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Role of plant phenology in mediating interactions between two biological control agents for spotted knapweed

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ABSTRACT

The role of spotted knapweed phenology on the attack rate of two seed-head insects *Urophora affinis* and *Larinus minutus* was assessed in a series of field studies at four study sites in south-eastern British Columbia, Canada. Slow or later developing knapweed plants had more seed heads that contained only single or multiple *U. affinis* whereas early or faster developing plants had more seed heads containing *L. minutus* alone or in combination with *U. affinis*. *L. minutus* did not distinguish between seed heads with or without *U. affinis* larvae when laying eggs. However, seed heads with multiple *U. affinis* present, produced fewer *L. minutus* adults than expected. The probability of single or multiple *U. affinis* galls being present increased with seed-head diameter but was not affected by seed-head height. Attack by *L. minutus* increased with seed-head diameters >5 mm and was lower at plant heights above 50 cm. These results demonstrate two mechanisms that enable *U. affinis* to successfully coexist with *L. minutus*: differences between the species in their response to the developmental phenology of knapweed heads, and increased survivorship of *U. affinis* in heads with multiple *U. affinis* galls through niche interference competition. These mechanisms provide a possible explanation for the persistence of *U. affinis* populations on spotted knapweed, in spite of high levels of within seed-head mortality that have been observed with increasing *L. minutus* populations.

1. Introduction

Spotted knapweed (*Centaurea stoebe* L. spp. *micranthos* (Gugler) Hayek) and diffuse knapweed (*Centaurea diffusa* Lam.) are two of the oldest targets for biological control in North America. Surveys for knapweed biocontrol agents began in 1961 in Europe (Jordan, 1995) and the first agent released for biological control of knapweeds in Canada was the seed head fly *Urophora affinis* Frfld. in 1970 (Harris, 1980). Forty years later, 12 biological control agents have been introduced in Canada (Bourchier et al., 2002) and the United States (Story et al., 2004) for biological control of knapweeds, with seven agents becoming relatively common. Recently various combinations of these biological control agents have been implicated in the successful biological control of both spotted knapweed (Story et al., 2006, 2008; Knochel and Seastedt, 2010) and diffuse knapweed (Seastedt et al., 2007; Myers et al., 2009).

As the number of established biocontrol agents occupying a common niche such as the seed head have increased, competition and the interactions between the biocontrol agents have been the subjects of considerable research (Berube, 1980; Story et al., 1987; Lang et al., 2000; Denoth et al., 2002; Smith and Mayer, 2005; Crowe and Bourchier, 2006; Seastedt et al., 2007). Of particular interest are potential interactions between *U. affinis* and the seed head weevil *Larinus minutus* Gyll. because both agents have been suggested to be important for the removal of spotted knapweed seed (Story et al., 2008) and *L. minutus* has been implicated to be the single agent responsible for suppression of diffuse knapweed (Seastedt et al., 2007; Myers et al., 2009).

Order of attack is critical for understanding the interaction between the two species. *U. affinis* attacks knapweed heads first in the season, laying its eggs into unopened, immature seed heads (Berube, 1980). Larvae form a hard gall from individual flower ovaries and multiple galls per head (between two and four galls) are common (Harris, 1980). *L. minutus* is univoltine and attacks newly opened knapweed seed heads (Kashefi and Sobhian, 1998) after *U. affinis*. *L. minutus* is commonly found as a single adult in a seed head and is capable of destroying all seeds in a single head (Groppe, 1990). Initial predictions regarding *L. minutus* were that it would be a complementary agent to *U. affinis* because galls of *U. affinis* were hardened prior to weevil attack and could not be harmed (Groppe, 1990). Subsequent field observations indicated that *L. minutus* destroyed, in some cases, large numbers of the fly galls (Lang et al., 2000; Smith and Mayer, 2005; Crowe and

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Bourchier, 2006; Seastedt et al., 2007) but that generally both species were capable of coexistence at knapweed sites.

The objectives of the experiments described in this paper are to: (1) evaluate the role of individual host–plant phenology on the attack rates of both *U. affinis* and *L. minutus*, and (2) to investigate the importance of the sequence of attack and resulting densities of *U. affinis*, for the competitive interaction between *U. affinis* and *L. minutus*.

2. Materials and methods

2.1. Field sites and insect phenology

Spotted knapweed phenology and recruitment for adult *U. affinis* and *L. minutus* were monitored from 5 June to 28 August 2002 at four sites, Heinz Cabin, (lat.: 49.05, long.: −117.41), Seven-Mile Reservoir (49.04,−117.49), Brilliant (49.32,−117.65), and Sanca Creek (49.39,−116.73) in the west Kootenay region of southeastern British Columbia, Canada. All four sites were relatively flat meadows, dominated by spotted knapweed, with established populations of *U. affinis* and *L. minutus* and were located in the Interior Cedar-Hemlock, dry warm or very-dry warm Bio-Geoclimatic zone (http://www.for.gov.bc.ca/hre/becweb/index.html). In 2006, seed from twenty spotted knapweed plants collected from Heinz Cabin, Brilliant and Sanca Creek were propagated and the identified as tetraploid *Centaurea stoebe* L. subsp. *micranthos* using methods outlined in Mráz et al. (2011). The Seven Mile Reservoir site could not be sampled in 2006 as it was destroyed during an upgrade to a dam in the area. In 2002 at all sites, sampling for adult flies and weevils was conducted repeatedly along five parallel transects that were located equidistant apart and covered the length of the knapweed patch. The size of the sampling patches ranged between 400 m^2 and 1500 m^2. Transect length, and the distance between transects, varied between sites in proportion to the dimensions of the patch. Adult insect recruitment was monitored between 10:00 am and 3:00 pm on a weekly basis, by collecting and counting adults with a sweep net at six sampling points, equidistant apart on each transect (between 5 and 10 m apart depending on site), for a total of 30 sampling points per site per week. At each sampling point, the sampler would make two sweeps, having a length of approximately 1 m, through the spotted knapweed canopy with a 30 cm diameter sweep net. Captured adults of both insect species were identified, counted, and released back into the patch.

2.2. Knapweed phenology and insect response

Spotted knapweed produces flowers indeterminately; monitoring of seed-head development commenced the week of 18 June, once the plants had bolted and bud development started, and continued until 28 August 2002. Knapweed seed head development was assessed each week on the same six plants, selected at equidistant intervals along each of five transects, located between each of the insect sweep transects, for a total of 30 plants at each site. Each week, seed heads on the selected plants were classified into one of six distinct development stages (similar to examples from Turgeon, 1986; Crawley and Akhteruzzaman, 1988; Floate, 1993; Connett et al., 2001): (1) Bud Initiation, (2) Bud Elongation, (3) Pre-flowering, (4) Flowering, (5) Wilted Florets, and (6) Seed Set. This phenology data permitted estimation of both average development and the proportion of seed heads at any given stage, each week of the study. Mean head-development for each of the 30 plants examined was calculated using the six categories of head development for each sampling week. These values were used as a measure of individual plant phenology at the both the plant and patch (site) levels. The week of 24 July was selected to assess the relative phenology of sample plants because this was the period of overlap between the end of the *U. affinis* flight period and the start of *L. minutus* oviposition at study sites. For the week of 24 July, the mean head development for each plant was calculated and plants from all four sites were sorted from slowest to fastest developing. Each plant was then classified into the <33% (slow), 34–66% (medium) and >67% (fast) quantile for development across sites.

During the week of 26 August 2002, when the adult phase of populations of both insect species was complete, all plants selected for phenological monitoring were harvested from each of the four sites. All of the seed heads on each of the plants were dissected in the laboratory, and eggs, larvae, and pupae of each species were identified and counted.

For all dissections of seed heads, within each plant, seed heads were classified (0) having no insects; (1) single *U. affinis* gall or attack; (2) more than one *U. affinis* gall or attack; (3) single *U. affinis* with *L. minutus* present; (4) more than one *U. affinis* with *L. minutus* present and (5) only *L. minutus* present. The relationship between proportion of seed heads in the categories of interest, and the predictors: (1) plant phenology (slow, medium or fast) and (2) site was assessed using generalized linear models (GLM) in R 2.10.1 (R Development Core Team, 2009) with quasibinomial errors to correct for overdispersion (Crawley, 2002). The head categories used as response variables were: (1) the proportion of heads attacked by *U. affinis* only (categories 1 and 2, including single and multiple galls) and (2) the proportion of heads attacked by *L. minutes* (categories 3, 4, 5). These head categories were of interest as they represented the sequence of attack of the knapweed heads during the season; first *U. affinis* followed by *L. minutus*. For all analyses, full models (main effects plus the two way interaction) were fitted initially. The significance of each term was then assessed by examining the estimated *P* value of the likelihood ratio statistic, associated with the change in deviance in the model, obtained by the removal of each term (Crawley, 2002). An *F*-test, accounting for overdispersion, was used to assess deletion of terms (Crawley, 2002) and the simplest significant model that explained the most deviance was then chosen.

2.3. Phenology of insect attack: Heinz Cabin

The seasonal pattern of insect attack on spotted knapweed was assessed in detail using additional weekly plant sampling and concurrent head dissections at the Heinz Cabin site. Attack rates of both species of biocontrol agents were estimated by randomly collecting 15 spotted knapweed plants from within the site on a weekly basis from 10 June to 28 August and a final sample on 19 October 2002. The diameter of each seed head at the widest point and its height on the plant were recorded prior to dissection. All seed heads on each of plant were dissected in the laboratory, and eggs, larvae, and pupae of each species, both alive and dead, including damaged galls were identified and counted. Insect species present in each seed head were classified into categories 0–5 as described above. Weekly dissections enabled comparison of *L. minutus* oviposition choice between seed heads with differing numbers of *U. affinis* (0, 1, 2, or 3+ flies per head) and to compare subsequent *L. minutus* survival across seed heads categorized by the number of *U. affinis* per head. For *L. minutus* oviposition choice, the distribution of *U. affinis* (0, 1, 2, or 3+ flies per head) for seed heads with weevil eggs (for the period when most weevil eggs were found, between 21 July and 10 August) was compared to the distribution of *U. affinis* per head for all seed heads dissected during this period. If females were choosing heads without *U. affinis* then these distributions would be significantly different using a χ^2 test. For *L. minutus* survivorship from the 19 October dissections, the distribution of *U. affinis* per seed head for heads from which adult *L. minutus* emerged was compared to the distribution