Influence of soil tillage on natural regulation of the cabbage root fly Delia radicum in brassicaceous crops

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Influence of soil tillage on natural regulation of the cabbage root fly *Delia radicum* in brassicaceous crops.

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Abstract

Ground dwelling predators provide regulation services of several insect pests. Enhancing these services may be a step toward integrated crop protection. Many studies have shown that soil tillage is deleterious to ground dwelling predators but pest regulation processes and services have rarely been measured. We performed an experiment to study whether simplifying soil tillage before the establishment of spring broccoli enhanced ground dwelling predator populations and the control they provide on *Delia radicum*. The direct effect of tillage on arthropods was assessed by comparing their emergence rates in plots differing in soil tillage management. The natural regulation service was assessed by comparing a control and an exclusion treatment in which predators were removed. The effect of soil tillage on carabids, spiders and staphylinids did not match the gradient of disturbance induced by tillage treatments. Tillage did not appear to affect the predators that likely contribute to *D. radicum* regulation. Consistently, the number of pests suppressed and the root injuries were unaffected by tillage treatments. The main deleterious effect of soil tillage was on the emergence of those carabid species that overwinter partly as larvae, suggesting that spring tillage could affect pest control in the following crops.

Keywords

ground dwelling predators; pest regulation service; cabbage root fly

1 Introduction

Farmers have long used soil tillage, mostly for weed management and refinement of the soil structure to favor crop germination (El Titi, 2003). However, many studies have shown that intensive tillage practices generally lead to a reduction in the abundance of soil-inhabiting macroorganisms including potential predators of weed seeds or arthropod pests, i.e. mainly spiders, carabid beetles and staphylinid beetles (Holland, 2004; Kendall, 2003). This reduction results from direct effects (e.g. direct mortality from the tilling, desiccation; Kendall, 2003) and/or from indirect effects, i.e. from different arthropods colonizing the field after tillage (e.g. in response to modified plant cover, to alternative prey availability; Petersen, 2002; Thorbek and Bilde, 2004). To date, most studies did not distinguish between these direct and indirect effects, mainly because it is difficult to design and carry out an experiment allowing these two effects to be disentangled (Thorbek and Bilde, 2004).

The effects of soil tillage on ground dwelling predators depend on several traits of the studied organisms including their development stage at tillage (Purvis and Fadl, 1996), burrowing depth (Lorenz, 1995) or body size (Hatten et al., 2007). Therefore, carabids, staphylinids and spiders respond differently to soil tillage: tillage generally does not have much of an impact on staphylinids whereas carabids and spiders are more affected (Wardle, 1995). Consequently, soil tillage is likely to modify pest natural regulation processes because ground dwelling predators abundance (Symondson et al., 2002) and traits (Wood et al., 2015) are involved in their predation potential.

Many articles reported the effect of soil tillage on pest predation using sentinel preys or predation cards (e.g. Petit et al., 2017; Tamburini et al., 2016), generally showing that reduced tillage had a positive impact on predation potential. However, such methods only give indications on the potential natural regulation obtained and have considerable limitations for several pests (Zou et al., 2017). Direct measurements, e.g. using exclusion protocols (Luck et al., 1988), are still needed to obtain a more realistic estimate of the effect of soil tillage on the natural regulation provided by pest predators. There is also a need to go further and to test whether tillage simplification leads to fewer injuries and/or damage to the crop in order to assess the potential of soil tillage simplification as a conservation biological control practice (Rusch et al., 2017).

The main pest of brassicaceous vegetables in northwestern Europe is the cabbage root fly *Delia radicum* L. (Diptera: Anthomyiidae), that may inflict 40-60% plant mortality (Estorgues, 2005) on spring vegetables without insecticide protection. The flies lay their eggs at the base of plant stems, on or slightly below ground (Hughes and Salter, 1959). Then the larvae develop below ground, feeding on, and thus inflicting injuries to plant roots. Metamorphosis occurs inside a pupa, also buried in the ground, near plant roots (Hughes and Salter, 1959). The cabbage root fly suffers high mortality rates between egg and pupa, mostly at the egg stage (about 80-90%; Hughes and Salter, 1959). Part of this mortality is due to predation by ground dwelling arthropods. The most significant predators are probably carabids (Andersen et al., 1983; Coaker and Williams, 1963) with a strong positive relationship between their body size and their regulation potential (Finch, 1996). Fewer staphylinid species are involved in *D. radicum* natural regulation but *Aleochara* (Gravenhorst) species are particularly efficient egg predators (Andersen et al., 1983). Moreover, they contribute to *D. radicum* natural regulation as parasitoids of the pupae (Hughes and Salter, 1959). Finally the role of spiders has never been reported but their significance as predators of arthropod eggs could be greater than currently expected (Nyffeler et al., 1990).

The aim of the present study was to compare the levels of natural regulation of *D. radicum* by ground dwelling predators under common tillage practices. We tested the following hypotheses: 1) intensive soil tillage significantly decreases the abundance of ground dwelling predators emerging inside the field; 2) The natural regulation provided by these ground dwelling predators decreases with soil tillage intensity and root injuries consequently increase. For this we monitored natural populations of *D. radicum* on broccoli *Brassica oleracea* var. *italica* Plenck (Brassicales: Brassicaceae) in an exclusion experiment performed under various tillage regimes.

2 Materials and methods

2.1 Study site and experimental design

The experiment was carried out in northwestern France during spring 2016, at the INRA experimental station ("Domaine expérimental de la Motte", UE 0787) of Le Rheu (48°07'N, 1°47'W), inside a 6.3 ha field sown with Triticale the previous year. The field was sown with an intercrop (mix Phacelia – buckwheat) in September 2015 and the intercrop was mown and the residues removed in February 2016. In the five years preceding the experiment, the field was under non-inversion tillage practices (i.e. no plowing). An area of 0.6 ha of the study field was split into four blocks separated by 10m bare ground passageways. Each block was split into three plots, each measuring 30m x 15m. Three tillage treatments were allocated to the plots in a randomized complete block design along a gradient of tillage intensity: plowed and harrowed, only harrowed or not tilled at all. Plowing was performed on March 21st using a four-bottom rollover moldboard plow equipped with skim coulters (Grégoire Besson SAS, Sèvremoine, France; depth ~ 25 cm) and harrowing was performed on March 22nd using a rotary harrow (RABE Agri GmbH, Bad Essen, Germany; rotors at 420 rpm, depth ~ 10 cm). On the 20th and 21st of April, the field was planted with broccoli (cv. 'Marathon') plants at the stage of 2-3 true leaves, every 0.50 m in rows 0.75 apart. The field was kept free of weeds at least until early June using herbicides (0.6 L/ha of metazachlore on April 29th followed by 1kg/ha of pyridate on May 20th) and by manual weeding in the experimental subplots on May 24th.

To minimize the homogenizing effect of ground dwelling arthropods migrating from surrounding habitats after soil tillage (Thorbek and Bilde, 2004), we excluded them in all treatments. Two "predator treatments" were implemented per plot. The control "exclusion" treatment, consisted of 25 m² square subplots surrounded by partially buried barriers (40cm below ground and 20cm above ground, Greenborder, Nortene ®). The "removal" treatment consisted of 9 m² square subplots in which 13 pitfall traps were added to remove the ground dwelling arthropods. This resulted in 1.4 traps per m² in "removal" subplots, a density exceeding most of the studies reporting efficient exclusion (e.g. 0.3 traps / m² on average in Chiverton, 1987 and in Holland, 1998), to ensure that we obtained an efficient removal of ground dwelling predators. Although subplot surfaces differed, plant density and plant growing conditions were identical as space within and between rows were the same. The traps were half-filled with water to which a few drops of odorless detergent were added and renewed weekly. Exclusion barriers and pitfall traps were set up immediately after soil tillage, on March 22nd and 23rd, to avoid arthropod immigration into experimental subplots.

2.2 Data collection

2.2.1 Soil tillage and ground dwelling arthropods emergence

Emerging arthropods abundance was monitored using emergence tents (60cm x 60cm x 60cm, MegaView Science Co.). Two tents were set up in each plot, immediately after tillage (between the 22nd and the 29th of March), so that there was a total of eight tents per tillage treatment. The lateral flaps of the emergence traps were buried vertically to a depth of about 10-15cm, as in Hanson et al. (2016), to avoid immigration or emigration of ground dwelling arthropods. Each tent included one pitfall and one aerial collector to collect ground dwelling and climbing or flying arthropods, respectively, emerging inside the tent. Both collectors were half-filled with water containing a few drops of odorless detergent. The trapping period began on March 30th and the traps were collected weekly until June 27th. Carabids, staphylinids and spiders were sorted and counted. The cumulative numbers of carabids, staphylinids and spiders trapped per tent from March 30th to June 27th were used as response variables in data analysis. Carabids were identified at the species level based on Roger et al. (2016) because: i/ their sensibility to soil tillage has been shown to depend on their overwintering stage (Purvis and Fadl, 1996) and ii/ they are thought to contribute much to *D. radicum* natural regulation (Coaker and Williams, 1963). Their overwintering stage (only as adults vs. partly or only as larvae) was assigned following Ribera et al. (1999) and the public database carabids.org (Homburg et al., 2014); and their mean body size was taken from Roger et al. (2016) (Table S1 in Supplementary materials). For each tent, mean carabid body length was calculated using community weighted means (CWM).

2.2.2 Soil tillage and Delia radicum *natural regulation*

Delia radicum egg laying was monitored using felt traps strapped at the base of broccoli stems (Freuler and Fischer, 1983). Four traps were set up in each removal subplot at planting, aligned along a diagonal to have two traps close to the barriers and two in the center of the subplots. They were recorded weekly until June 20th. Eggs laid on the traps were counted, removed and the trap was then replaced around the same plant. Plants used for egg monitoring were not used for any other measurement. The cumulated number of eggs laid per plant throughout the experiment (i.e. from April 21st to June 20th) was used as a response in data analysis.

When eggs had theoretically reached the pupal stage (about 300 degree days between egg laying and pupation; Collier and Finch, 1985; i.e. about 27 days after the end of egg laying in our experiment), soil samples (12cm in diameter, 13.5 ± 0.4cm in depth) were taken from around plant roots (including the root system after cutting the broccolis at the base of plant stem) and the number of pupae was counted after washing the samples through a 1mm x 1mm square mesh sieve. Ten samples were taken in every subplot on June 30th, on randomly selected plants, making a total of 240 samples, i.e. 40 per combination of tillage treatment – predator treatment. The same plants were used to assess root injuries caused by *D. radicum* larvae feeding following the qualitative ordinal notation proposed by Dosdall et al. (1994): 0 = no root damage; 1 = small feeding channels on the root comprising less than 10% of the root surface area; 2 = 11-25%; 3 = 26-50%; 4 = 51-75%; and 5 = 76-100% of the tap root surface area injured. In figures, this qualitative ordinal scale was transformed into a continuous variable representing the fraction of the tap root surface injured (using the median of each class) to facilitate the interpretations.

2.3 Data analysis

We tested the effect of soil tillage on ground dwelling predators abundance and on carabid traits and we tested the effects of soil tillage, predator treatment and of their interaction on the number of *D. radicum* eggs laid, on the number of pupae and on root injuries. All statistical analyses were performed using R software (R core team, 2017). We used generalized linear mixed models with a distribution appropriate to the type of the response variable (functions ‘lmer’, ‘glmer’ or ‘glmer.nb’ of the package ‘lme4’; Bates et al., 2015). Considered distributions were: negative binomial (response variables: cumulative density of carabids, staphylinids and spiders; number of *D. radicum* eggs per plant; number of *D. radicum* pupae per plant), binomial (response variable: proportion of carabids overwintering partly as larvae) and Gaussian (response variable: carabid body length). In every model, the experimental block was added as a random factor. Finally, the qualitative ordinal variable of root injuries was analyzed with a cumulative link mixed model (function ‘clmm’, package ‘ordinal’; Christensen, 2015). The significance of the fixed effects was tested using type II Wald chi-square tests, except for root injuries where a likelihood-ratio chi-square test was used (function ‘Anova’, package ‘car’; Fox and Weisberg, 2011 and package ‘RVAideMemoire’; Hervé, 2017). When the effect of tillage treatment was significant, pairwise comparisons of the estimated marginal means of each treatment were performed using the Tukey method (function ‘emmeans’ from the ‘emmeans’ package; Lenth, 2017).

3 Results

3.1 Soil tillage and ground dwelling arthropods emergence

Between March 30th and June 27th, we trapped 380 spiders, 5171 staphylinids and 1069 carabids in the emergence tents (see Table S2 and Figure S1 in Supplementary materials for details on species composition and emergence dynamic). The cumulative number of carabid beetles and the cumulative number of spiders trapped per tent did not differ between tillage treatments (Table 1) but tillage treatment had a significant effect on the number of staphylinids, which was significantly lower in the “no-tillage” treatment than in the other two (Table 1). There was no effect of tillage treatment on the CWM carabid body length but the proportion of carabids overwintering at least partly as larvae was significantly lower in the tents set up in the “only harrowed” treatment than in the two others (Table 1). *Trechus* gr. *quadristriatus* (85.9% ± 1.4%) was highly dominant in this group of carabid species that overwintered at least partly as larvae.

3.2 Soil tillage and Delia radicum *natural regulation*

The number of eggs laid per plant did not differ significantly between tillage treatments ($\chi^2 = 4.52$, df = 2, P = 0.104). We collected significantly more pupae in the “removal” than in the “exclusion” treatment ($\chi^2 = 6.26$, df = 1, P = 0.012; Figure 1A). There was no significant effect of tillage treatment on the number of collected pupae ($\chi^2 = 1.97$, df = 2, P = 0.372) and the interaction between the removal and tillage treatments was not significant ($\chi^2 = 0.81$, df = 2, P = 0.667). The number of pupae was reduced on average by 22.7% in the presence of ground-dwelling predators.

D. radicum larvae inflicted significantly more root injuries in the “removal” than the “exclusion” treatment ($\chi^2 = 11.43$, df = 1, P < 0.001; Figure 1B) but there was again no significant difference among soil tillage treatments ($\chi^2 = 0.73$, df = 2, P = 0.693) and no interaction between these two factors ($\chi^2 = 3.51$, df = 2, P = 0.173).

4 Discussion

Arthropod emergence in the three tillage treatments tested did not fit the expected gradient of disturbance. Our results are therefore not in agreement with the general consensus that ground dwelling arthropod abundance decreases when soil tillage intensity increases (Rusch et al., 2017; Wardle, 1995). However, most studies published to date did not distinguish between the direct and indirect effects of soil tillage. The discrepancy between our results and previous reports may then come from the removal of indirect effects in our experimental setup. Using similar methods on the same taxa, Thorbek and Bilde (2004) also found that the direct effects of soil tillage were minimal. Nevertheless, among carabids we showed that the proportion of species overwintering at least partly as larvae was lowest when plots had been only harrowed. This suggests that overwintering larvae are more sensitive than adults to the rotary harrow, but not if the field is previously plowed. The higher sensibility of larvae had already been pointed out in carabids (Purvis and Fadl, 1996) but our results further indicate that the direct effects of soil tillage probably depend on the sequence of applied practices: the negative effect of harrowing was suppressed when applied after plowing. The deepest tillage operation (i.e. often plowing) is thus not necessarily the most injuring and could even have a protective effect on overwintering larvae against more damaging practices. This is in line with the fact that some overwintering larvae live at a shallow depth, as shown for *Pseudoophonus rufipes* for instance (10-15cm in Hartke et al., 1998), and thus could have been buried by plowing (Roger-Estrade et al., 2001) and protected from the harrow. Finally, it should be noted that some effects of tillage such as modified soil structure (Bronick and Lal, 2005) and modified soil organic matter distribution in the tilled horizon (Balesdent et al., 2000) are not immediate. They may have consequences on microorganisms (Bronick and Lal,

2005) and on higher-order trophic levels (such as the predators monitored here) in the long term that we could not assess due to the short duration of tillage treatments differentiation. To date the response, over several years, of ground dwelling predators following tillage changes remains largely unexplored (but see Wardle et al., 1999).

Regardless of tillage practice, we found that the number of pupae per plant and the intensity of root injuries were reduced in the presence of locally emerging ground dwelling predators. On the other hand, the number of pests suppressed or the level of root injuries did not differ between tillage treatments. The ground dwelling predators that overwintered inside the field therefore provided the same level of regulation in every tillage condition. This is consistent with the limited effect of soil tillage on the emergence of the ground dwelling arthropods. Especially, soil tillage had no effect on two characteristics of carabid communities which potentially determine their predation potential: i/ body size, which is a key factor in prey – predator relationships in general (Brose et al., 2006) and in this biological system in particular (Finch, 1996); and ii/ their abundance, especially at the beginning of egg laying, which is probably the appropriate time for *D. radicum* regulation (Mesmin et al., 2019). Conversely, the two features of ground dwelling arthropods emergence that were impacted by soil tillage were unlikely to lead to different levels of predation. First, the observed effect on overwintering traits of carabids was related to the massive emergence of *T.* gr. *quadristriatus*, a species that emerged too late to contribute to *D. radicum* egg predation. Secondly, although emerging staphylinids were not determined to species, there were very probably few *Aleochara* among them as only 63 *D. radicum* pupae out of 1713 were parasitized by *Aleochara* spp. (results not shown), a weak abundance that is usual in the region studied (e.g. Lamy et al., 2016). Other potential *D. radicum* predators may also have rapidly dispersed by air after emergence as most staphylinids have good flying abilities (Levesque and Levesque, 1995).

To conclude, soil tillage before crop establishment did not have a significant impact on the natural regulation of *D. radicum* in spring broccoli. This finding is consistent with the fact that the ground dwelling predators that are likely to provide the service in spring did not suffer from soil tillage. However, we showed that shallow tillage affected the species that overwinter partly as larvae, suggesting that the natural regulation services these species provide later in summer and autumn crops could be weakened. Furthermore, the harmlessness of plowing vs. the deleterious effect of harrowing on these taxa raises the question of the relative impact, on ground dwelling predators, of one deep soil disturbance vs. the multiple shallow disruptions that can be necessary to control weeds in no-plow systems.

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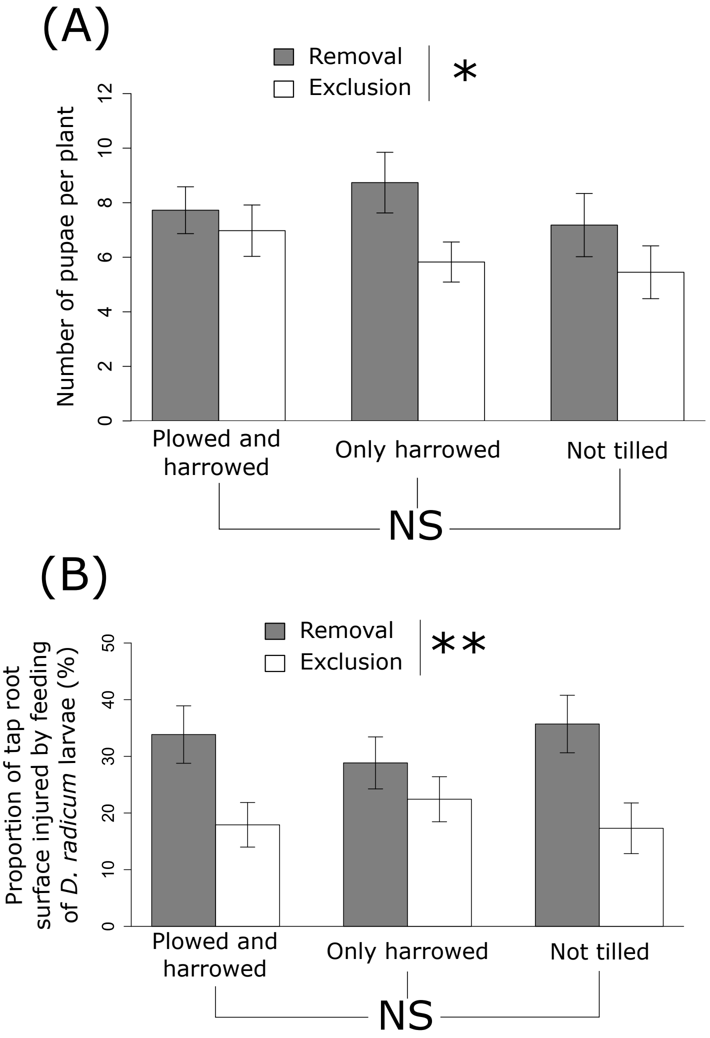
Table 1: Values and significance of Type-II Wald chi square tests performed on the cumulative number of carabids, spiders and staphylinids trapped per tent from March 30th to June 27th and on two functional traits of carabids trapped during this period: the proportion of each overwintering stage and the community weighted mean body length. The back-transformed estimated marginal means (± SE) are given for each tillage treatment (PH: plowed and harrowed, OH: only harrowed, Ø: not tilled) and the results of pairwise comparisons are shown when chi square tests were significant: tillage treatments denoted with different letters are significantly different ($p < 0.05$).

Figure 1: Mean number (± SE) of pupae collected per plant at harvest (A) and mean proportion (± SE) of the tap root surface attacked by *D. radicum* larvae (B) in all tillage treatments. Grey bars indicate the removal treatment and white bars the exclusion treatment. The asterisks and “NS” indicate the significance of differences between treatments (**<0.010<*<0.050<NS).


(A)
Removal
Exclusion
*
Number of pupae per plant
0
2
4
6
8
10
12
Plowed and harrowed
Only harrowed
Not tilled
NS
(B)
Removal
Exclusion
**
Proportion of tap root surface injured by feeding of *D. radicum* larvae (%)
0
10
20
30
40
50
Plowed and harrowed
Only harrowed
Not tilled
NS

Response variable		χ^2	df	P	Tillage	Estimate (± SE) and group	
Abundance	Carabids	5.68	2	0.058	PH	59.9 ± 9.4	-
					OH	37.1 ± 6.3	-
					Ø	44.6 ± 7.6	-
	Spiders	5.67	2	0.059	PH	12.5 ± 2.4	-
					OH	16.0 ± 3.2	-
					Ø	24.0 ± 4.7	-
	Staphylinids	19.87	2	< 0.001	PH	259.4 ± 24.7	a
					OH	268.7 ± 26.8	a
					Ø	168.1 ± 17.2	b
Functional traits of carabids	Proportion of carabids overwintering only as adults	44.33	2	< 0.001	PH	0.3 ± 0.1	a
					OH	0.7 ± 0.1	b
					Ø	0.4 ± 0.1	a
	Body length	2.33	2	0.311	PH	4.1 ± 0.2	-
					OH	4.0 ± 0.2	-
					Ø	4.4 ± 0.2	-