Effects of reduced water availability and insecticide on damage caused by cabbage root fly larvae

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INTRODUCTION

The incidence of horticultural pests in combination with increased frequency of extreme weather events, like summer drought, can compromise crop production in Nordic agricultural systems during the already short summer growing season. In particular, rootfeeding insects can severely damage plants when combined with water deficits because root herbivores reduce plant water and nutrient uptake (Zvereva & Kozlov 2012).

The cabbage root fly Delia radicum (L.) (Diptera: Anthomyiidae) is a major pest of cauliflower Brassica oleracea (L.) (Brassicales: Brassicaceae) crops in Iceland (Halldorsson 1989). Its larvae feed on roots of brassica plants, initially on root hairs and then by tunnelling into the taproot. Cabbage root flies overwinter as pupae in diapause in the soil around brassica plant roots (Bažok et al. 2012). In Iceland, adults emerge in June and females lay eggs between late June and early July (Halldorsson 1989). Eggs are laid on the soil surface around the base of the stems of brassica plants and are relatively resistant to desiccation (Lepage et al. 2012). In contrast, survival of first-instar larvae is compromised at low levels of soil moisture (Lepage et al. 2012).

The aim of this study was to investigate the potential effects of reduced water supply on damage by cabbage root fly on cauliflower plants using a field experiment in Iceland. Specifically, we assessed the effect of reduced water supply on larval densities and on early-season growth of cauliflower plants. We expected reduced biomass production in plants that were not treated with insecticides; we hypothesized that this effect would be aggravated during periods of reduced water availability because the water deficit imposed by root damage would be stronger when combined with reduced water supply.

METHODS

The field experiment was carried out at the headquarters of the Soil Conservation Service of Iceland in Gunnarsholt, South Iceland $(64^{\circ}05'N; 19^{\circ}50'S)$. The climate is maritime, with average annual temperature $<3.7^{\circ}C$ and annual precipitation >1000 mm (IMO 2017); the summer of 2017 was relatively dry (Figure S1). Soils are Andosols with silty loam texture and weak structure; due to their volcanic origin, these soils have low particle cohesion, high water holding capacity and low bulk density. The experiment was set up close to a field that had had a heavy infestation of cabbage root fly larvae the previous year (2016; GH personal observation).

The experimental field was divided into four blocks, each with four 1x1 m plots 0.5 m

apart (Figure S2). Eight-week old cauliflower seedlings (var. botrytis, cultivar Flamenco) were obtained from a local plant nursery (Flóragarðyrkjustöð, Hveragerði). Four seedlings were planted 30 cm apart in each plot (64 plants total) on 26 June 2017, and fertilized initially with Blákorn (N 12%, P 12.6%, K 14.1%; 25 g/plant). The experimental treatments were randomly assigned to each plot and consisted of the combined effects of reduced water, regular watering and the presence or absence of the insect pest (application of insecticide). The four treatment levels were: reduced water and no insecticide (RN), reduced water and insecticide (RI), watering and no insecticide (WN), and watering and insecticide (WI). Plants in the experimental treatments did not differ in size at the beginning of the experiment (Supplementary materials S3).

The insecticide treatments were applied on 11 July, after the peak in egg laying (Supplementary materials S4). The plants were treated with 50 ml/plant of 0.05% concentrated dimethoate solution (Perfection 400 EC, BASF AG, Germany) around the root collar. Water availability was manipulated by covering half of the plots (hereafter 'reduced water plots') with transparent plastic sheets ~20 cm above the soil surface, with the sides open to secure free air circulation and avoid excessive warming. Plastic sheets excluded precipitation during a 10-day rainy period (17-26 July; precipitation during this period was 23.5 mm, IMO 2017). Watered plots were watered on July 10 (2 l/plot) and July 26, 27, 28 and 30 (4 l/plot); the total watering was therefore 18 l/plot, equivalent to 18 mm of precipitation. The natural precipitation during the experiment (June 26- August 2) was 74.2 mm (IMO 2017). Thus, precipitation received by plots with reduced water supply was 50.7 mm (total precipitation minus precipitation during the period they were covered, 74.2-23.5 mm), comparable to a dry year (Figure S1), and 92.2 mm for watered plots (total precipitation plus additional watering, 74.2+18 mm). An acrylic sheet covered all plots to prevent differential egg-laying by cabbage root flies on plots covered by the plastic sheets. All sheets were removed on July 26.

The cauliflower plants were harvested on 2 August (37 days after planting). Soil was carefully removed from the roots and all larvae found were counted. The fresh and dry weights of plants (including flower heads) were recorded to the nearest mg, using a Sartorius Top-loading Balance (ED62025). Dry weights were recorded after oven-drying at 80°C for 48 h.

The effects of the experimental treatment (reduced water, watering and insecticide application, and their combined effect) on the number of larvae found on cabbage roots and on plant biomass production were analysed using mixed effects models, including block and plot identity as nested random effects. One of the plants died before the experiment started and was not included in the analyses. To assess statistical significance, models with and without the interaction were compared using Chi square values, and non-significant interactions were dropped. Modelling assumptions were checked by visual inspection of the residuals. All statistical analyses were performed in R 3.4.1 (R Development Core Team 2017) using the libraries *lme4* to build mixed effects models and *lmerTest* to calculate Satterthwaite approximations of p-values. Means and standard errors are reported.

RESULTS

In total 648 larvae were found on the roots of harvested plants, feeding on the surface of plant roots. The application of insecticide strongly reduced the number of larvae (an average of 0.13±0.25 (total 2 larvae) were found in RI plots, and none in WI plots). Reduced water plots without insecticide had more larvae (28.25±13.98 larvae per plot; total 452 larvae) than watered plots (12.13±6.23 larvae per plot; total 194 larvae), and there was a marginally interaction significant between reduced water supply and insecticide treatment (Chisquare=4.191, p=0.04; Figure 1a).

The interaction between reduced water and insecticide application was not significant in the model for plant biomass (Chi-square=1.13, p=0.29). Both reduced water (t-value=2.786,

b) Total dry plant biomass

Figure 1. Effect of treatments on: (a) the number of larvae found on roots of harvested plants, and (b) on the total dry plant biomass at harvest. RN: reduced water and no insecticide, RI: reduced water and insecticide, WN: water, no insecticide, WI: water and insecticide. Small case letters indicate significant differences (p<0.1) between treatments. Bars show means with standard errors.



a) Cabbage root fly larvae

p=0.019) and insecticide application (t-value=3.682, p=0.004; Figure 1b) had a positive effect on plant biomass. The mean biomass of plants treated with insecticide was nearly double that of plants not treated with pesticide (26.42 g vs 14.90 g respectively). Plants under reduced water produced more biomass than watered plants (24.98 g vs 16.01 g). Interestingly, the number of larvae found around roots of plants at harvest and plant biomass were not significantly correlated (r=-0.09, p=0.51).

DISCUSSION

Insecticide application effectively reduced the number of larvae on roots of harvested cauliflower plants. Contrary to our expectation, the reduction in biomass production in the presence of larvae (i.e. plants not treated with insecticide) was not aggravated under reduced water availability.

The results of this short study suggest that larvae of cabbage root fly were most numerous around roots of plants exposed to less water. Water stress can enhance the survival of some root-feeding insects (Staley et al. 2007) and can limit the populations of their natural enemies (Grant & Villani 2003). However, the increased abundance of larvae in the reduced water plots found in our study did not translate into a stronger reduction of plant biomass. This



might be because plants were harvested when larval damage was at an early stage and larvae were still feeding superficially on the roots. Due to logistic constraints, the duration of cultivation in the present experiment (37 days) was short compared to other experiments [70 days (Kage et al. 2004), 90-110 days (Bažok et al. 2012)], although root damage by cabbage root fly larvae on plants has been reported as early as 4-5 weeks after planting in the field (Joseph & Martinez 2014) and should be easiest to detect and have the greatest effect at this stage. In Iceland, cauliflower is usually harvested after 2-2.5 months, and larvae are not fully developed until September (Halldorson 1989). The duration of our study might mean that cauliflower plants were not mature at the time of harvesting, and a longer duration might have led to a stronger reduction of plant biomass by cabbage root fly larvae.

The plant biomass was significantly higher in reduced water treatments (Figure 1b), suggesting that plants were not water-stressed. We cannot discount the possibility that the plastic sheet that was supposed to cause water stress unintendedly increased temperatures and enhanced plant growth. These ameliorated growth conditions could explain the lack of interaction between the experimental treatments (reduced water*insecticide). In the presence of larvae (i.e. non-insecticide treated plants) cauliflower plant biomass production was lower but did not differ between watered and reduced water treatments, suggesting that water was not a limiting factor.

Our study showed that both reducing water and insecticide application can significantly affect the density of cabbage root fly larvae on cauliflower roots. The potential combined effect of higher loads of larvae and less mitigation of root damage under water-limited conditions may pose a challenge in plant protection. Increased use of insecticides is costly and can have important environmental consequences, so there is a need for alternative methods to reduce the presence of cabbage root flies. These could involve the use of natural enemies (Messelink & Slooten 2004) or simple physical barriers to egg-laying (Skinner & Finch 1986). In addition, cultural control and prevention methods can be used to reduce infestation by the cabbage root fly, such as crop rotation, delayed planting, or the use of intercrops (Bažok et al. 2012). If the use of insecticides is the only option, optimizing the timing and dosage of the application should consider temporal patterns of abundance of the insect pest specific to each region.

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