Assessing the relationship between pest density and plant damage: a case study with the belowground herbivore Delia radicum (Diptera: Anthomyiidae) on broccoli

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Abstract

For many crops, we have poor knowledge about the relationship between pest density and damage. However, investigating pest harmfulness is particularly relevant currently in the search for alternative crop protection strategies that are unlikely to totally suppress pest populations. Here, we assessed the harmfulness of *Delia radicum* (L.) on broccoli (*Brassica oleracea* var. *italica* Plenck). We worked inside insect-proof cages set up in the field with additional pitfall traps to remove ground dwelling predators. Plants were manually infested with 10 levels of pest density ranging from 0 to 100 individuals per plant, following a natural infestation pattern. Surprisingly, no plants died but almost 100% of the pests introduced died over the course of the experiment. However, all broccoli development and growth traits were negatively correlated with pest density and broccoli head mass at harvest decreased linearly with pest density. The observation over time of development and growth traits showed evidence of plant compensation, suggesting that the head mass of individual plants may have reached similar values if allowed to fully mature. The relationship between pest density and damage, together with forecast models of pest population dynamics could be used to develop decision support tools assessing the relevance of preventative treatments.

Keywords

Crop losses; plant traits; plant injury; damage; plant compensation.

Introduction

Throughout the history of agriculture, the development of pest management strategies has led to dramatic decreases in crop losses to pests (Oerke 2006). However, the predominant crop protection method, that is pesticide usage, has various negative consequences, notably on human health and the beneficial fauna of agro-ecosystems (Desneux et al. 2007; Geiger et al. 2010). Alternatives are therefore urgently needed, but in contrast to pesticides, most of these alternatives (push-pull, insect netting, intercropping…) are likely to have partial efficiency. For example, conservation biological control, based on the enhancement of natural enemy populations, is thought to be an interesting method for reducing crop losses (Eilenberg et al. 2001) but is incompatible with the complete suppression of pest populations because by

definition, predators cannot maintain their populations without prey. It is therefore necessary to reconsider the relationship between pest density, plant injuries (i.e. the symptoms of pest development on the physiology of the host; Pedigo et al. 1986) and damage (i.e. the decrease in yield quantity or quality; Zadoks 1985; Pedigo et al. 1986). In this context, pest populations should reach an intermediate size that is both i/ high enough to allow the build up of efficient natural enemy communities, including several specialists depending on this specific resource (Devictor et al. 2010) and ii/ low enough to result in acceptable losses for the farmer, a level that is likely to depend on the production situation (*sensu* Aubertot and Robin 2013) (International Conference on Global Crop Losses 2017). In many cases, plants are able to tolerate moderate levels of pest infestation (Poston et al. 1983; Fenemore 1984; Pedigo et al. 1986), i.e. to withstand injuries without significant damage (Verdugo et al. 2016), so that this intermediate pest population size could be innocuous in several pest/crop systems (e.g. Rogers and Brier 2010).

In general, injuries and damage due to below ground pests have been less well studied than those inflicted by above ground pests (Hunter 2001). This may be due to methodological constraints because recording freshly caused injuries below ground necessarily involves destructive sampling, thus preventing the evaluation of subsequent damage. However, the effects of pest development below ground are not restricted to root tissue loss and indirect effects of root injuries could also occur above ground (Teixeira et al. 1996; Murray et al. 1996; Hunter 2001). Indeed, the physiological and morphological consequences of root injuries can include reduced leaf surface (Cardona et al. 1982), plant height and above ground biomass (Boica Junior et al. 2015), as well as an increase in lignin content (Hopkins et al. 1995), and soluble nitrogen concentration in the sap (Gange and Brown 1989). Gange and Brown (1989) suggested that due to root removal, below ground pests could have a similar effect as drought on plants. These indirect effects which can be assessed by monitoring plant growth and development above ground, may or may not lead to a significant decrease in final yield (Rosenheim et al. 1997; Brandelero et al. 2016).

Delia radicum (L.) (Diptera: Anthomyiidae) is the main pest of *Brassica* vegetables in northwestern Europe. The main cause of damage due to this pest in leaf and flower vegetables is early plant mortality, which can reach 40-60% without insecticides (Estorgues 2005). Females lay their eggs on the ground, within a distance of 5cm from plant stems (Hughes and Salter 1959). The larvae then develop by feeding on plant roots throughout three life stages. They thereby inflict injuries to the roots with several potential consequences on the plants (see above) among which the disturbance of water and nutrient uptake (Gange and Brown 1989). Several studies have been performed to quantify the damage inflicted by this pest on two *Brassica* crops: cauliflower and oilseed rape. Turnock et al. (1992) did not find a relationship between injuries caused by root maggots and yield of oilseed rape, hence suggesting that cropped plants fully compensated for injuries, at least up to the maximal tested pest density (25 eggs per plant). In cauliflower, El Titi (1979) showed a correlation between egg density and plant mortality for infestation levels ranging from 10 to 60 eggs per plant. This study was used by Bligaard et al. (1999) to define an economic threshold of *D. radicum* on cauliflower, suggesting that intervention was needed if more than one egg was found per felt trap (used for monitoring egg laying) and per day two to four weeks after planting. This threshold is still used by farmers and advisors in France to evaluate the intensity of pest infestation, but it is no longer

relevant in terms of decision making because: i/ the chlorfenvinphos used by El Titi (1979) is now prohibited and ii/ the current protection strategy against the cabbage root fly is preventative and consists of drenching the roots of all leaf and flower *Brassica* vegetables with spinosad (Success™ 4) before planting. Also in cauliflower, Bligaard (1999) performed several experiments with varying times of infestation and found that plant biomass decreased when egg density rose from 0 to 25 eggs per plant, but final yield was not monitored. Only one study showed reduced production of surviving cauliflowers in response to *D. radicum* attack (El Titi 1977). Finally, a methodological limitation in these previous studies was that the eggs were always introduced all at once. This pattern is unrealistic compared to natural infestation (Estorgues 2005) and may have had consequences on pest survival, plant-pest interactions and therefore on the subsequent damage: Finch and Skinner (1988) showed increased *D. radicum* survival for instant egg inoculation compared to protracted inoculation.

The objective of the present study was to assess *D. radicum* harmfulness on broccoli *Brassica oleracea* var. *italica* Plenck (Brassicales: Brassicaceae), a crop for which we expected a similar tolerance as that previously observed for cauliflower. For this, we worked on caged plants grown in the field to ensure natural root development and we controlled pest density using artificial egg infestations. We expected broccoli to show a tolerant response to *D. radicum* (Poston et al. 1983), with compensation for low injury levels, hence no damage; and damage resulting from greater levels of injury, ranging from yield reduction to plant mortality.

Materials and methods

Study site and experimental design

The experiment was conducted in an 850m² plot at INRA's experimental station (UE0787, Domaine expérimental de la Motte au Vicomte), in Le Rheu, France (48°06'N; 1°47'W) during spring 2016. The field used for this study has a deep soil (80-120cm), hydromorphic from the surface, originating from shale and wind deposited loam (classified as luvisol following the international soil classification system of the IUSS Working group WRB 2015). Previous crops were maize in 2012, 2013, 2014; fava beans in 2015 and meadow for seven months before this study started. Six insect-proof cages (6m long × 3m wide × 2m high; 300 * 300µm mesh; Diatex ®) were set up on the plot. On the 5th of April 2016, 30 untreated broccoli plants (cv. 'Marathon') at the stage of two true leaves were planted in each cage, every 0.50m in rows 0.75m apart. Insect-proof cages prevented plant colonization by flying insects, whether they were females from surrounding cabbage root fly populations, other *Brassica* pests or *D. radicum* natural enemies. PET barriers (Greenborder, Nortene®) buried all around the cages (60cm high, 40 of which below ground) prevented hypogeic and epigeic organisms from gaining access. Two sticky panels (29.7 * 42cm), a yellow and a blue one, were hanged inside each cage in order to catch flying arthropods that could emerge from the soil. Twenty four pitfall traps were also set up in each cage (i.e. 1.3 per m²) to reduce predation by ground dwelling arthropods on immature fly stages. Pitfall traps were half-filled with water and a few drops of odorless detergent. Barriers and traps were set up 13 days before planting, and the cages, the day before planting.

Artificial plant infestation

Eggs were manually deposited on plants inside insect-proof cages. The timing of egg additions was designed to mimic the natural egg laying dynamics of *D. radicum*, which is generally centered on a peak that represents about 40% of the total amount laid (calculation based on previous experiments performed under natural infestation conditions). The first natural egg laying peak is quite stable over the years in north-western France and typically occurs between early and mid-May when transplanting is done at the beginning of April (Estorgues 2005). We hence spread the infestation out over three consecutive weeks with a peak on the 2nd of May representing 40% of the total amount of eggs applied on each plant. The two other inputs were made on the 25th of April and on the 9th of May, each representing 30% of the total amount of eggs applied per plant. We defined ten pest densities, summarized in Table 1, ranging from 0 (control) to 100 eggs per plant, a range similar to egg counts made in production fields from the same region (Josso et al. 2013).

The eggs were obtained in the laboratory from a strain of *D. radicum* originally collected in fields of the same experimental station in early summer 2015 and reared as described in Lamy et al. (2017). Females were offered slices of swede placed on a filter paper for egg laying. The eggs were then brought to the field and directly used for infesting the plants. They were deposited at the base of plant stalks with a fine paintbrush. At each date of plant infestation, an additional batch of about 300 eggs was placed in a Petri dish, on a moistened filter paper and kept in a climate-controlled room (16:8h photoperiod and 20°C). After seven days, natural egg mortality was estimated as the proportion of unhatched (i.e. dead) eggs. Infestation levels were distributed to broccoli plants in a randomized complete block design: inside each cage, egg density was attributed randomly to each plant; there were three broccoli plants for each egg density in each cage, thus a total of 18 plants per egg density in the experiment.

At broccoli harvest, i.e. on June 21 and 22, a soil sample (12cm in diameter and 13.5 ± 0.4cm (mean ± SE) in depth, ensuring the collection of more than 70% of the pupae; Hughes 1960; Finch et al. 1978) was taken around each broccoli root with a motorized auger. The number of *D. radicum* larvae and pupae was counted after washing the samples through a 1mm * 1mm mesh sieve.

Plant development and growth

We evaluated plant development and plant growth weekly for every broccoli plant from April 12 to June 14 (i.e. during 10 weeks). The development of each plant was assessed by recording the leaf numbers, the presence / absence of at least one lateral sprout and the presence / absence of a visible inflorescence. These measurements match the three main stages of broccoli development (Feller et al. 1995). Plant mortality was also recorded. Plant growth was evaluated via two measurements: the product of the largest leaf length and width (thereafter called “relative leaf area”), known to be highly correlated with leaf area in *Brassica* crops (Olfati et al. 2010; Cargnelutti Filho et al. 2015; Tartaglia et al. 2016) and the height above ground of the apical meristem (thereafter simply called “plant height” in the text), often used to give complementary information on plant growth (Kloen and Altieri 1990; Brandelero et al. 2016).

Root injuries and final head mass

As the first broccoli had reached their optimal development regarding marketable standards (buds tight and close to opening), we harvested every plant in a row on the 21st and 22nd of June. Stalks were cut about 1cm below the insertion of the first branch of the head and heads were weighed with a spring scale (precision = ± 5g).

Injuries caused by *D. radicum* larvae feeding on plant roots was assessed by visual examination using the scale defined by Dosdall et al. (1994): 0 = no injury; 1 = slight feeding, < 10% of tap root surface injured; 2, 3, 4 and 5 respectively 10-25%, 26-50%, 51-75%, 76-100% of root surface injured.

Data analysis

Generalized linear mixed models were used to analyze every dimension of plant development, growth and production. We generated a model for every dimension of plant development (i.e. number of leaves, proportion of plants with at least one lateral shoot and proportion of plants with an inflorescence) and growth (i.e. relative largest leaf area, plant height) including the number of eggs (quantitative), the sampling session (factor), and their interaction as fixed effects as well as the cage and plant identifiers as random effects to account respectively for potential spatial correlation among data obtained inside the same cage and for the fact that the same plants were monitored in each session (Faraway 2006). The analysis of lateral shoots and inflorescences was restricted to the dates when shoots or inflorescences were recorded, respectively. For the latter, only two measurement sessions were analyzable which made it impossible to use the plant identifier as a random factor. This proportion was therefore analyzed at the cage scale and the random effect set for the cage identifier to account for repeated measures (Faraway, 2006). Pearson correlation coefficients were computed among all development and growth traits over their respective period of analysis (Table S1 in Supplementary materials).

The effect of the number of eggs on the proportion of plants displaying root injuries (injury class > 0) and on final head mass was assessed, using a random factor set for the cage identifier to consider potential spatial correlation among data obtained inside the same cage.

All statistical analyses were performed using R software (R core team 2017). The models described above were fitted using generalized linear mixed modeling (functions ‘lmer’ or ‘glmer’ of the package ‘lme4’; Bates et al. 2015) with a distribution and link function adapted to the data analyzed: identity-link Gaussian (response variables: log transformed number of leaves; log transformed relative largest leaf area; square root transformed plant height; final head mass) or logit-link binomial (response variables: proportion of plants with at least one lateral shoot; proportion of plants with an inflorescence; proportion of plants with root injuries). When necessary, an additional random factor set for the statistical individual was used to account for data overdispersion. The significance of the fixed effects was tested using type II Wald chi-square tests (function ‘Anova’, package ‘car’; Fox and Weisberg 2011).

Post-hoc tests were performed on the models fitted on plant development and growth traits. We studied the effect of egg density on plant traits as a function of time: for each date

(factor level), we estimated the marginal slope of the linear trend between egg density and plant traits (in cm/egg for plant height for instance; function 'emtrends' with back-transformation of the slopes to the response scale; package 'emmeans'; Lenth 2017). Then we extracted the slope of the steepest relationship between each plant trait and egg amount. These values provided estimates for the strongest effects of pests on plant traits observed in our experimental design.

Thirty plants (i.e. 17%) suffered from slug attack or lost their apical meristem so that it was not possible to record their largest leaf length and width or plant height on at least one occasion. All data collected on such plants were discarded.

Results

Plant survival and mortality of D. radicum *immature stages*

The 150 broccoli plants survived the entire experiment. The proportion of unviable eggs among the three batches used for artificial infestations was similar with an overall mean of 18.9% (± 1.3%). At the end of the experiment, a total of only two pupae was recovered from the soil samples taken around the plant roots, suggesting a high mortality of the 6065 introduced eggs before reaching their final developmental stage.

Plant development and growth

The relative largest leaf area, plant height and the number of leaves were highly correlated, with Pearson correlation coefficients exceeding 90%. In contrast, none of the data had a correlation coefficient exceeding 63% with the proportion of plants with an inflorescence and coefficients did not exceed 37% for correlations with the proportion of plants with at least one lateral shoot (Table S1 in Supplementary materials).

Except for the proportion of plants with at least one lateral shoot, all development and growth traits were negatively correlated with an increase in pest density (Table 2). The interaction between measurement dates and the number of eggs deposited was significant for growth traits, but not significant for development traits.

Plant trait responses to pest attack followed a similar pattern (Fig. 1) of 1) no pre-existing trend in development and growth traits prior to pest input; then 2) a transient negative effect becoming significant after a delay varying from a couple of days to several weeks after the first infestation, depending on the trait, and finally 3) a waning of the effect with no significant trend at the end of the experiment. Hence, even the highly infested plants recovered and showed the same final development and growth states as those of non-infested plants, as measured by our parameters. The exception was plant height, which remained negatively correlated with egg density until the end of the experiment. All plants produced an inflorescence and 73% produced at least one lateral shoot by the end of the experiment. The time course of negative effects of egg density (Fig. 1) indicates that these organs appeared later when plants suffered high pest densities.

The date at which the most negative trend between egg density and development or growth trait was observed varied depending on the trait considered. It ranged from 23 days to 50 days after the first artificial egg infestation for the proportion of plants with lateral shoots and the plant height respectively (Fig. 1). Focusing on this particular date, the models indicated

linear relationships between plant traits and egg density (Fig. 2), with only slight deviations from linearity for the proportion of plants with at least one lateral shoot or with an inflorescence, which may simply be due to the binary nature of these response variables.

Root injuries and final head mass

All plants harvested were in injury class 0 or 1 (i.e. less than 10% of the tap root surface injured). The proportion of plants with root injuries due to *D. radicum* larvae feeding increased linearly with the number of eggs deposited ($\chi^2 = 13.0$; df = 1; $P < 0.001$; Fig. 3).

All plants produced a broccoli head, with an overall mean yield of 295.9 ± 13.8g per plant. Plant production was negatively and linearly correlated with the amount of eggs deposited on the plant ($\chi^2 = 11.1$, df = 1, $P < 0.001$; Fig. 3). For a rise from 0 to 100 eggs, the predicted drop in broccoli mass was 136.1 g, for an attainable mass of 346.8 g, i.e. a loss of 39% of the attainable mass.

Discussion

The two main results of this study are that 1) all plant development and growth traits, as well as the final mass of broccoli heads, were negatively correlated, at least transitorily, with *D. radicum* egg density and 2) no plants died and all produced a harvestable head despite a maximum pest density expected to lead to plant death.

D. radicum *affects all development and growth traits and reduces plant production*

All development and growth traits were negatively and linearly correlated with pest density, at least for one measurement date. Additionally, broccoli mass decreased linearly with the amount of eggs deposited, when the first plants were ready for harvest. Our results therefore confirm that development delays and reduced plant growth during the growing season can lead to significant drops in head mass at harvest, at least when all plants are harvested in a row (Brandelero et al. 2016). This result is interesting because plant mortality is currently regarded as the main damage caused by the cabbage root fly on broccoli (El Titi 1979; Estorgues 2005). Similarly, El Titi (1977) found a negative correlation between *D. radicum* pupae density and vegetable mass in cauliflower, suggesting that this type of relationship between pest density and yield might be found for other *Brassica* crops. Our results indicate that (at least when no plant mortality occurs) the impact of *D. radicum* on plants is linear, with no threshold: even the lowest pest densities limit plant growth and head mass. This is not exactly consistent with the findings of Bligaard (1999), which suggested threshold effects in the relationship between the biomass (a growth trait) of cauliflowers and *D. radicum* egg density. Our results therefore suggest that *D. radicum* can be harmful even when it does not kill the plants.

However, all development and growth traits, except above ground plant height, were no longer correlated with pest density at the time of the final measurement. Thus it appears that the plants were compensating for the injuries caused by the pest. We therefore suggest that the production potential of each plant, i.e. the attainable yield if every plant had been harvested at individual maturity, may not have been modified by pest density in our experimental conditions, but that the plants had not yet fully compensated for the pest attack by the end of our experiment. The plants may thus have been able to tolerate the pest attacks (Strauss and Agrawal 1999;

Verdugo et al. 2016), as shown in many non-crop plants (e.g. Heichel and Turner 1983; Fowler and Rausher 1985; Karban and Courtney 1987; Maschinski and Whitham 1989) but also for instance in *Aphis gossypii* infesting cotton (Rosenheim et al. 1997). In the latter study, comparing infested and non-infested plants, the authors showed that leaf area was transitorily reduced by 58% when plants were infested with aphids but then by harvest the cotton plants had fully recovered so that yield was not affected (Rosenheim et al. 1997). In our crop/pest system, the consequences of pest infestation in terms of damage are likely to depend on the production situation. If the harvest is spread over several weeks, e.g. in the context of market gardening where produce is harvested only in time for sale, plants may reach their potential and *D. radicum* infestation may not affect final yield. In contrast, when the aim is to reduce the number of harvests to limit the costs, e.g. in the context of field vegetable production where the volumes are large and the associated costs for harvest are high, *D. radicum* is likely to add to the natural variability of the time needed to reach plant maturity (Dufault 1997; Grevsen 2000; Lindemann-Zutz et al. 2016). Thus, an infestation may lead to either additional harvests being performed, thereby increasing production costs, or to an increased amount of unharvested plants (because not fully mature at harvest), decreasing the income.

The absence of plant mortality and D. radicum *mortality*

A surprising result of our experiment is that no plants died. Two hypotheses may explain this: 1) the maximum egg densities were not high enough to kill plants in our growing conditions or 2) the maximum egg densities should have killed the plants but the unexpected high developmental mortality of *D. radicum* led to an underestimation of the real harmfulness of this pest. In previous studies on cauliflower, El Titi (1979) and Bligaard (1999) reported significant plant mortality with much lower egg densities (respectively 60 and 25 eggs per plant), introduced when plants were at a similar developmental stage (~ 4 leaves). However, the link between pest density and damage is also likely to depend on plant growing conditions. Generally, good growing conditions increase plant compensating abilities (Fenemore 1984; Pedigo et al. 1986; Maschinski and Whitham 1989). More specifically, as root-feeding pests induce symptoms similar to those of drought (Gange and Brown 1989; Foggo and Speight 1993), the availability of water may be a crucial factor determining the ability of plants to tolerate a pest attack (Godfrey and Yeargan 1985; Dunn and Frommelt 1998). In the present study, the spring was particularly wet: the meteorological station nearby (1.6km) recorded 181.5mm throughout the experiment. Such precipitation levels are very unlikely to induce water stress in broccoli: in warmer conditions, Erdem et al. (2010) showed significant water stress only when spring broccoli received as little as 130mm, but no stress when it received 193mm or more. In addition, the windbreak effect of the cages probably limited desiccation. These favorable conditions may partly explain the absence of mortality.

Although the level of *D. radicum* mortality is usually high (~ 80-90%; Hughes and Salter 1959; Meyling et al. 2013), it was extreme in our experiment as a hundred percent mortality has never been reported. First, it cannot be ruled out that some pupae were beyond the area covered by the auger used (i.e. 12cm in diameter). Following Hughes (1960) and Finch et al. (1978) the area prospected was enough to collect more than 70% of the pupae. Given that we collected only two pupae within this area, it is unlikely that the pupae missed would have

substantially changed the estimation of *D. radicum* mortality rate. Then, some mortality occurred during the egg stage: we showed that between 10 and 25% of the eggs were unviable, which is consistent with previous studies conducted with this biological model (Neveu et al. 1997). Actual egg mortality in the field could have been higher due to one supplementary handling, to deposit the eggs at the base of plant stems. Additional egg mortality could be attributable to predation by ground dwelling arthropods. Indeed, Fig. S1 in Supplementary materials shows that our exclusion setup did not completely suppress the ground dwelling arthropod fauna, at least during the weeks of plant infestation. The carabid family, that dominated this fauna, was essentially represented by two species *Metallina lampros* and *Phyla obtusa*. These share functional traits which likely determine the efficiency of ground dwelling predators on *D. radicum*: small body size and carnivorous diet (Purtauf et al. 2005). *Metallina lampros* is thought to be specialized on *D. radicum* and to contribute largely to its natural regulation (Hughes 1959; Coaker and Williams 1963; Andersen et al. 1983).

On the other hand, the results discussed above concerning the decreased plant growth and yield show that at least some of the eggs survived and produced harmful larvae. As almost no pupae were found around broccoli roots, some pest mortality also appears to have occurred during the larval stage. Based on previous studies performed in open fields, predation by ground dwelling arthropods on buried materials (here, *D. radicum* larvae) seems rather unlikely (Finch and Elliott 1994; Lee and Edwards 2012). However, using insect-proof cages meant that any trapped ground dwelling predators had virtually no alternative resources on which to prey besides the *D. radicum* eggs (no other *Brassica* herbivores were observed inside the cages). We also recorded high numbers of ants at the beginning of May (Fig. S1, in Supplementary materials), i.e. during larval development of the first introduced eggs. Several authors showed that many ant species are significant predators of below ground pests (Carroll and Janzen 1973; Yadav et al. 2012; Pacheco et al. 2017). For instance, Yadav et al. (2012) showed that ants caused 60% mortality of *Galleria mellonella* larvae that were being used as below ground sentinel prey. The unusually high mortality recorded here may therefore be related to the unusual presence of ants. Also, as mentioned above, the spring when the study took place was particularly wet and the soil was hydromorphic. High soil moisture is known to decrease larval survival (Finch and Skinner 1988) and could have contributed to the high mortality observed. Finally, the favorable growing conditions in our experiment may have increased the constitutive and induced resistance mechanisms of the plant and caused additional larval mortality. Induction of plant defense following infestation may explain some of the *D. radicum* mortality as well as some of the effects on plant traits, through a trade-off between resistance (i.e. mobilizing energy and matter to produce defensive compounds) and growth, i.e. an allocation cost (Strauss et al. 2002).

Conclusion

In our experiment, the introduced *D. radicum* suffered high mortality rates, thus we could not establish a clear relationship between pest density and plant mortality. As a systematic insecticide treatment at planting (Spinosad, authorized in conventional and organic production) is currently used to prevent plant mortality, the re-assessment of this relationship is urgently needed. However, we showed that plant mortality is not the only cause of damage in this

crop/pest system and that sublethal effects can lead to a 40% decrease in broccoli mass at harvest even in conditions where the survival of the pest is not optimal. Based on our findings concerning pest harmfulness coupled to forecast models of population dynamics (e.g. Collier et al. 1991) it should be possible to develop decision support tools evaluating the relevance of the treatment, based on the expected pest infestation and its expected impact on crops. As predation by ground dwelling arthropods was probably a mortality factor of prime significance in the present study, it may be very valuable to take them into account in such models.

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Author contribution statement

All authors conceived and designed research. MV, LD and XM performed the field work. XM analyzed data. XM, ALR, AMC, VF and YT were involved in writing of the manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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607

608 **Table 1** Pattern of artificial infestation of the broccoli plants with cabbage root fly eggs
609

1st infestation (3 weeks after planting)	0	1	3	6	9	12	15	18	24	30
2nd infestation (4 weeks after planting)	0	3	4	8	12	16	20	24	32	40
3rd infestation (5 weeks after planting)	0	1	3	6	9	12	15	18	24	30
Total	0	5	10	20	30	40	50	60	80	100

610

611 **Table 2** Effect of the number of *D. radicum* eggs deposited per plant and its interaction with
612 the date of measurement on the development and growth traits recorded for the broccoli plants.
613 Bold p-values show significant differences at $\alpha = 5\%$
614

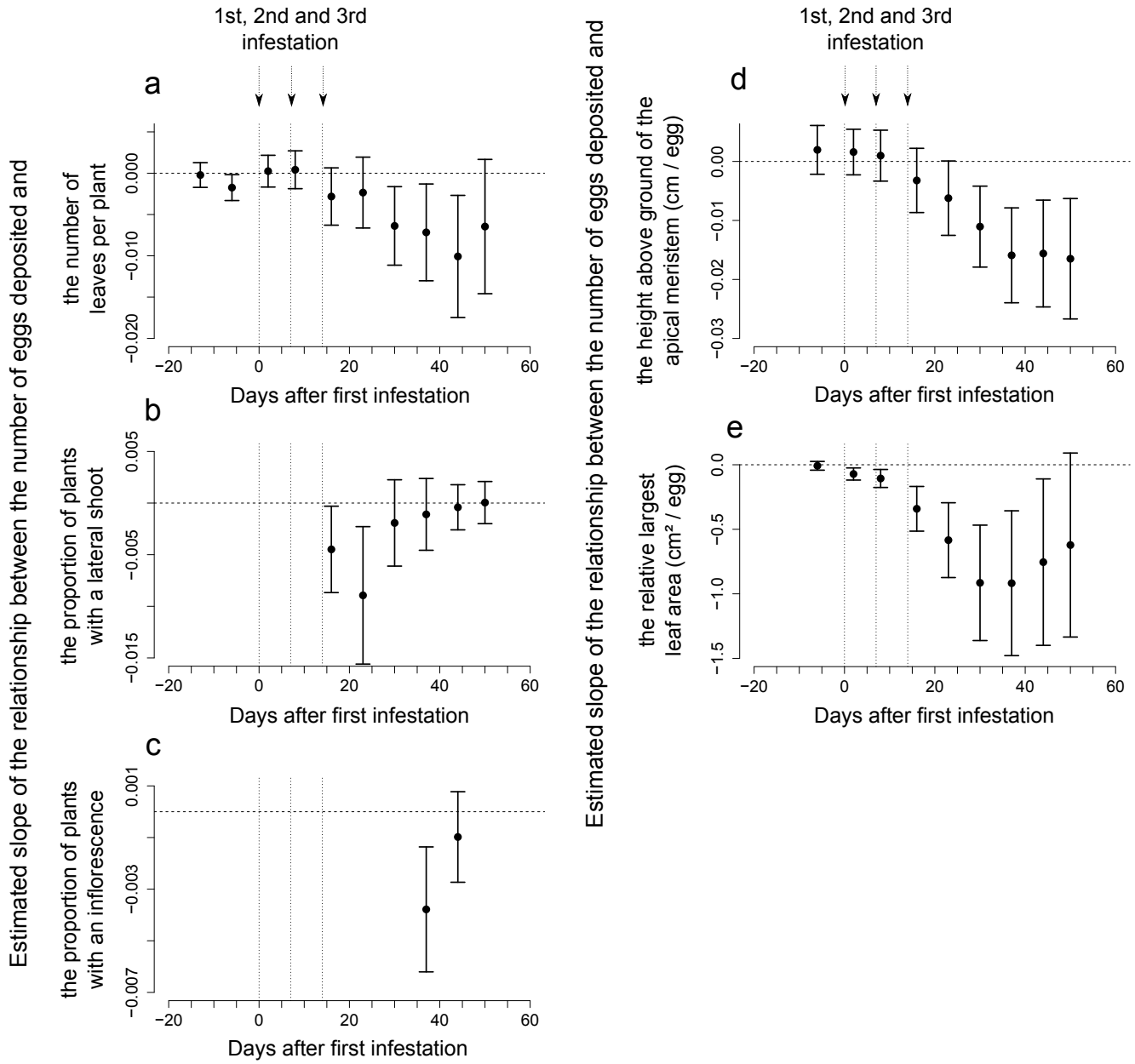
	Number of eggs				Interaction "number of eggs: date"		
	χ^2	DF	P-val	Trend	χ^2	DF	P-val
Number of leaves	6.61	1	**0.010**	↘	15.17	9	0.086
Proportion of plants with at least one lateral shoot	2.36	1	0.125		8.64	5	0.124
Proportion of plants with a visible inflorescence	8.25	1	**0.004**	↘	2.34	1	0.126
Relative largest leaf area	13.66	1	**< 0.001**	↘	24.16	8	**0.002**
Height above ground of the apical meristem	5.09	1	**0.024**	↘	54.82	8	**< 0.001**

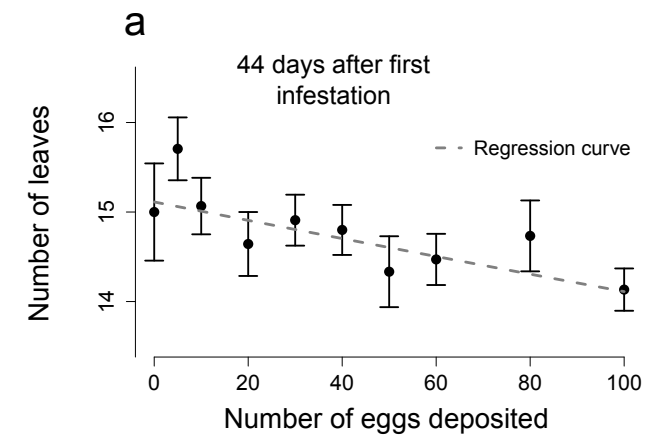
615

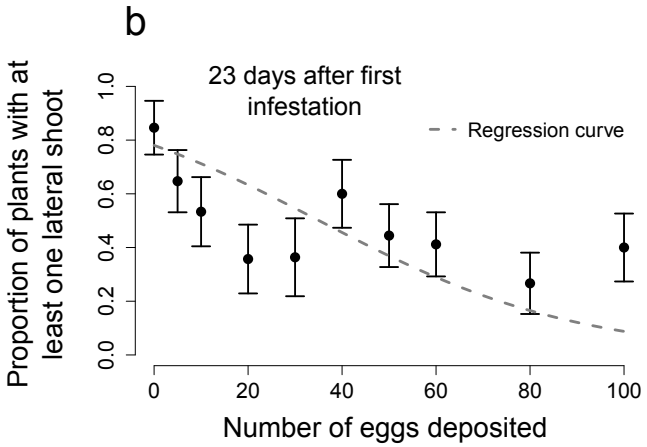
Fig. 1 Evolution through time of the linear trend between plant traits (a: number of leaves; b: proportion of plants with at least one lateral shoot; c: proportion of plants with an inflorescence; d: height above ground of the apical meristem in cm and e: relative largest leaf area in cm^2) and the number of eggs deposited. Each point and associated error bar represents the estimate ± 95% confidence interval of this trend, obtained with function 'emtrends' (Lenth 2017). A negative value indicates that plant development or growth is negatively correlated with the number of eggs deposited at the time of measurement. The trend is significant (i.e. the effect of the number of eggs on plant trait is significant at a given time) if the error bar does not cross the horizontal dashed line in 0. Vertical dotted lines represent the three artificial infestations of broccoli plants

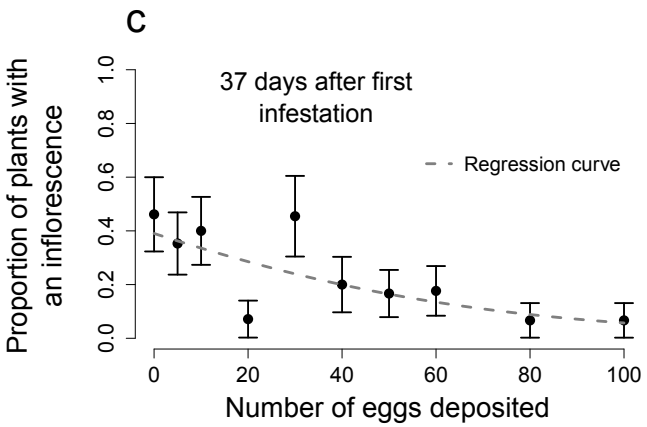
Fig. 2 Plant development (a: leaf number, mean ± SE; b: proportion of plants with at least one lateral shoot, prop ± SE and c: proportion of plants with a visible inflorescence, prop ± SE) and growth traits (d: height above ground of the apical meristem in cm, mean ± SE; e: relative largest leaf area in cm^2, mean ± SE) as a function of the number of *D. radicum* eggs deposited per plant for the measurement date indicated above the graphs, corresponding to the time when the correlation between plant trait and egg density was the most negative (Fig. 1). The dashed grey lines present the regression curves obtained with the coefficients of the models presented in the text and in Table 2

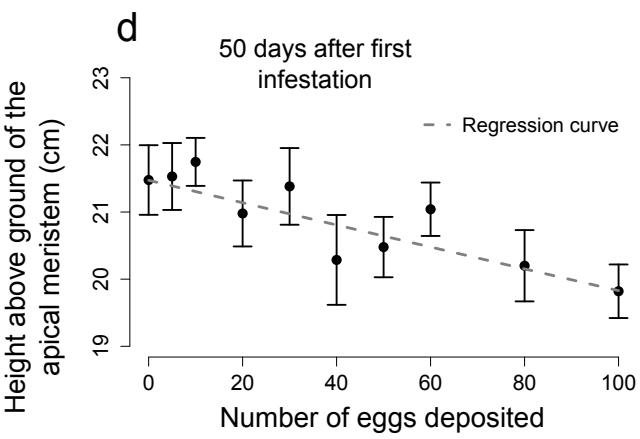
Fig. 3 Proportion of plants showing root injuries (prop ± SE; a) and broccoli mass at harvest (in grams, mean ± SE; b) according to the number of *D. radicum* eggs deposited per plant

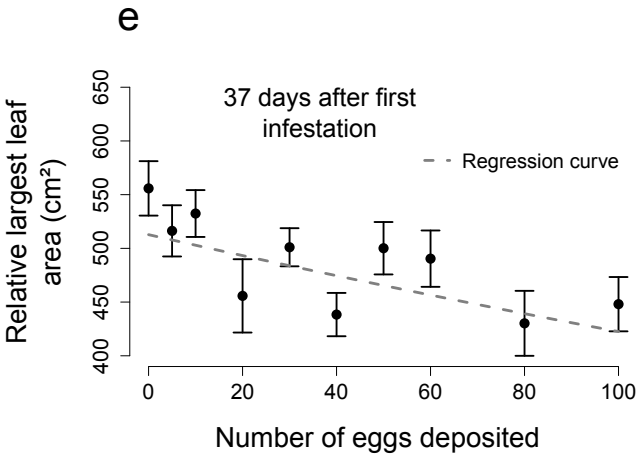

1st, 2nd and 3rd infestation
a
the number of leaves per plant
0.000
−0.010
−0.020
b
the proportion of plants with a lateral shoot
0.005
−0.005
−0.015
c
the proportion of plants with an inflorescence
0.001
−0.003
−0.007
−20
0
20
40
60
Days after first infestation
Estimated slope of the relationship between the number of eggs deposited and
d
the height above ground of the apical meristem (cm / egg)
0.00
−0.01
−0.02
−0.03
e
the relative largest leaf area (cm² / egg)
0.0
−0.5
−1.0
−1.5

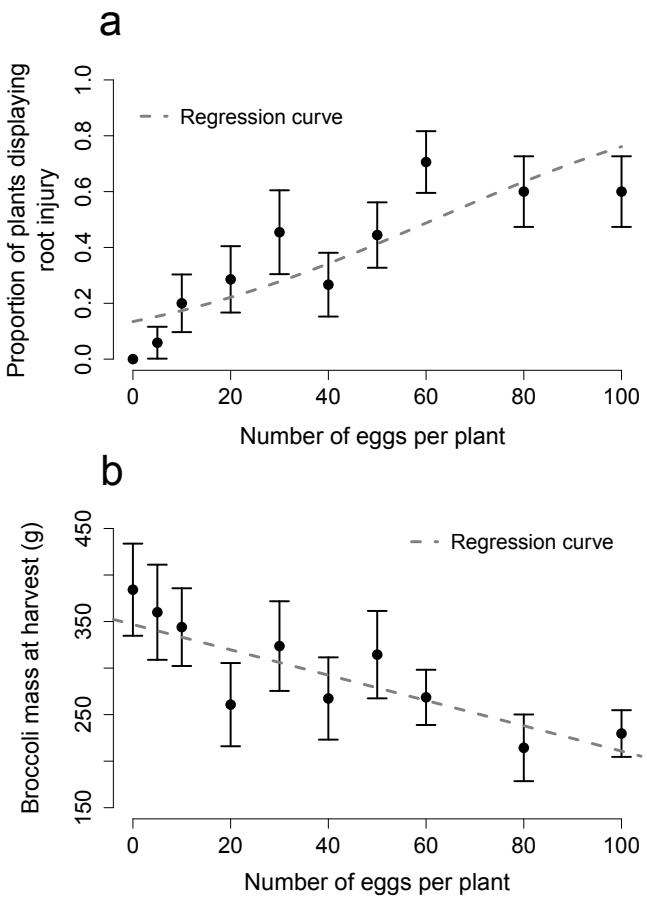

a
44 days after first infestation
Regression curve
Number of leaves
14
15
16
0
20
40
60
80
100
Number of eggs deposited


b
23 days after first infestation
Regression curve
Proportion of plants with at least one lateral shoot
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
Number of eggs deposited


c
37 days after first infestation
Regression curve
Proportion of plants with an inflorescence
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
100
Number of eggs deposited


d
50 days after first infestation
Regression curve
Height above ground of the apical meristem (cm)
19
20
21
22
23
0
20
40
60
80
100
Number of eggs deposited


e
37 days after first infestation
Regression curve
Relative largest leaf area (cm²)
400
450
500
550
600
650
0
20
40
60
80
100
Number of eggs deposited


a
Proportion of plants displaying root injury
0.0
0.2
0.4
0.6
0.8
1.0
Regression curve
0
20
40
60
80
100
Number of eggs per plant

b
Broccoli mass at harvest (g)
150
250
350
450
Regression curve
0
20
40
60
80
100
Number of eggs per plant