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Does gall midge larvae cause pre-dispersal seed mortality and limit cornflower population growth?

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ABSTRACT

Many kinds of pests can reduce seed production. Some directly attack seeds before they are released, and some are hosted by the fruit and impact seed ripening and viability indirectly. Pre-dispersal seed mortality may have strong effects on plant population dynamics and evolution. Our goals were to determine to what extent insect-mediated pre-dispersal seed mortality contributes to population-level declines of cornflower, Centaurea cyanus L. We recorded occurrence and abundance of seed-feeding insects on flower heads in twelve cornflower populations. We measured flower head size, number of disc florets, seed production, and seed viability and germination. Larger flower heads had proportionally fewer healthy seeds. Although we observed no visible damage to the C. cyanus seed, the presence of gall midge (Cecidomyiidae) larvae inside the flower head correlated with four times fewer viable seeds. It seems that gall midges could have a significant impact on ovule fertilization, seed abortion and viability of fully developed cornflower seeds. The higher rate of aborted seeds in the presence of gall midge larvae could have been because the larvae extracted resources from the seeds, or because the larvae repelled pollinators. The viability of apparently healthy seeds was 40% lower in flower heads that contained larvae and/or aborted seed. Insect-mediated pre-dispersal mortality could select against evolution toward larger flower head, and have detrimental consequences on seed number, viability and germination, all of which could limit the spread of C. cyanus populations.

1. Introduction

Seed set of insect-pollinated, self-incompatible species benefits from high pollinator abundance and richness. Pollinator declines in recent decades has reduced plant reproduction (Thomann et al., 2013). This trend is potentially compensated by evolutionary selection for larger floral display that attract more pollinators (Thomann et al., 2013, 2015), but the evolutionary trajectory for flower size is driven by conflicting selection pressures (Celedon-Neghme et al., 2007). One of them is due to other insects like leaf consumers that reduce the health of the whole plant (Munzbergova and Skuhrovec, 2013), pollen beetles that consume pollen grains and even flowers (Larson et al., 2001), and pests that directly attack the seeds before they are released (Skuhrovec et al., 2008; Munzbergova et al., 2015). Pest impacts on seed production that fall into the last category are described under the generic term "pre-dispersal seed predation".

Pre-dispersal seed predation may have strong effects on plant population dynamics by reducing recruitment and lowering population growth (Kolb et al., 2007). However, it is highly variable both spatially and temporally (Kolb et al., 2007; Boieiro et al., 2010). Pre-dispersal seed predation appears to increase with population size, plant density, flower and seed numbers (see examples in Kolb et al., 2007), but this is not always the case (Boieiro et al., 2010). In most of the Asteraceae, it has been shown that pre-dispersal seed predation is positively correlated with flower head size both within and among species (Fenner et al., 2002), but it is still not clear whether it can exert significant selection on plant reproductive strategies in natura. In Centaurea cyanus L. (also Cyanus segetum Hill), the iconic cornflower, it has been recently shown that flower head responded to selection by climate warming and pollinator decline by earlier flowering and larger flower heads with longer receptivity (Thomann et al., 2015). C. cyanus is an arable-dependent, over-wintering weed in cereal and oilseed rape fields. It is already severely affected by agricultural intensification, pollinator decline and habitat fragmentation, and nearly endangered in

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western Europe (Bellanger et al., 2012). As a self-incompatible species with high sensitivity to inbreeding depression (Bellanger et al., 2014, 2015), it is highly dependent on insect visit. Flower heads of *Centaurea* species, including *C. cyanus,* are known to host a few Tephritidae (Diptera) species (Merz, 1994; Koprdova and Martinkova, 2006; Munzbergova et al., 2015) and *Cecidomyiidae* (Skuhravá and Skuhravý, 1993; Munzbergova et al., 2015). Here we study whether pre-dispersal seed predation could contribute to population-level declines in *C. cyanus*, including through interference on fertilization, seed viability and flower head size.

2. Materials and methods

The plants of *C. cyanus* were harvested from 12 sites in the Burgundy region, France (Table 1) from the end of June until the middle of July 2008. Flowering of *C. cyanus* is a continuous process: when sprouting from the rosette, the main stem has leaves and a terminal flower head; secondary branches containing leaves and a terminal flower head are produced at every stem/leaf angle, and so on for successive branching orders. The sampling was done as soon as possible in each location to get the former matured flower heads but in quantity enough for the study, which resulted in the presence of a few flower heads from the main stem while most belong to secondary axes. Such a procedure reinforces the sampling homogeneity since the position of flower heads has effect on pre-disperal predation (Koprdova and Martinkova, 2006). When the last, central disc florets of a flower head finish flowering, the bracteas tighten and the flower head get nearly closed inside the bracteas. Seed get ripe around 12 days later (Darmency et al., 2015), and then the flower head opens and leave almost instantaneously seed shedding by gravity. Sampling earliest ripe seeds provides a snapshot of the initial predation phase and secures comparison among populations. Five complete and ripe flower heads of 20 plants from each population were each put in paper bags and stored under dry room conditions (20 °C, 40% relative humidity): such a sampling procedure was shown to provide sound basis for further statistical and biological interpretation (Skuhrovec et al., 2008; Munzbergova et al., 2015). Four months after collecting, the diameter of the receptacle and length of the bract of each flower head was measured with a digital caliper. The presence and number of well-known pre-dispersal seed predators Tephritids, Cecidomyiidae, Tortricidae, Curculionidae, and some Carabidae (see Skuhrovec et al., 2008; Munzbergova et al., 2015) were recorded. Identification has been confirmed by specialists. Immature stages have been stored in alcohol only when they have been already dead. Cecidomyiidae larvae were dry so that it was necessary to macerate them in 10% KOH solution and wash them to make the

larvae skin brighten so they could be accurately identified. The number of disc florets per flower head (disc florets are the only fertile florets and have one unique ovule each) were counted and classified by whether or not they were fertilized. Then the seeds were examined and classified as previously described (Bellanger et al., 2014): 1) damaged; 2) aborted (apparently undamaged but white and often somewhat flat); 3) or healthy (undamaged, full and bluish).

A sample of 56 flower heads was taken from all the populations by random draw without replacement of the plant in order to test the viability of the purported aborted and healthy seeds. The origin of the seeds was carefully noted (i.e. from attacked or completely healthy flower heads, and from flower heads with a mixture of pure aborted and healthy seeds or with only healthy seeds). They were then deposited on germinating paper in Petri dishes with pure water at 15/25 °C under a 12 h photoperiod for two weeks, and then supplemented by giberellic acid 0.3 g/L for an additional week. The remaining, ungerminated seeds were tested for viability by dissection: the seed was deemed viable if the embryo appeared white, and dead if the embryo was brown or absent. Two-sample t-test was used to compare groups of flower heads of first versus second order stems, and with or without cecidomyiidae larvae. This, Pearson's chi-square test (Chi^2) and linear and non-linear regressions, were done using Systat 10.2 software (2002).

3. Results

3.1. Plants

The number of disc florets per flower head ranged from 1 to 51. In general, it increased linearly with the diameter of the flower head, both when all plants were considered together (y = 7.5 + 2.75x, with x in mm, P < 0.001, R^2 = 0.17; Fig. 1A) and when only the 12 population mean values were included in the regression (y = 4x, P = 0.01, R^2 = 0.47, Fig. 1B). This could suggest that there are both environmental and genetic causes involved here. There was also a trend towards proportionally fewer healthy seeds in larger flower heads, but with a wide dispersion of values (y = 0.7−0.044x, P < 0.001, R^2 = 0.02). This was not found when the regression included only the 12 population means (P = 0.10).

From a total of 1200 flower heads analysed, only 55 were the first head to flower on a plant. These flower heads were not different from those of secondary axes; they had similar diameter, bract length and number of disc florets (24.7 versus 23.0, t-test P = 0.09). However, the proportion of healthy seeds was three times lower in the first flower heads (0.15 versus 0.47, t-test P < 0.001), which corresponds to a higher proportion of unfertilized florets

Table 1
Locations sampled and total number of flower heads with *C. cilicrus* larvae, total number of *C. cilicrus* larvae and other insects found in 100 flower heads. Populations of *C. cyanus* plants are described with WGS84 international coordinates, altitude, field context (with the number in parentheses indicating the width in meters of the field edge where the plants were concentrated, otherwise plants were throughout the field), and apparent population size (dividing into three classes of number of individuals).

Locality	WGS84	Altitude (m)	Crop (width of population, m)	Pop. Size	Nb. flower heads with *C. cilicrus*	Nb. *C. cilicrus*	Nb. other insects
Heuilley sur Saone	47°33/5°45	190	Strip line between rye and mustard	<50	32	110	0
Epagny	47°45/5°06	336	Oilseed rape (5)	>>100	41	249	5
Pagny-la-Ville	47°06/5°18	181	Winter wheat (1)	>>100	31	168	3
Buxerolles	47°81/4°93	399	Organic wheat (1)	>>100	18	81	2
Montigny sur Vingeanne	47°57/5°44	239	Oilseed rape (1)	<50	76	701	2
Thury	47°02/4°32	402	Oilseed rape (5)	50−100	45	200	3
Fleurey sur Ouche	47°19/4°51	333	Winter barley (2)	50−100	33	245	8
St Martin du Mont	47°43/4°79	548	Winter wheat (1)	<50	7	103	0
Chamboeuf	47°23/4°90	474	Winter wheat	<50	9	103	3
Meursauge	47°66/4°63	401	Winter wheat	50−100	29	232	2
Chatel-Gérard	47°/63/4°10	314	Winter wheat	50−100	11	64	11
Perrigny	47°27/5°01	232	Oilseed rape (1)	50−100	35	230	0