *D. radicum* infestation also does not affect larval survival and development time of *P. rapae*, but larval weight was positively affected by *D. radicum* infestation. *C. rubecula* sex ratio was female biased in hosts feeding on *D. radicum* infested plants and male biased in hosts feeding on uninfested plants. Even though *C. rubecula* was shown to have a clear preference for hosts feeding on *D. radicum* infested plants, no advantage but also no penalty is found for the performance of *C. rubecula* developing in hosts feeding on *D. radicum* infested plants. However, a female biased sex ratio in hosts feeding on *D. radicum* infested plants might be a second indication that *P. rapae* feeding on infested plants have an advantage as a host to *C. rubecula*. Further research will be necessary to find a link between the shown behaviour of *C. rubecula* and its performance.

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Introduction

Aboveground and belowground plants are confronted to an array of attackers, where herbivorous insects represent their main threat. Plant defence systems act directly or indirectly and can be induced by herbivory. This induced defence can be expressed at the site of attack or, when the induced defence is systemic, the response can spread to other (undamaged) parts of the plant (reviewed by Bezemer and van Dam 2005). Because plants have organs aboveground as well as belowground, using systemic induced defence systems, a herbivore attack belowground can induce a response aboveground (Wäckers and Bezemer 2003) and vice versa (reviewed by Bezemer and van Dam 2005). Belowground and aboveground living organisms can in this way be linked by induced plant defence.

Changes in secondary plant compounds in response to a belowground attack seem to play a large role in influencing the performance of different aboveground trophic levels (Bezemer et al. 2003, Bezemer et al. 2005, van Dam et al. 2005, van Dam & Raaijmakers 2006). Not only performance, but also behaviour of organisms belonging to different trophic levels can alter in response to root herbivory, through root herbivory induced changes in plant volatiles (Soler et al. 2007a). Different studies found an effect of root feeders on the performance and behaviour of aboveground herbivores (Moran and Whitman 1990, Masters et al. 2001, Bezemer et al. 2003, Bezemer et al. 2005, Soler et al. 2005, Soler et al. 2007a, Soler et al. 2007b) and the directions of these effects differ. Masters et al. (2001), for example, found a positive effect of root herbivory on seed feeders (Masters et al. 2001), while Moran and Whitman (1990) found a neutral effect of a root feeding aphid on a leaf feeding aphid (Moran and Whitman 1990) and Bezemer et al. (2003) found a negative effect of a root feeder on a foliage feeder (Bezemer et al. 2003). Studies on the effect of root feeders on the behaviour and performance of higher aboveground trophic levels also revealed significant effects in different directions. Bezemer et al. (2005), for example, showed a positive effect of soil community on the performance of parasitoids of aphids, while the aphids themselves experienced a negative effect (Bezemer et al. 2005). Contrary, herbivory by the root-feeding insect *Delia radicum* negatively affected the performance of both a primary parasitoid of *Pieris brassicae* caterpillars, *Cotesia glomerata*, and its hyperparasitoid *Lysibia nana*, via changes in plant quality of *Brassica nigra* (Soler et al. 2005). The behaviour of the parasitoid *C. glomerata* was also proved to be influenced by the presence of root herbivory (Soler et al. 2007a).

Soler et al. (2005) studied a system consisting of a root feeder (*Delia radicum*), a leaf feeder (*Pieris brassicae*), its primary parasitoid (*Cotesia glomerata*) and its hyperparasitoid (*Lysibia nana*),

with *Brassica nigra* as host plant. The aim of this study was to find whether root herbivory by *D. radicum* can influence the development of the four aboveground trophic levels through induced changes in the levels of primary and secondary plant compounds. At an intermediate level of root herbivory (five *D. radicum* larvae/plant), they found an increased larval development time of both the leaf herbivore *P. brassicae* and its parasitoid *C. glomerata*. They also found a reduced cocoon weight of the parasitoid *C. glomerata* and a reduced adult weight of its hyperparasitoid *L. nana*, while the pupal weight of *P. brassicae* was not affected compared to the control group. In this study also plant responses to root herbivory were measured. Foliar glucosinolate concentrations (sinigrin) were found to increase significantly (about 50%) in the presence of an intermediate level of root herbivory. Although specialist herbivores of crucifers as *P. brassicae* are thought to be well adapted to high levels of glucosinolates, this study suggests that increased levels of glucosinolates could be a possible mechanism explaining the negative influence of root herbivores on the development of the aboveground herbivore, its primary parasitoid and a hyperparasitoid (Soler et al. 2005). Subsequently, Soler et al. (2007a) studied whether host acceptance and plant preference of the parasitoid *C. glomerata*, which was the most affected trophic level, was affected by belowground herbivory by *D. radicum*, and whether this could be caused by root herbivore induced changes in plant volatiles. In a semi-field experiment, it was found that *C. glomerata* significantly prefers to oviposit in hosts feeding on control plants over hosts feeding on plants with root herbivory. In a two-choice flight-cage experiment, *C. glomerata* also has a preference to search for hosts on root undamaged plants. The volatile blend of the root-infested host-plant complexes showed higher levels of toxic sulfides and lower levels of attractants, compared to the volatile blend of host-plant complexes without root infestation (Soler et al. 2007a). It is therefore likely that these differences in volatile blends are exploited by *C. glomerata* to distinguish between *D. radicum* infested and uninfested plants.

In the current study I use a model system related to the one of Soler et al. (2005) described above. This model system comprises the root feeder *Delia radicum* (Diptera: Anthomyiidae), the host plant *Brassica nigra* (Brassicaceae), the foliar feeder *Pieris rapae* (Lepidoptera: Pieridae) and its parasitoid *Cotesia rubecula* (Hymenoptera: Braconidae). Although the species are closely related, there are some differences between the herbivore-parasitoid model system *P. rapae* - *C. rubecula* used in this study and the model system *P. brassicae* - *C. glomerata* used by Soler et al. (2005). *P. brassicae* lays clusters of 10-100 eggs (gregarious) while *P. rapae* lays single eggs (solitary), but both use the same host plants (from the *Brassica* genus) (Davies and Gilbert 1985). *C. glomerata* parasitizes both *P. brassicae* and *P. rapae*, with a strong preference for *P. brassicae*, while *C. rubecula* is specialized on *P. rapae* (Brodeur and Vet 1995). Both species are koinobiont endoparasitoids

(Harvey et al. 1999, Soler et al. 2005). The host of a koinobiont parasitoid continues to feed and grow after parasitism (Harvey et al. 1999).

In a two-choice flight-cage experiment similar to that in Soler et al. (2007a), *C. rubecula* was expected to behave similar to *C. glomerata* (Soler et al. 2007a), by preferring hosts feeding on uninfested host plants over hosts feeding on plants infested with a root herbivore. However, *C. rubecula* surprisingly showed a clear preference for hosts feeding on host plants infested with the root herbivore *D. radicum* over hosts feeding on uninfested host plants (unpublished data R. Soler). Assuming a link between preference and performance, the observed preference of *C. rubecula* for hosts on *D. radicum* infested plants is expected to be correlated with a positive effect of *D. radicum* infestation on the performance of *C. rubecula* progeny.

In order to investigate whether there is a link between the observed preference of *C. rubecula* for hosts feeding on infested plants and its performance when developing in these hosts, I first address the question whether root herbivory by *D. radicum* influences the performance of the herbivore *P. rapae* through induced changes in the host plant *B. nigra*. Differences in foodplant quality are known to affect development time and pupal mass of *P. rapae* (Gols et al. 2008). An increased defence reaction of *B. nigra* in response to *D. radicum* infestation, could therefore negatively affect the performance of *P. rapae*. Secondly, I address the question whether the performance of the parasitoid *C. rubecula* is influenced when the performance of its host *P. rapae* is affected. Larval survival of *C. rubecula* could be affected by the relatively strong encapsulation reaction of *P. rapae* (Brodeur and Vet 1995). This encapsulation reaction might be negatively affected by increased glucosinolate levels in the plant (Bukovinszky et al. 2009). As root herbivory by *D. radicum* induces aboveground glucosinolate production of *B. nigra* (Soler et al. 2005), *D. radicum* infestation might also affect the encapsulation reaction of *P. rapae* towards *C. rubecula*. If the performance of *P. rapae* is negatively influenced, the survival of *C. rubecula* could be higher because of a lower encapsulation reaction by *P. rapae*. Weight and development time of parasitoids can be affected differently by host quality, depending on parasitoid species. Koinobiont parasitoids attacking exposed hosts, like *C. rubecula*, are thought to favor decreased development time over increased adult size (Harvey & Strand 2002, Harvey et al. 2004). This is seen in *C. rubecula* by Gols et al. (2008) where differences in foodplant quality for hosts were only translated into adult size differences of *C. rubecula*, development time was not affected. If the development time and weight of *P. rapae* are negatively influenced by *D. radicum* infestation, the development time of *C. rubecula* is not expected to change, but the weight of *C. rubecula* would be influenced in the same direction.

Materials and methods

The experiment consisted of two parts with both two treatments. In part I the effects of herbivory on the roots of *B. nigra* by *D. radicum* on the performance of the foliar herbivore *P. rapae* was studied. In part II the effects of root herbivory on the roots of *B. nigra* by *D. radicum* on the performance of the parasitoid *C. rubecula* was studied. The experimental design is shown in figure 1.

Part	Treatment	# <i>B. nigra</i> plants	<i>D. radicum</i> +/-	<i>P. rapae</i> +/-	<i>C. rubecula</i> +/-
I	A	24	+	+	-
	B	24	-	+	-
II	C	48	+	+	+
	D	48	-	+	+

Figure 1. Experimental design

Brassica nigra

B. nigra plants were germinated on sterile glass beads, after one week the seedlings were transplanted to 1.1 L pots filled with 450 g mixture of potting soil and gravel (80%/20%) and a top layer of 330 g sand. The plants were grown in a greenhouse, with a temperature of 21 ± 2 °C (day) and 16 ± 2 °C (night), a relative humidity of 60% and a photoperiod of 16:8h (day/night). Plants were watered daily and three weeks after transplanting 100 cc plant nutrient solution was given per plant.

Delia radicum

D. radicum larvae were obtained from a rearing maintained at the Netherlands Institute of Ecology (NIOO-KNAW), Heteren, The Netherlands. *D. radicum* was cultured on yellow turnip (*Brassica napobrassica*) and adults were fed with a mixture of sugar, yeast extract and powdered milk (1:1:1).

Pieris rapae

P. rapae larvae were obtained from a rearing maintained at the Laboratory of Entomology, Wageningen University, Wageningen, The Netherlands. In order to obtain *P. rapae* eggs, two *B. nigra* plants infested with *D. radicum* were placed in one cage of the *P. rapae* butterfly rearing and two uninfested plants in a second cage of the rearing. The plants were staying in the cages for seven hours, after three hours the plants were changed between butterfly cages.

Cotesia rubecula

C. rubecula adults were obtained from a rearing maintained at the Netherlands Institute of Ecology (NIOO-KNAW), Heteren, The Netherlands. *C. rubecula* was cultured on *P. rapae*.

Experimental set-up

Four weeks after transplanting *B. nigra* seedlings, each plant of treatment A and C was infested with four second instar *D. radicum* larvae by carefully uncovering about one cm of the plant main root and placing the larvae next to the root with a brush. When the larvae successfully crawled into the soil, the root was covered again. Six days after *D. radicum* infestation two newly hatched *P. rapae* larvae were introduced by placing them on the first two mature leaves of the plant. Four days later the *P. rapae* larvae of treatment C and D were weighed on a Mettler Toledo Microbalance (accuracy $\pm 1\mu\text{g}$) and subsequently parasitized. For parasitizing, *C. rubecula* females were individually offered one first instar *P. rapae* larva in a plastic vial. Only one oviposition per larva was allowed, up to a maximum of ten *P. rapae* larvae were offered per *C. rubecula* female. To each *C. rubecula* female, larvae from only one treatment were offered. Parasitized *P. rapae* larvae were placed back on the first two mature leaves of the plant. When an introduced *P. rapae* larva was not found back on the plant, a new larva was weighed, parasitized and introduced to the plant. Seven and ten days after emergence, *P. rapae* larvae of treatment A and B were weighed and afterwards placed back on the first two mature leaves of the plant. Eight and eleven days after emergence a similar proceeding was executed for treatment C and D.

Ten days after *P. rapae* introduction, the experimental tables were flooded with water to prevent *P. rapae* larvae from moving among plants. Fourteen days after *P. rapae* introduction, the soil of plants in treatment A and C was covered with gauze to prevent emerging *D. radicum* from dispersing in the greenhouse.

P. rapae and *C. rubecula* larval development was checked two times a day. Fresh *P. rapae* pupae were carefully removed with a clean and sharp scalpel within 16 hours after pupation. Pupae were weighed on a microbalance and per plant placed in a labelled plastic cup covered with gauze and placed in a climate chamber with a temperature of 21 °C and a photoperiod of 16:8h (day/night). Development time of *P. rapae* larvae was determined as the mean time in hours from egg laying to pupation. Fresh *C. rubecula* cocoons were carefully removed with a flexible forceps within 16 hours after pupation and per cocoon placed in a labelled Petri dish (5 cm diameter) in a climate chamber with a temperature of 21 °C and a photoperiod of 16:8h (day/night). Development time of *C. rubecula* larvae was determined as the mean time in hours from parasitizing to pupation.

P. rapae and *C. rubecula* pupal development was checked four times a day, with exception of one day when it was only checked once. Newly emerged *P. rapae* adults were removed from the cup, placed individually in polyethylene bags and immediately placed in a freezer (-18°C). Newly emerged *C. rubecula* adults were immediately placed in a freezer (-18°C). After at least one day, *P. rapae* and *C. rubecula* fresh adult weight was determined on a microbalance and sex was determined by eye. To measure the dry weight, adults of both *P. rapae* and *C. rubecula* were dried for two days (70°C) and weighed on a microbalance. Development time of both *P. rapae* and *C. rubecula* adults was determined as the mean time in hours from egg laying/parasitizing to adult emergence.

Root damage was checked on the main root of each infested plant, to ensure root herbivory by *D. radicum*.

Statistical analysis

Development time and weight of *P. rapae* and *C. rubecula* was analysed employing restricted maximum likelihood (REML) in Genstat 10. Larval survival and sex ratio was analysed employing multiple Chi-square tests in SPSS 17.0

Results

Larval survival

D. radicum infestation did not affect unparasitized and parasitized *P. rapae* larval survival, *C. rubecula* larval survival is also not affected by *D. radicum* infestation (table 1; fig. 2).

Table 1. Larval survival of *P. rapae* unparasitized, *P. rapae* parasitized and *C. rubecula* (Pearson Chi-square tests, $\alpha=0.05$).

Larval survival				
	<i>P. rapae</i> unparasitized		<i>P. rapae</i> parasitized	<i>C. rubecula</i>
Factor	d.f.	P	P	P
Root herbivory	1	0.334	0.231	0.599

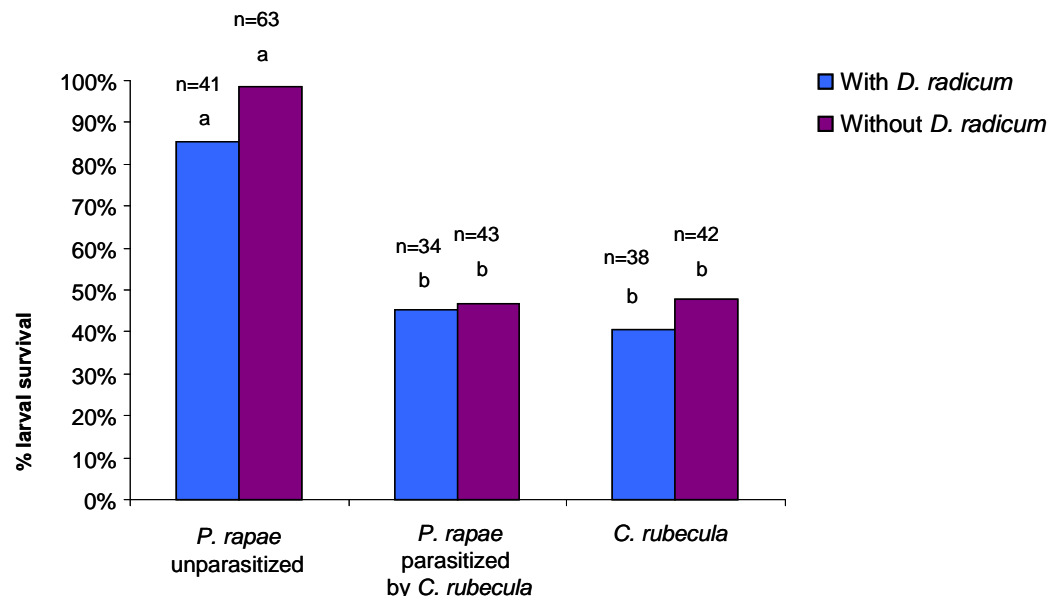


Figure 2. Larval survival, *P. rapae* unparasitized, *P. rapae* parasitized by *C. rubecula* and *C. rubecula*. Columns with identical letters are not significantly different based on Pearson Chi-square tests, $\alpha=0.05$. Per column the sample size is indicated.

Weight

D. radicum infestation significantly affected unparasitized *P. rapae* larval weight seven days after emergence (table 2; fig. 3a). *P. rapae* feeding on *D. radicum* infested plants have a 27.3% higher larval weight than *P. rapae* feeding on control plants. Figure 3a and table 2 show no effect of root herbivory on larval weight ten days after emergence, however when two outliers were removed from the dataset, the mixed model analysis did show a significant difference between treatments ($F=$

5.108; $P=0.026$). No effects of *D. radicum* infestation are found on *P. rapae* larval weight four days after *P. rapae* emergence, or on the fresh and dry adult weight (table 2; fig. 3). Parasitized *P. rapae* larval weight was not affected by *D. radicum* infestation (table 3; fig. 4).

The groups of *P. rapae* measured at seven and ten days after *P. rapae* emergence had evenly distributed sex ratios. The sex ratio of the groups measured at four, eight and eleven days after emergence is not known. The groups measured at four days after emergence are different from the groups measured at seven and ten days after emergence. The two groups of *P. rapae* measured at ten days after emergence are similar to the groups measured at seven days after emergence, but with 15 extra *P. rapae* larvae included in the treatment without *D. radicum*. All *P. rapae* larvae were held at the same moment and under the same conditions.

C. rubecula fresh and dry adult weight of both sexes is not affected by *D. radicum* infestation (table 4; fig. 5).

Table 2. Approximate *F*-test of fixed effects from the REML analysis of the effect of root herbivory on unparasitized *P. rapae* weight 4 days, 7 days and 10 days after *P. rapae* emergence and adult fresh and dry weight.

Weight		<i>P. rapae</i> unparasitized									
		4 days after emergence		7 days after emergence		10 days after emergence		Adult fresh		Adult dry	
Factor	d.f.	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Root herbivory	1	1.657	0.200	11.278	0.001	3.844	0.053	1.268	0.263	0.845	0.360

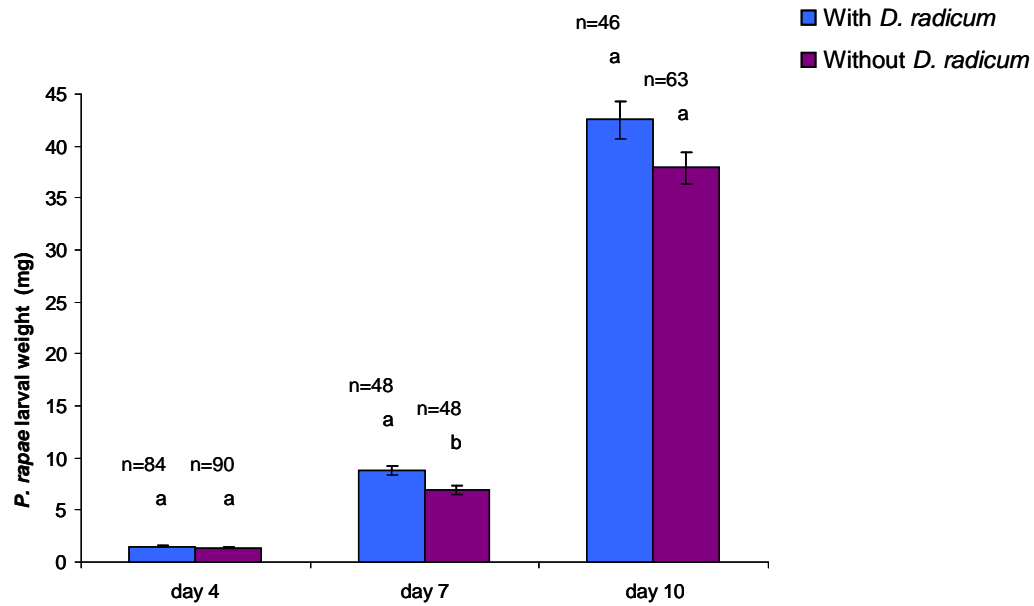
Table 3. Approximate *F*-test of fixed effects from the REML analysis of the effect of root herbivory on parasitized *P. rapae* weight 8 days and 11 days after *P. rapae* emergence and adult fresh and dry weight.

Weight		<i>P. rapae</i> parasitized							
		8 days after emergence		11 days after emergence		Adult fresh		Adult dry	
Factor	d.f.	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Root herbivory	1	0.632	0.428	0.234	0.629	0.322	0.573	0.030	0.864

Table 4. Approximate *F*-test of fixed effects from the REML analysis of the effect of root herbivory on *C. rubecula* weight of adult male and female fresh and dry weight.

Weight		<i>C. rubecula</i>							
		Male fresh		Female fresh		Male dry		Female dry	
Factor	d.f.	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Root herbivory	1	0.117	0.735	1.804	0.187	3.240	0.081	0.003	0.958

(a)



(b)

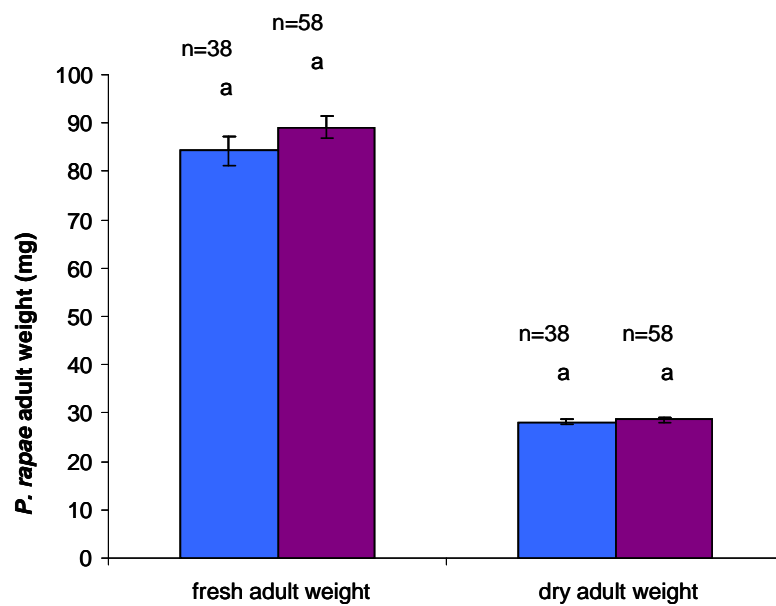


Figure 3. *P. rapae* not parasitized by *C. rubecula*. Mean (\pm SE) larval weight 4 days, 7 days and 10 days after emergence (a) and mean (\pm SE) fresh and dry adult weight (b). Within pairs, columns with identical letters are not significantly different (mixed model analysis, $\alpha=0.05$). Per column the sample size is indicated.

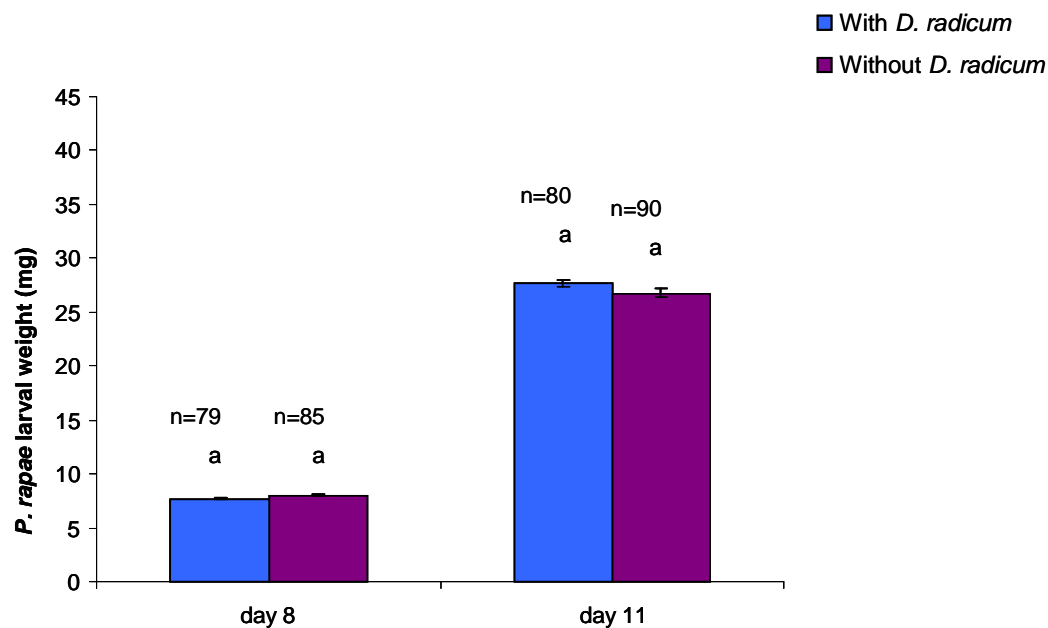


Figure 4. *P. rapae* four days after emergence parasitized by *C. rubecula*. Mean (\pm SE) larval weight 8 days and 11 days after emergence. Within pairs, columns with identical letters are not significantly different (mixed model analysis, $\alpha=0.05$). Per column the sample size is indicated.

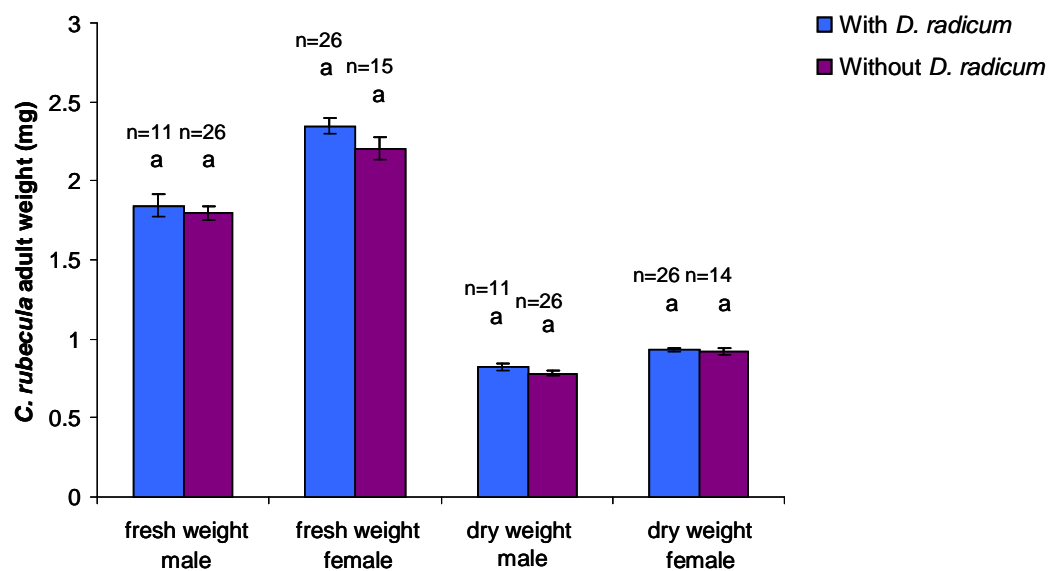


Figure 5. *C. rubecula* mean (\pm SE) male and female fresh and dry adult weight. Within pairs, columns with identical letters are not significantly different (mixed model analysis, $\alpha=0.05$). Per column the sample size is indicated.

Development time

No effect is found of *D. radicum* infestation on *P. rapae* development time from egg laying to pupation or from egg laying to adult emergence (table 5; fig. 6). *D. radicum* infestation did not affect *C. rubecula* development time from parasitizing to pupation, or from parasitizing to male and female adult emergence (table 6; fig. 7).

Table 5. Approximate *F*-test of fixed effects from the REML analysis of the effect of root herbivory on unparasitized *P. rapae* development time from egg laying to pupation and from egg laying to adult emergence.

Development time		<i>P. rapae</i> unparasitized			
		Egg – pupa		Egg – adult	
Factor	d.f.	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Root herbivory	1	0.474	0.493	0.011	0.917

Table 6. Approximate *F*-test of fixed effects from the REML analysis of the effect of root herbivory on *C. rubecula* development time from egg laying to pupation and from egg laying to male and female adult emergence.

Development time		<i>C. rubecula</i>					
		Egg – cocoon		Egg – adult male		Egg – adult female	
Factor	d.f.	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Root herbivory	1	1.187	0.279	1.073	0.307	1.970	0.168

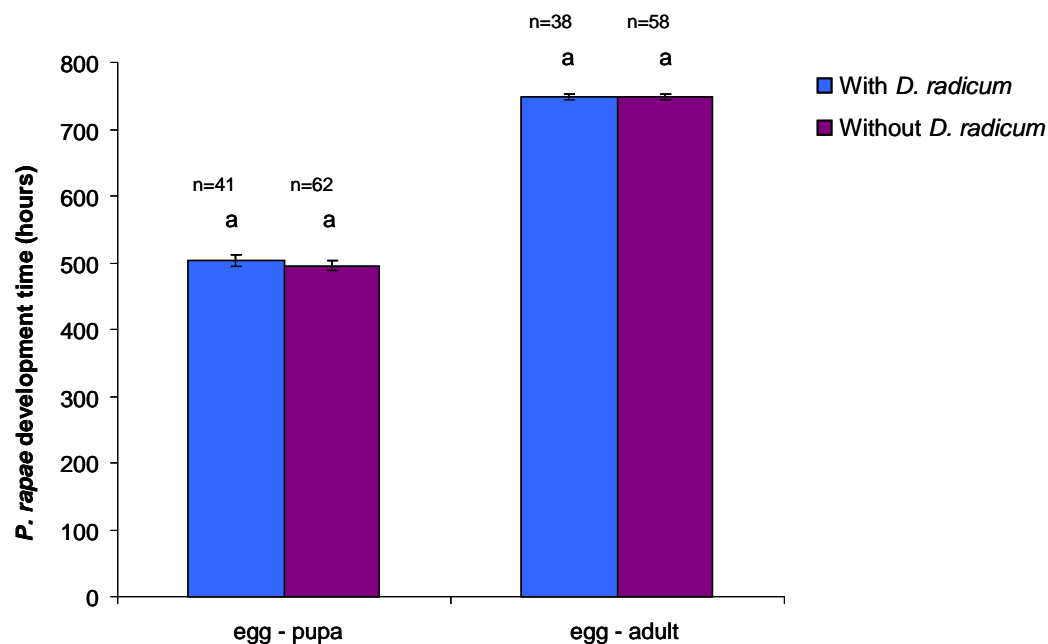


Figure 6. Mean (\pm SE) *P. rapae* development time from egg laying to pupation and from egg laying to adult emergence. Within pairs, columns with identical letters are not significantly different (mixed model analysis, $\alpha=0.05$). Per column the sample size is indicated.

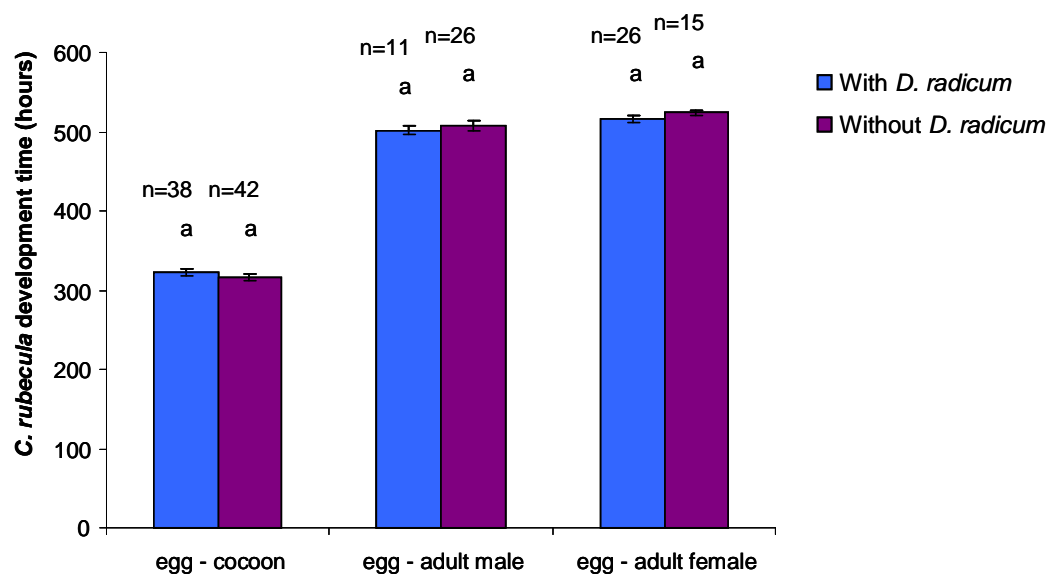


Figure 7. *C. rubecula* mean (\pm SE) development time from parasitizing to pupation and from parasitizing to male and female adult emergence. Within pairs, columns with identical letters are not significantly different (mixed model analysis, $\alpha=0.05$). Per column the sample size is indicated.

Sex ratio

C. rubecula adults emerging from hosts feeding on uninfested plants showed a significant male biased sex ratio, while parasitoids emerging from hosts feeding on *D. radicum* infested plants showed a significant female biased sex ratio (table 7; fig. 8).

Table 7. Percentages of *C. rubecula* females within treatments.
P-values are given for observed sex ratio compared to 1:1 sex ratio
 (Chi-square tests, $\alpha=0.05$)

Sex ratio <i>C. rubecula</i>			
Treatment	d.f.	% female	<i>P</i>
With <i>D. radicum</i>	1	70.3%	0.014
Without <i>D. radicum</i>	1	34.9%	0.047

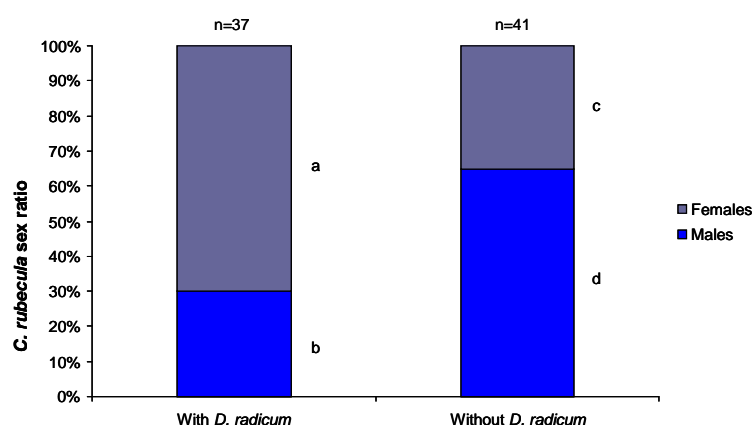


Figure 8. *C. rubecula* sex ratio. Columns with identical letters are not significantly different (Chi-square tests, $\alpha=0.05$). Per column the sample size is indicated.

All main roots of infested plants were damaged by root herbivory (appendix 1). Additional results are shown in appendix 2. A brief overview of the results is shown in appendix 3.

Discussion

The results of this study show that root herbivory via the shared host plant has a neutral effect on the development of *Cotesia rubecula* when developing in its host *Pieris rapae*. Root herbivory was found to have a neutral to slightly positive effect via the shared host plant on the development of *Pieris rapae*.

The larval survival of *C. rubecula* and of both parasitized and unparasitized *P. rapae* is not influenced by the root herbivore *D. radicum*. No effect of *D. radicum* is shown on larval survival of *C. rubecula*, which means the hypothesized decreased immune defence reaction of *P. rapae* when feeding on *D. radicum* infested plants is not observed. The encapsulation reaction (Brodeur & Vet 1995) could have occurred in both treatments, as 40.5% (with *D. radicum*) and 47.8% (without *D. radicum*) of parasitized *P. rapae* larvae survived at least until pupal stage with no egressing *C. rubecula* larvae.

However, from a study of M. Kruidhof (unpublished results) conducted during the same period and testing *P. rapae* from the same rearing as in the current study, it is known that 38.5% (with *D. radicum*) and 34% (without *D. radicum*) of parasitized *P. rapae* larvae did not contain a *C. rubecula* egg. Of the *P. rapae* larvae only 4.9% (with *D. radicum*) and 6.4% (without *D. radicum*) encapsulated a *C. rubecula* egg. This suggests that the majority of the successful developed parasitized *P. rapae* larvae in the current study did not receive an egg from the ovipositing parasitoid and only a minority encapsulated the *C. rubecula* egg. The by M. Kruidhof observed high percentage of *P. rapae* without an egg (unpublished results) could be caused by low larval quality, *C. rubecula* females might have noticed this and did not oviposit an egg.

The weight of unparasitized *P. rapae* larvae seven days after emergence was positively influenced by root infested *B. nigra* plants. At four and ten days after emergence this effect was not seen. Also no effect of *D. radicum* infestation is shown on cocoon or adult weight of *C. rubecula*, pupal or adult weight of unparasitized *P. rapae*, nor on larval, pupal and adult weight of *P. rapae* parasitized by *C. rubecula*. None of the measured development times of *C. rubecula* or (un)parasitized *P. rapae* was affected by *D. radicum*. The shown higher larval weight of *P. rapae* feeding on *D. radicum* infested plants might be caused by induced changes in levels of primary or secondary plant compounds which could have had a positive effect on larval growth. It could also be a possibility that the host plants, because of root herbivory, have a deficiency of primary or secondary plants compounds which are necessary for larval development. In order to obtain the desired quantity of these compounds, *P. rapae* larvae might have to feed in higher quantities resulting in a higher weight than *P. rapae*

feeding on plants without *D. radicum* infestation. The positive effect of *D. radicum* infestation on *P. rapae* weight is not yet shown at four days after emergence, the day the larvae were parasitized in this experiment, but as can be seen from the data, there might be a trend that is not significant yet at four days after emergence but female *C. rubecula* might already distinguished between different sized hosts. Assuming this ability to distinguish at four days after emergence, when the positive effect on *P. rapae* larval weight has a positive effect on *C. rubecula* performance, this could make the link between the performance of *C. rubecula* and its shown behaviour to prefer hosts feeding on *D. radicum* infested plants over hosts feeding on uninfested plants (unpublished results R. Soler). However, no effect of *D. radicum* is shown on the performance of *C. rubecula*. This can be explained by a suggestion made by Harvey et al. (1999). It says that for parasitoids that do not consume all host tissue before pre-pupal egression, like *C. rubecula*, host quality varies independently of host size at oviposition (Harvey et al. 1999). Koinobiont parasitoid fitness is also argued to be more complex than just a positive linear function of host size (Harvey et al. 2004). So in this case, even when it is assumed *C. rubecula* is able to detect a larger host four days after host emergence, the host will not per se be of higher quality resulting in a higher parasitoid performance. Host quality should instead be described by “immunological, metabolic and nutritional interactions between host and parasitoid” as suggested by Harvey et al. (1999). Even when a higher larval weight of the host in any way is an advantage to *C. rubecula*, it is assumed to be quite minor as there is no positive effect found of *D. radicum* infestation on the performance of *C. rubecula*. This minor advantage is not expected to influence the behaviour of *C. rubecula* such that it significantly prefers to oviposit in *P. rapae* hosts feeding on *D. radicum* infested plants as found by R. Soler (unpublished results).

It was hypothesized that if *P. rapae* development time and weight would be negatively affected by root herbivory, development time of *C. rubecula* would not change, but *C. rubecula* weight could be affected in the same direction. The results show that *D. radicum* infestation positively influenced *P. rapae* weight during a short period in larval stage. No effect was found of *D. radicum* infestation on *C. rubecula* cocoon and adult weight or on *C. rubecula* development time. It is assumed that *C. rubecula* development time was already optimal and so was not affected by the presence of *D. radicum*. It would be expected that if *P. rapae* weight is positively influenced and *C. rubecula* development time is already optimal, *C. rubecula* weight would be positively influenced too. As this is not observed, the observed increase in *P. rapae* larval weight is assumed not to have been enough to affect *C. rubecula* performance. Besides, the increased *P. rapae* larval weight was not actually observed in *P. rapae* larvae parasitized by *C. rubecula*. Although, this neutral effect of *D. radicum* on parasitized *P. rapae* larval weight might be explained by the different days of weighing; the parasitized larvae were weighed at eight and eleven days after emergence, while the unparasitized

larvae were weighed at four, seven and ten days after emergence. The effect of *D. radicum* on unparasitized *P. rapae* larvae was observed at seven days after emergence and not any more at ten days after emergence. The weight difference might only appear in the period before eight days after emergence, so that it was not observed when the parasitized *P. rapae* larvae were weighed at eight days after emergence.

The sex ratio of *C. rubecula* emerging from *P. rapae* larvae feeding on *D. radicum* infested *B. nigra* plants was female biased, while the sex ratio on plants without *D. radicum* infestation was male biased. A female biased sex ratio on infested plants could be caused by an effect of *D. radicum* infestation on male mortality, though larval survival was not affected by *D. radicum*. Sex ratio of most parasitoid species is female biased on hosts of higher quality and male biased on hosts of lower quality (reviewed by Godfray 1994) but this is not studied for *C. rubecula*. According to the results and the assumption that also for *C. rubecula* a female biased sex ratio occurs in high quality hosts, *P. rapae* hosts feeding on *D. radicum* infested plants are of higher quality to *C. rubecula* than *P. rapae* hosts feeding on uninfested plants. Which, in accordance with the results of R. Soler (unpublished) that *C. rubecula* females prefer to oviposit in *P. rapae* hosts feeding on *D. radicum* infested plants, can be another indication that *P. rapae* feeding on infested *B. nigra* plants have some kind of advantage as a host for *C. rubecula*. This second indication even shows that difference in quality between hosts can be detected by *C. rubecula* females in absence of the plant and its volatiles. Though, two remarks on this result have to be made. First, the *C. rubecula* females parasitizing the *P. rapae* larvae were allowed to oviposit in up to ten larvae. This means that the two treatment groups of *P. rapae* both were parasitized by about only ten *C. rubecula* females. These two times ten females determined the sex ratios. The second remark is that all parasitizing females only parasitized *P. rapae* larvae from one treatment. The females were not exposed to both treatments. Because of these remarks the conclusion has to be made very tentatively.

No link between the performance and the by R. Soler (unpublished results) shown behaviour of *C. rubecula* was found, as no advantage, but also no penalty was shown for the performance of *C. rubecula* parasitizing *P. rapae* hosts feeding on *D. radicum* infested *B. nigra* plants. This is not in accordance with the by *D. radicum* negatively influenced preference and performance of *C. glomerata* as shown by Soler et al. (2005, 2007a). When the performance of *C. rubecula* shows no differences between developing in hosts feeding on plants with and without *D. radicum* infestation the question remains: What is the advantage for *C. rubecula* of parasitizing hosts feeding on *D. radicum* infested plants? Further research might give answer to this question.

In further research it is recommended to do an experiment first to confirm the *C. rubecula* sex ratio results obtained in the current study. In this experiment again two groups should be made; *P.*

rapae larvae feeding on *B. nigra* plants infested with *D. radicum* and *P. rapae* larvae feeding on *B. nigra* plants without *D. radicum* infestation. At least twenty *C. rubecula* females per treatment should oviposit in these *P. rapae* hosts. Alternating, hosts from both treatments should be offered to single *C. rubecula* females to give the females the opportunity to distinguish between hosts feeding on *D. radicum* infested and uninfested plants. When this first recommended experiment confirms the results obtained in the present research, it might be interesting to do an analysis of all chemical compounds produced in *B. nigra* in reaction to root herbivory by *D. radicum*. Soler et al (2005) already measured foliar glucosinolate concentrations, nitrogen, phosphorus, potassium and the carbon/nitrogen ratio of *B. nigra* with and without root herbivory by *D. radicum*. But other primary and secondary plant compound concentrations could also change in reaction to root infestation, which might tell more about the differences in *P. rapae* host quality. When the first recommended experiment shows that the sex ratio of *C. rubecula* on *D. radicum* infested plants does not differ from the sex ratio on control plants, the preference of *C. rubecula* for *D. radicum* infested plants could be explained by a preference for leaf damaged host plants over undamaged host plants (Geervliet et al. 1996). Within the leaf damaged plants, *C. rubecula* does not discriminate between plants infested with different host and non-host species (Geervliet et al. 1996). This is in accordance with the finding that volatiles emitted by the tested *Brassica* species (*B. oleracea*), in reaction to feeding by different herbivore species, are very similar (Geervliet et al. 1997). According to this, root damaged host plants might also be more attractive to *C. rubecula* than undamaged host plants, because of the aboveground plant volatiles that might also be very similar to the volatiles emitted in reaction to leaf herbivory.

A second study can consist of a similar performance experiment as executed in the present study, but with the herbivore - parasitoid combinations *P. brassicae* - *C. rubecula* or *P. rapae* - *C. glomerata* on *D. radicum* infested and uninfested host plants. Testing these combinations in performance experiments it can be tested if the known effects of *D. radicum* on parasitoid performance are specific to parasitoid or specific to host (e.g. when *C. rubecula* shows a negatively influenced performance when developing in *P. brassicae* feeding on *D. radicum* infested plants, the negative effects shown on both parasitoids are specific to the host).

In the two-choice preference test executed by R. Soler (unpublished results) experienced *C. rubecula* females were tested for their host preference. Here *C. rubecula* females trained on hosts that were feeding on plants with *D. radicum* infestation preferred to oviposit in hosts feeding on infested plants. Around 50% of the *C. rubecula* females trained on hosts feeding on uninfested plants still preferred hosts feeding on the infested plants. Although this shows a clear preference of *C. rubecula* for hosts feeding on *D. radicum* infested plants, it is not known whether this is instinctive or

learned. To complement the two-choice preference test executed by R. Soler (unpublished results), an experiment can be executed in which naïve *C. rubecula* females are tested in a similar two-choice experiment to show an instinctive host choice.

In summary, root herbivory by *D. radicum* has no effect on the performance of *C. rubecula* when parasitizing a *P. rapae* host feeding on a *B. nigra* host plant. To find a link between the observed behaviour (R. Soler, unpublished results) and performance of *C. rubecula*, further research will be necessary.

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Appendix

1. Root herbivory damage
2. Additional results
3. Poster as presented at Netherlands Annual Ecology Meeting (NERN, NECOV)

Appendix 1. Root herbivory damage

(a)



(b)



Figure 1. Root damaged by herbivory of *D. radicum* (a) and a undamaged root (b).

Appendix 2. Additional results

Survival egg to adult

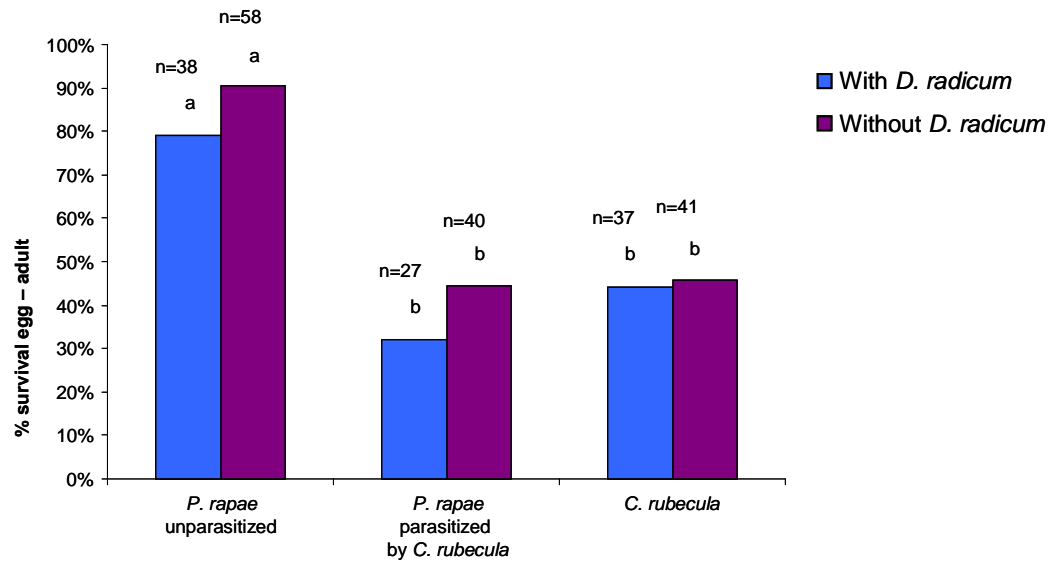


Figure 2. Percentage of survival from egg to adult, *P. rapae* unparasitized, *P. rapae* parasitized by *C. rubecula* and *C. rubecula*. Columns with identical letters are not significantly different based on Pearson Chi-square tests, $\alpha=0.05$. Per column the sample size is indicated.

Weight

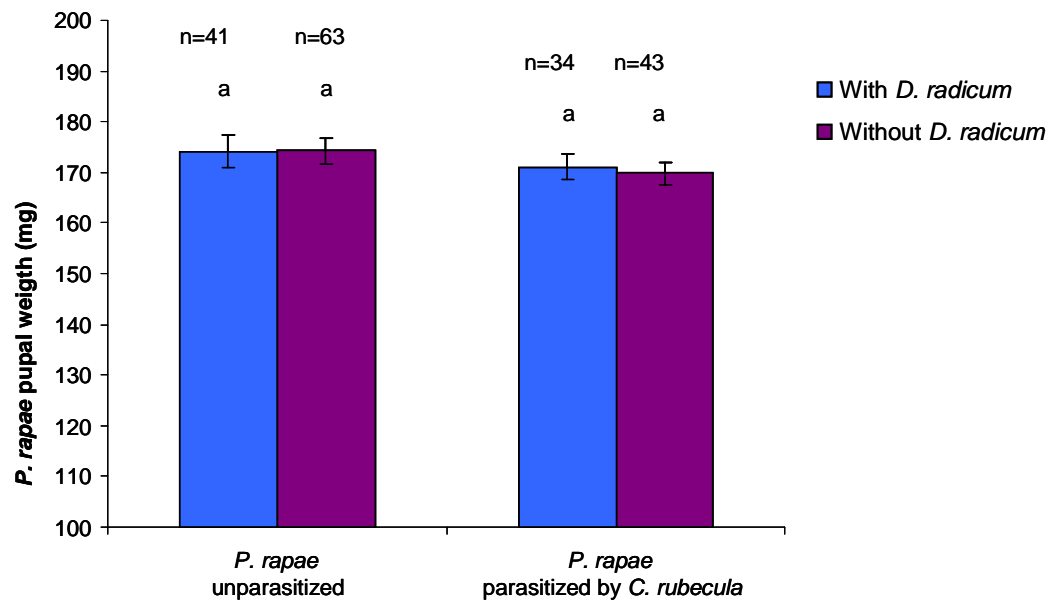


Figure 3. *P. rapae* mean (\pm SE) pupal weight, *P. rapae* unparasitized and *P. rapae* parasitized by *C. rubecula*. Columns with identical letters are not significantly different (mixed model analysis, $\alpha=0.05$). Per column the sample size is indicated.

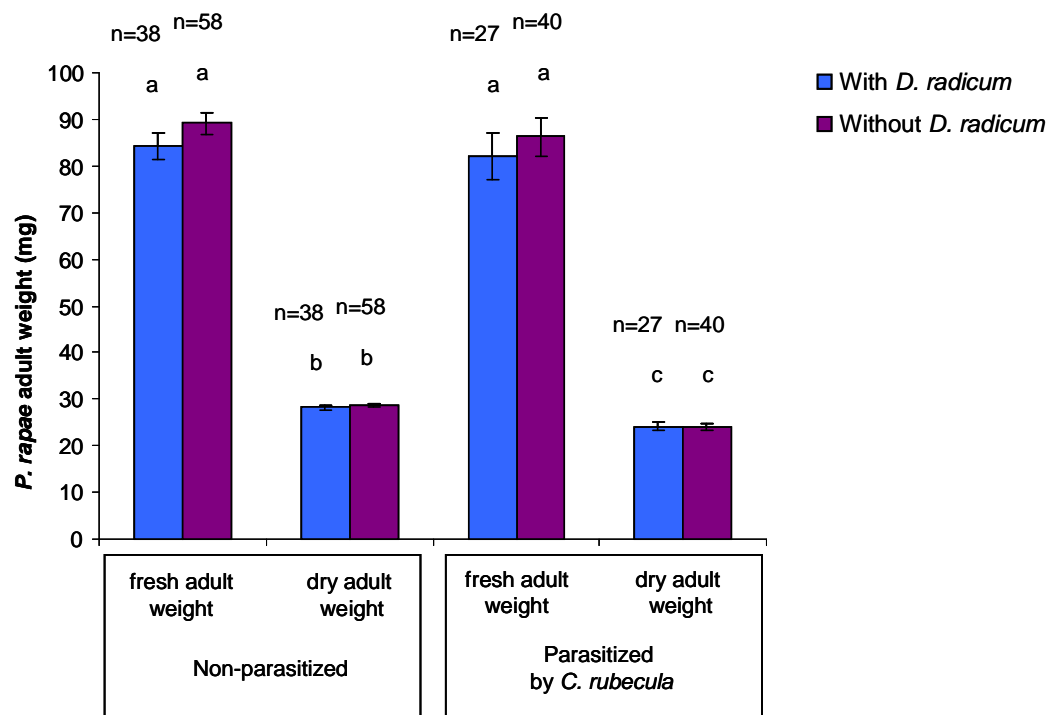


Figure 4. *P. rapae* mean (\pm SE) fresh and dry adult weight, *P. rapae* unparasitized and *P. rapae* parasitized by *C. rubecula*. Columns with identical letters are not significantly different (mixed model analysis, $\alpha=0.05$). Per column the sample size is indicated.

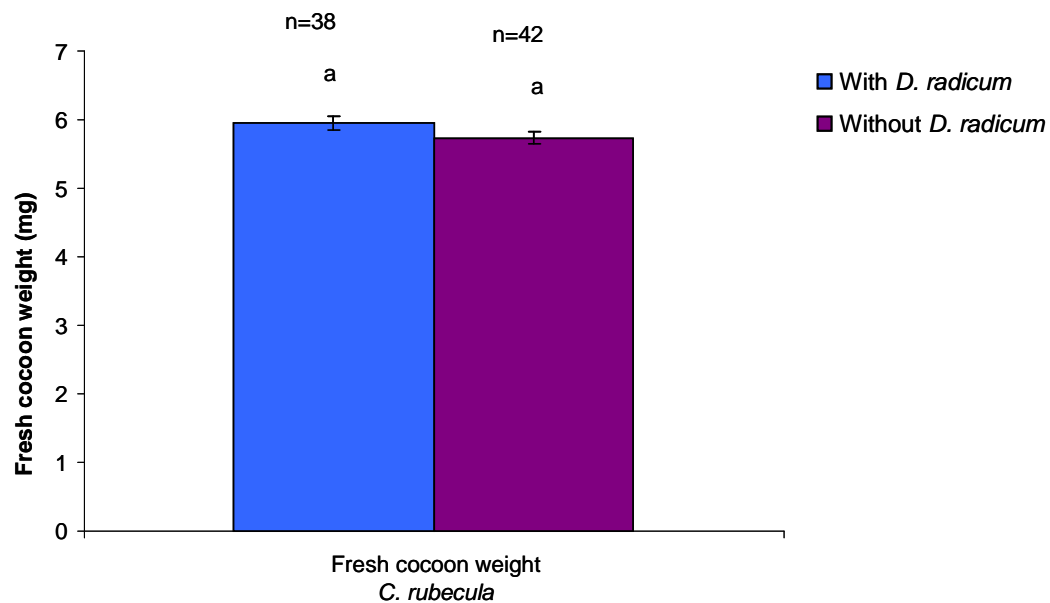


Figure 5. *C. rubecula* mean (\pm SE) fresh cocoon weight. Columns with identical letters are not significantly different (mixed model analysis, $\alpha=0.05$). Per column the sample size is indicated.

Development time

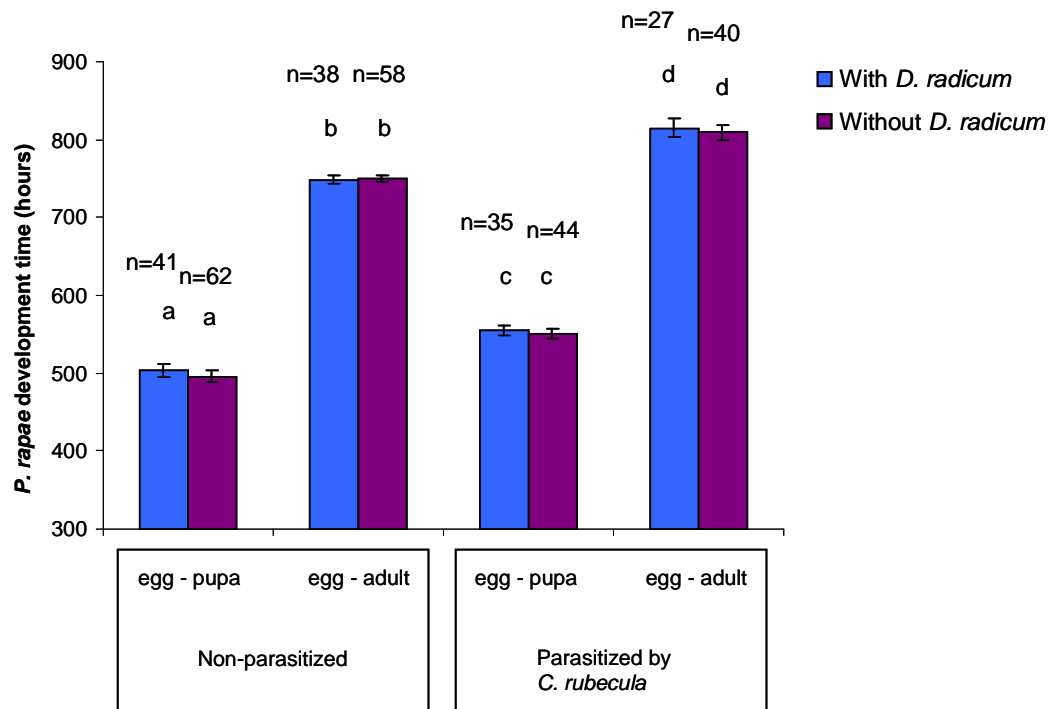


Figure 6. Mean (\pm SE) *P. rapae* development time from time of egg laying to fresh pupa and from time of egg laying to adult emergence, *P. rapae* unparasitized and *P. rapae* parasitized by *C. rubecula*. Columns with identical letters are not significantly different (mixed model analysis, $\alpha=0.05$). Per column the sample size is indicated.

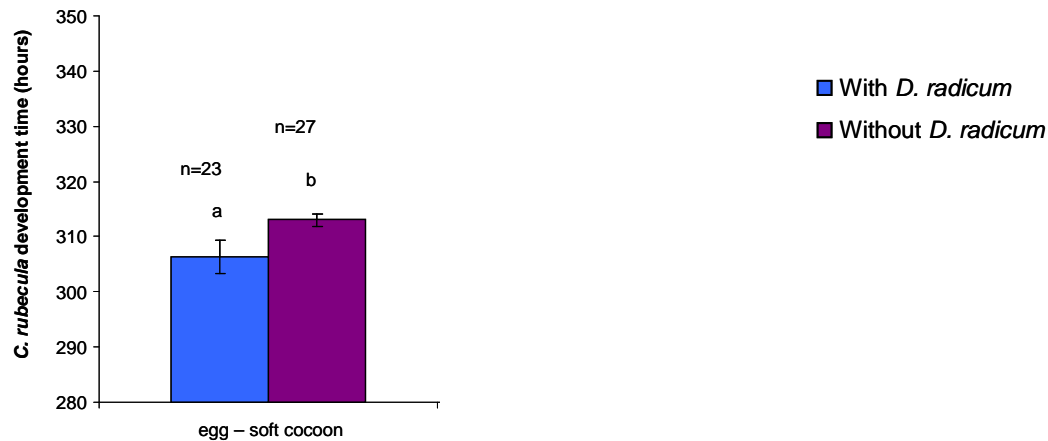


Figure 7. *C. rubecula* mean (\pm SE) development time from time of parasitizing to time of soft cocoon. Columns with identical letters are not significantly different (mixed model analysis, $\alpha=0.05$). Per column the sample size is indicated.

Plant buds

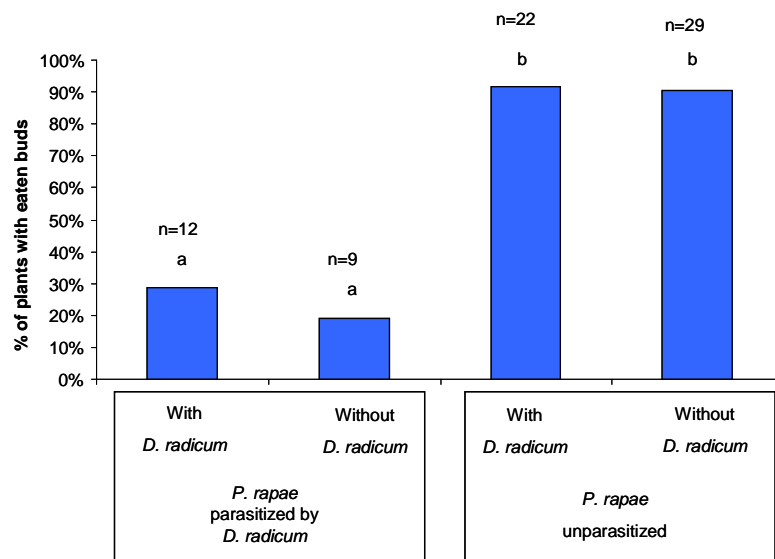


Figure 8. Percentage of plants with eaten buds 15 days after *P. rapae* introduction. Columns with identical letters are not significantly different based on Pearson Chi-square tests, $\alpha=0.05$. Per column the sample size is indicated.

Appendix 3. Poster as presented at Netherlands Annual Ecology Meeting (NERN, NECOV)

