

Two Shoot-Miners, *Ceutorhynchus alliariae* and *Ceutorhynchus roberti*, Sharing the Same Fundamental Niche on Garlic Mustard

ESTHER GERBER,^{1,2} HARIET L. HINZ,¹ BERND BLOSSEY,³ AND SVEN BACHER⁴

Environ. Entomol. 41(5): 1086–1096 (2012); DOI: <http://dx.doi.org/10.1603/EN11335>

ABSTRACT A combination of observational and experimental methods, in both the laboratory and field, were used to assess niche partitioning between *Ceutorhynchus alliariae* Brisout and *C. roberti* Gyllenhal (Coleoptera: Curculionidae), two coexisting shoot-boring weevils on garlic mustard, *Alliaria petiolata* (M. Bieb.) Cavara and Grande (Brassicaceae). We compared their morphology, oviposition behavior, larval development, distribution, abundance, and attack rates in their sympatric range, and of *C. alliariae* when found alone and in sympatry with *C. roberti*. Results indicate only very small differences in the fundamental niches of the two species. Comparison of *C. alliariae* in the range it occurs alone with the range where it co-occurs with *C. roberti* revealed some evidence for competition between the two species, i.e., attack levels of *C. alliariae* were reduced in areas where it co-occurred with *C. roberti*. However, the study showed no character displacement in regard to adult size or shoot choice of *C. alliariae* and we found no indication for superiority of either of the two species. Clearly, manipulative experiments would be necessary to unambiguously test for competition between the two species. Our results, based on a subset of niche dimensions known to be important in other systems, suggest that *C. alliariae* and *C. roberti* may present one of the rare cases, in which niche differentiation is not the main mechanism underlying coexistence.

KEY WORDS *Ceutorhynchus alliariae*, *Ceutorhynchus roberti*, niche partitioning, niche overlap, competitive coexistence

The concept of ecological niche is central to community ecology to understand species interactions and ultimately species coexistence. A species niche can be understood as a multidimensional hypervolume within which the species can maintain a viable population (Hutchings 1957). This abstract concept embraces all requirements (niche dimensions) of an organism, which are infinite in number and include abiotic environmental factors, such as temperature, humidity, or light intensity, as well as biotic factors (i.e., the resources the species requires). The largest ecological niche that a species can occupy is called its fundamental niche (Begon et al. 1990). However biological constraints (e.g., the presence of predators or competitors) restrict organisms to their realized niche, in general smaller than the fundamental niche. Niche requirements of coexisting species can differ in all dimensions, but most frequently, differences in habitat and food type requirement are observed (Schoener 1974). Specialized herbivores feed and develop on a restricted number of host plant species, and therefore are obliged to share an important component of their niche. They frequently use different parts

of the plant (e.g., Wilson et al. 1990, Gianoli 2000, Blossey et al. 2001, Tóth and Cagán 2005, Cripps et al. 2006) and in cases where they share the same plant structures, the larvae tend to develop in different parts within this structure or vary in their temporal usage (Zwölfer 1980, Vayssières and Wapshere 1983, West 1985, Bacher 1993, Forrester 1993, Tschamke 1993, Hinz and Müller-Schärer 2000). Traditionally, high niche overlap has been associated with interspecific competition, and niche partitioning is seen as a way to avoid competition and to explain the coexistence of similar species (Hardin 1960). However, the general premise that two species sharing the same niche dimensions cannot coexist is being challenged. For instance, species coexistence on a metapopulation level can be based on a competition-colonization trade off with one species being the superior competitor, whereas the other species is a better colonizer (Wilson et al. 1999, Amarasekare and Nisbet 2001, Harrison et al. 2001). Coexistence also has been shown to be promoted through aggregated distributions of the superior competitor (Shorrocks et al. 1979, Atkinson and Shorrocks 1981, Hanksi 1981). According to this model, coexistence of competing species on a patchy, ephemeral resource is facilitated by independently aggregated spatial distributions that reduce interspecific interactions relative to intraspecific interactions. Through aggregation of the superior competitor, patches with few or no individuals arise, which can

¹ CABI, Rue des Grillons 1, 2800 Delémont, Switzerland.

² Corresponding author, e-mail: e.gerber@cabi.org.

³ Department of Natural Resources, 202 Fernow Hall, Cornell University, Ithaca, NY 14853.

⁴ University of Fribourg, Department of Biology, Unit of Ecology & Evolution, Chemin du Musée 10, 1700 Fribourg, Switzerland.

serve as refuge for the weaker competitor. *Ceutorhynchus alliariae* Brisout and *C. roberti* Gyllenhal (Coleoptera: Curculionidae) are two congeneric shoot-borers on garlic mustard, *Alliaria petiolata* (M. Bieb.) Cavara and Grande. The two species occur both geographically isolated from (allopatric) and associated with each other (sympatric) in Europe (Fowler 1891, Hoffmann 1954, Dieckmann 1972, Heijerman 1993, Strejček 1996, Schott 2000, Rheinheimer and Hassler 2010). Adults can be distinguished using size (*C. roberti* is slightly larger), by tarsal color (*C. alliariae* has red, *C. roberti* has black tarsi), and aedeagus morphology (Dieckmann 1972, Strejček 1996). In the past, *C. alliariae* was regarded by taxonomists as a subspecies of *C. roberti* based on morphological characteristics (Hoffmann 1954). Today, their species status generally is accepted by taxonomists (Dieckmann 1972), supported by different aedeagus shapes and sympatric populations (Strejček 1969). However, no crossbreeding experiments were conducted so far to confirm their species status. According to literature records, both species appear on garlic mustard from mid-April onwards and lay eggs into the developing shoots of their host plant. Larvae mine shoots and leave the shoot to pupate in the soil. Emergence of new generation adults for *C. alliariae* is recorded in July and August (Dieckmann 1972). Both species overwinter in the leaf litter. *Ceutorhynchus alliariae* has been reported to prefer shady and *C. roberti*, open, sunny habitats (Dieckmann 1972). Both species have been recorded to be attacked by a solitary ectoparasitoid, *Stenomalina gracilis* (Walker) Pteromalidae (Kuhlmann et al. 2006, Yu 2009). No other information is available on these two species in the literature.

Their host plant, garlic mustard, is an obligate cool-season biennial herb that prefers humid, shady habitats such as forest margins, but it also occurs in more xeric and more exposed locations (Hegi 1986). Seedlings emerge in early spring and form rosettes that overwinter. Plants typically produce 1–12 shoots (up to 1.5 m tall) in spring of the second year, set seed by midsummer, and die. The native range of garlic mustard includes much of Europe (Tutin 1964) but the species also has invaded temperate North America, where it is the target of a biological control program (Blossey et al. 2001).

A subset of fundamental and realized niche dimensions known to be important in other systems was explored for *C. alliariae* and *C. roberti*. We first established whether the two taxa are indeed two reproductively isolated species (using the species definition by Mayr 1942), by conducting a crossbreeding experiment. A combination of observational and experimental methods, in both the laboratory and field, were used to assess niche partitioning of *C. roberti* and *C. alliariae*. We compared their egg, larval, and adult morphology and measured distribution, abundance, host plant attack rate, and parasitism of both species at a regional and local level in the sympatric range (where both species occur) as well as in the range where only *C. alliariae* occurs. In addition, experiments to examine their oviposition behavior, larval

development, and adult phenology were conducted to determine temporal and spatial niche overlap.

Materials and Methods

All experiments were conducted in the native range of both weevil species at CABI in Delémont, Switzerland (47° 21' N, 7° 22' E), from 1998 to 2002.

Crossbreeding. To see whether *C. alliariae* and *C. roberti* would interbreed and produce viable offspring, two methods were used:

1) Newly emerged, unmated adults were transferred onto potted gauze-covered rosettes of garlic mustard in the summer of 1999 and overwintered outdoors. Female *C. alliariae* were overwintered with *C. roberti* males (henceforth called *C. alliariae* mixed-species) and *C. roberti* females with *C. alliariae* males (henceforth called *C. roberti* mixed-species). On 12 March 2000, we established 15 (*C. alliariae*) and 12 (*C. roberti*) mixed species pairs (i.e., female–male pairs) in Perspex cylinders (16 cm in height, 11 cm in diameter) kept in an outdoor shelter at ambient temperatures. Weevils were provided with fresh plant material that was replaced every 2–5 d, and shoots were dissected under a dissecting microscope to record the number of eggs until oviposition ceased. Eggs were placed on moist filter paper in petri dishes, kept at room temperature, and hatching larvae were counted and removed daily. For comparison, 10 single species pairs per species were established (see below, oviposition).

2) In the summer of 2000, newly emerged unmated adults from the rearing colony were transferred as mixed species pairs (10 replicates) onto gauze-covered rosettes and kept in a garden bed over winter. Single species pairs (five replicates per species) were established identically to verify that experimental conditions permitted normal weevil development. Elytrae of released adults were marked with a spot of white nail varnish to distinguish between released weevils and their offspring. All plants were thoroughly searched for resident weevils before the experiment started. As a control to test how successfully resident weevils were removed, five plants were kept under gauze without adding weevils. Marked weevils were retrieved on 14 May 2001, and on 2 July all plants were dissected under a dissecting microscope. Pots where plants showed larval feeding were kept covered with gauze and checked regularly for emerging weevils.

Morphology. We measured length of the elytra under a dissecting microscope fitted with a micrometer of 50 *C. roberti* (25 females, 25 males) and 52 *C. alliariae* (21 from the sympatric range (i.e., eight females and 13 males, and 31 from the range where it occurs alone, i.e., 18 females and 13 males). Males can be distinguished from females by the presence of an indentation on their ventral abdomen (E.G. and H.L.H., unpublished data). Size differences were analyzed using a two way analysis of variance (ANOVA) with species and sex as factors. We also measured the size of eggs and larval head capsule diameters obtained when single species were reared on plants in a com-

mon garden (see below) to compare the two species, but also to find out how many larval instars exist.

Oviposition. Single pairs of *C. alliariae* or *C. roberti*, respectively, were placed into Perspex cylinders ($n = 10$ replicates per species) on 12 March 2000. Tests were identical in design and timing to crossbreeding experiments described above. Adults still alive after their first oviposition period were overwintered on gauze-covered garlic mustard rosettes. We repeated the oviposition experiment in spring 2001 with 10 pairs per species of surviving adults.

On 13 April 2001, pairs of *C. alliariae* or *C. roberti*, respectively, were released onto bolting garlic mustard plants to test for potential oviposition preferences for certain shoot parts. Because oviposition behavior might depend on oviposition pressure (i.e., female density, different densities (1, 2, and 8 pairs) were used ($n = 5$ replicates/species/treatment).

Adults were removed on 25 April 2001 and shoots were cut at ground level. Each shoot was measured and divided into 10 equally long segments, and the number and location of each egg found during shoot dissections under a dissecting microscope were recorded. The spatial distribution of eggs on plants was analyzed separately for each species with two way ANOVAs by using release density and shoot segment as factors. Because no eggs were laid into the lowest and only one egg by *C. roberti* into the second lowest segment, these were excluded from the analysis to meet assumptions underlying ANOVA.

Development and Spatial Distribution of Larvae. To check for potential differences in phenology and distribution of larvae within plant shoots between the two species, potted, gauze-covered garlic mustard plants were infested with individual pairs of *C. alliariae* (15 replicates) or *C. roberti* (16 replicates) from 1 to 8 April 1999. One or two randomly selected plants were dissected every 2 wk until 27 May 1999. Developmental stage and the number and location of each instar were recorded.

Field observations have shown that both species can also lay eggs into large petioles of rosettes (E. G. and H.L.H., unpublished data). To investigate potential differences in the ability of *C. alliariae* and *C. roberti* larvae to complete development in rosette petioles, single pairs of each species were placed on gauze-covered rosettes, with petioles >15 cm in length, for 10–11 d in May 2002 ($n = 10$ /species). On 23 July and 12 August 2002, all rosettes were searched for emerged adults and then dissected for signs of attack.

Distribution, Abundance, and Adult Phenology. We used a combination of literature searches, personal communications with European taxonomists, and targeted field surveys to assess distribution and abundance of *C. alliariae* and *C. roberti*. Between mid-February and mid-November in the years 1998–2002, insects were sampled at 46 sites in the sympatric range, i.e., Switzerland ($n = 42$) and southern and southeastern Germany ($n = 4$), and at 32 sites in the range where only *C. alliariae* occurs, i.e., northeastern Germany ($n = 30$) and in eastern Austria ($n = 2$). All captured weevils were identified by tarsal color at the

laboratory. Data from field sites visited repeatedly (2–18 times) were pooled. Each site in the sympatric range in Switzerland was categorized according to light conditions (shady: plants growing under tree cover; intermediate: plants growing at the edge of a forest, trees, or a hedge; sunny: plants growing in open, sun exposed habitats). The influence of light condition on species composition and the proportion of each species in field sites (percentage of frequency) were tested using paired sample *t*-tests. Seven field sites with abundant weevil populations in the sympatric range were selected and repeatedly recorded from 2000 to 2002. Changes in the proportion of *C. roberti* at field sites sampled over these 3 yr were analyzed with repeated measures of ANOVA.

To assess attack levels in the sympatric range ($n = 10$ sites) and in the range where only *C. alliariae* occurs ($n = 6$ sites), as well as co-occurrence of larvae of the two weevil species in individual shoots in the sympatric range, at least 12 bolting plants were collected randomly along transects, crossing the site at its largest width. Collections were made just before the moment when most mature larvae would leave the plant to pupate in the soil. Plants were returned to the laboratory and the length, basal diameter, and number of larvae and exit holes were recorded for each shoot. Because larvae of the two species cannot be distinguished, we reared them through to adulthood. Mature larvae were transferred individually into plastic vials (21 mm in diameter, 65 mm in height) filled two-thirds with sifted soil for pupation. Vials were kept in an underground insectary (i.e., a shelter dug underground, providing similar conditions for insect larvae as in soil), and checked every second day for emergence of adults. Based on these emergence data, we compared pupation length and the size of shoots from which the two species were successfully reared. We further tested whether distributions of *C. alliariae* and *C. roberti* larvae on a shoot level were independent of each other in sympatric sites by using a χ^2 test.

For each field site, we calculated attack rates (percentage of attacked shoots) and an average attack level (i.e., number of larvae per attacked shoot). In the sympatric range these attack levels represent a mixture of both species. To compare attack levels for *C. alliariae* in the sympatric range and the range where it occurs alone, the proportion of larvae attributable to *C. alliariae* in the sympatric range was estimated indirectly using the proportion of *C. alliariae* adults collected, because larvae of the two species cannot be differentiated (see below). We had found that the proportion of *C. alliariae* adults collected at a field site is closely correlated with the proportion of *C. alliariae* adults reared from larvae collected at the same field site (Spearman's rank correlation: $r^2 = 0.82$, $n = 9$; $P = 0.007$).

To investigate adult phenology of both species, newly emerged adults of each species were kept in transparent plastic cylinders (11 cm in diameter, 15 cm in height) and provided with cut shoots or leaves of garlic mustard, depending on plant phenology. Food was changed regularly and feeding activity (i.e., the

Table 1. Oviposition and fertility of *C. alliariae* and *C. roberti* in single and mixed species combinations and during their first and second oviposition period

Combination	Species	No. of eggs per female (mean ± SE)		Oviposition period (mean ± SE)		% eggs eclosed	
		First	Second	First	Second	First	Second
Single	<i>C. alliariae</i>	98.5 ± 8.1 a (n = 10)	47.7 ± 8.9 a (n = 7)	54.5 ± 3.1 a (n = 10)	59.9 ± 3.6 a (n = 7)	68 (n = 122)	47.3 (n = 38)
	<i>C. roberti</i>	89.1 ± 7.5 a (n = 10)	36.7 ± 12.0 a (n = 10)	47.8 ± 2.2 a (n = 10)	37.0 ± 7.7 b (n = 10)	60 (n = 114)	59.2 (n = 54)
Mixed	<i>C. alliariae</i>	9.4 ± 3.7 (n = 8)	—	17.6 ± 5.1 (n = 8)	—	3.8 (n = 41)	—
	<i>C. roberti</i>	33.7 ± 15.3 (n = 7)	—	27.3 ± 8.4 (n = 7)	—	0.6 (n = 152)	—

Statistical comparisons were made between *C. alliariae* and *C. roberti* single species pairs by using independent sample *t*-tests. Means followed by the same letter are not significantly different; no statistical tests were conducted for percentage of eggs eclosed. —, not investigated.

presence of feeding holes) recorded until September. To estimate overwintering survival, weevils were kept outside on gauze-covered garlic mustard rosettes (1–10 pairs per rosette) and survival rates (percentages) were recorded the next spring.

Parasitism. We recorded the stage and number of weevil larvae parasitized by ectoparasitoids in each dissected shoot and transferred attacked hosts with the parasitoid larvae attached into petri dishes lined with moist filter paper for pupation and adult parasitoid emergence. Vials kept for pupation of weevils (see above paragraph) that failed to produce adults were checked for presence of endoparasitoid cocoons. All cocoons encountered were left in the vials until the next spring to collect emerging adult endoparasitoids. All parasitoids were forwarded to a specialist for identification.

Ectoparasitoids were found on second- and third-instar larvae only. Therefore, we calculated parasitism rates based on the total number of second- and third-instar larvae found upon dissection.

No information on the larval stages attacked by the solitary, koinobiontic ichneumonid endoparasitoid recorded (see Parasitism section below) is available from the literature. Because of observations of this species early in the season we assume that all larval stages can be attacked and we therefore calculated parasitism rates as the number of parasitoid cocoons divided by the number of all larval instars found upon dissection.

Results

Crossbreeding. In the first set up, all females in single-species pairs laid eggs, whereas for mixed species pairs, eight of 15 *C. alliariae* females and seven of 12 *C. roberti* females laid eggs (see Oviposition section below). The number of eggs laid was strongly reduced for mixed species pairs (Table 1; independent sample *t*-test: *C. alliariae*: *t* = 10.372, *df* = 16, *P* < 0.001; *C. roberti*: *t* = 3.714, *df* = 15, *P* = 0.002). In contrast to single-species pairs, only a small percentage of eggs of mixed-species pairs were fertile and eclosed (Table 1).

In the second approach to check crossbreeding, we found no attack on plants infested with *C. roberti*

mixed-species combinations, and only one out of 10 plants onto which *C. alliariae* mixed-species combinations had been released was attacked. In contrast, all plants infested with single-species pairs of *C. roberti* and all but one plant infested with *C. alliariae* were attacked. In addition, one control plant (no weevils released) showed mining damage, indicating that some contamination from weevils naturally occurring at the Center’s garden might have been present and could explain offspring recorded from the one *C. alliariae* mixed-species plant. The two weevils that emerged from this plant showed the morphological characteristics of *C. alliariae*.

Morphology. Adult *C. roberti* were larger than adult *C. alliariae*, and in both species females were larger than males (species: *C. alliariae*: 1.86 ± 0.02 mm; *C. roberti*: 2.08 ± 0.2; *F*_{1,98} = 112.97, *P* < 0.001; sex: females: 2.02 ± 0.02 mm; males: 1.92 ± 0.2; *F*_{1,98} = 22.99, *P* < 0.001). However, size was variable (range: *C. alliariae*: 1.69–2.13 mm, *C. roberti*: 1.72–2.29 mm) and cannot be used reliably to separate the two species. The presence of *C. roberti* had no effect on body size of *C. alliariae* (i.e., *C. alliariae*, collected in the sympatric range and in the range where it occurs alone, were equal in size (independent sample *t*-test: *t* = 0.711, *df* = 32.718, *P* = 0.482). Head capsule measurements revealed three larval stages (Table 2). Egg size and head capsule diameters overlapped between species, and therefore cannot be used as reliable characters to distinguish the two species.

Oviposition. Under seminatural conditions, females of both species started to lay eggs at the same time in mid-March. This corresponded well with observations in the field and coincided with bolting garlic mustard plants. By the beginning of June, oviposition ceased in both species. The number of eggs laid during the first and second oviposition periods was not significantly different (Table 1). The length of the oviposition period did not differ between the species in the first period, but was slightly longer for *C. alliariae* in the second period (Table 1).

Because we found no differences in spatial distribution of eggs laid on plants between different release densities (density × segment: *C. alliariae*: *F*_{14,96} = 1.050, *P* = 0.412; *C. roberti*: *F*_{14,96} = 2.750, *P* = 0.086)

Table 2. Size of eggs and head capsule diameters of larvae of *C. alliariae* and *C. roberti*

Stage	n	<i>C. alliariae</i>	Range (mm)	n	<i>C. roberti</i>	Range (mm)
		Mean (mm) ± SE			Mean (mm) ± SE	
Eggs	35			42		
Length		0.588 ± 0.007	0.512–0.678		0.601 ± 0.007	0.512–0.702
Width		0.378 ± 0.005	0.309–0.440		0.398 ± 0.007	0.309–0.488
Instars						
L1	27	0.356 ± 0.004	0.321–0.393	34	0.344 ± 0.004	0.286–0.368
L2	21	0.471 ± 0.005	0.440–0.512	37	0.485 ± 0.004	0.428–0.547
L3	16	0.634 ± 0.004	0.606–0.655	48	0.663 ± 0.005	0.595–0.726

we pooled data for each species across densities. Neither species distributes their eggs regularly over shoot segments (*C. alliariae*: $F_{7,96} = 6.934$, $P < 0.001$; *C. roberti*: $F_{7,96} = 9.787$, $P < 0.001$). Most eggs were laid into the upper portions of shoots and the shoot base was avoided (Fig. 1). Females of *C. roberti* tended to lay a higher proportion of eggs into the top 20% of shoots compared with females of *C. alliariae* (Fig. 1, independent t -test: $t = -1.863$, $df = 58$, $P = 0.067$), whereas *C. alliariae* laid a higher proportion in the next two segments (21–40%) (Fig. 1, independent t -test: $t = -3.867$, $df = 58$, $P < 0.001$). While females of *C. alliariae* laid most of their eggs singly, females of *C. roberti* laid nearly 40% of their eggs in clusters of up to eight eggs.

Development and Spatial Distribution of Larvae. Larval development on potted plants was similar in both species (Fig. 2) requiring approximately 7 wk from egg to mature larva. Number of larvae mining (independent sample t -test: $t = -0.472$, $df = 11$, $P = 0.646$) and feeding patterns of both species in the shoots were identical. Larvae of both species mined the entire shoot and occasionally extended feeding into the root crown. Mature larvae of both species left the plant to pupate in the soil.

All rosettes established for development tests showed signs of mining; however, successful develop-

ment was observed only for one adult in each species. Garlic mustard rosettes are an inferior phenostage for development of the two shoot-mining species.

Distribution, Adult Phenology, and Occurrence at Field Sites. *Ceutorhynchus alliariae* occurred further north and east than *C. roberti* and was the only species collected around Lübeck (Germany), Berlin (Germany), and Vienna (Austria) (Fig. 3). Both species co-occurred in central Europe. *Ceutorhynchus roberti* was more common at most field sites around Delémont (Switzerland) and Müllheim (Germany), whereas *C. alliariae* dominated sites around Lake Neuchâtel (Switzerland) (Fig. 3). The frequency of each species varied greatly among field sites; however, across all sites in the sympatric range neither of the two species appeared to be more frequent than the other ($t = 0.344$, $df = 45$, $P = 0.732$). At the field sites sampled in the sympatric range, *C. alliariae* and *C. roberti* were equally frequent regardless of light condition ($t = -1.608$, $df = 15$, $P = 0.129$). The frequency of *C. roberti* at most field sites fluctuated from 2000 to 2002, but no significant trend was found (repeated measures ANOVA for effect of year: $F_{2,12} = 1.99$, $P = 0.179$), with some populations showing an increase, and others showing a decrease.

Adults of both species appeared and can be observed mating on garlic mustard at the beginning of March, and numbers declined by the end of May. Field sex ratio was 1 ♀ : 1.17 ♂; ($n = 2,027$) for *C. alliariae* and 1 ♀ : 0.98 ♂; for *C. roberti* ($n = 2,780$). F1-generation adults of *C. alliariae* and *C. roberti* emerged from the end of May to the end of July, and there was no obvious difference in emergence patterns between the two species.

Larvae of *C. alliariae* and *C. roberti* were reared from shoots with similar base diameters and heights (independent sample t -test: base diameter: $t = -1.771$, $df = 76$, $P = 0.081$; height: $t = -1.262$, $df = 76$, $P = 0.211$), and we found no difference in shoot size requirements for *C. alliariae* between the sympatric range and the range where it occurs alone (independent sample t -test: base diameter: $t = 1.550$, $df = 61$, $P = 0.126$; height: $t = 1.267$, $df = 61$, $P = 0.210$).

Shoot collections showed that at sites with larval densities between 2.2 and 5.9 per shoot, larvae of *C. alliariae* and *C. roberti* occurred independently of each other in shoots ($P > 0.05$). However, at two field sites with high attack (90–100% of shoots mined, larval densities of 4.8 and 8.3 per shoot, respectively), larvae of the two species occurred more often to-

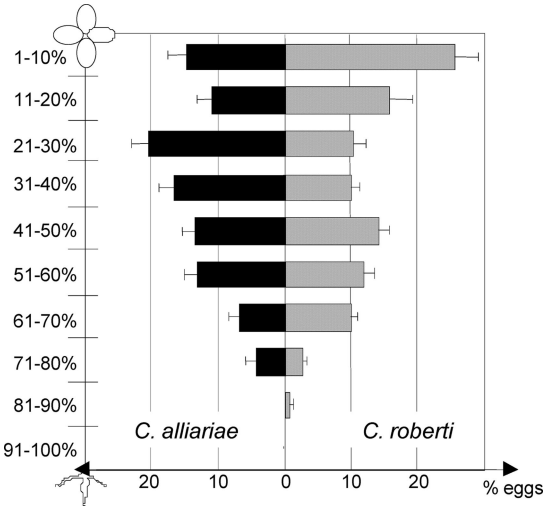


Fig. 1. Distribution of eggs of *C. alliariae* ($n = 407$) and *C. roberti* ($n = 834$) in shoots of garlic mustard. Each shoot was divided into 10 segments of equal length.

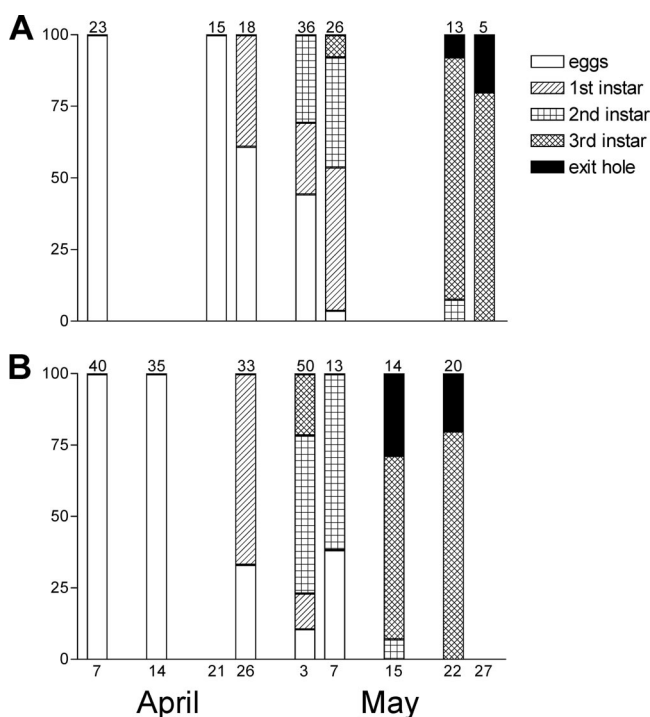


Fig. 2. Phenology of immature stages of *C. alliariae* (A) and *C. roberti* (B) on potted plants of garlic mustard infested at the Center. Each bar represents data of one or two dissected plants. Numbers on top of bars indicate the total number of stages found per dissection date.

gether in the same shoot than expected by chance (site 9: $\chi^2_{df=1} = 5.32$, $P = 0.048$; site 10: $\chi^2_{df=1} = 20.90$, $P < 0.001$).

There was no difference in overall attack rates between the sympatric range and the range where only *C. alliariae* occurs, with an average of $78.0\% \pm 5.9$ ($n = 10$) and $74.7\% \pm 8.6$ ($n = 6$) of shoots attacked, respectively. Up to 100% of shoots can, however, be attacked in both ranges, and we found up to 24 larvae mining a single shoot. Overall, attack levels (i.e., the number of larvae mining in a shoot), were higher in the sympatric range (Fig. 4; Mann-Whitney: $z = -2.558$, $P = 0.011$). The resulting estimated attack levels attributable to *C. alliariae* in the sympatric range was lower than its attack levels in the range where it occurs alone (Mann-Whitney: $Z = -14.315$, $P < 0.001$). Attack levels of *C. alliariae* in the sympatric range reached only 42.5% of the levels reached where *C. alliariae* occurred without *C. roberti*.

After emergence, weevils kept in cylinders fed on leaves of garlic mustard, but stopped feeding by the end of August. In the field we never observed adults during summer and only on rare occasions in the fall. Winter survival in captivity was generally high for both species; 86–94% for *C. alliariae* and 82–98% for *C. roberti* in 1999 and 2000.

Parasitism. *Ceutorhynchus alliariae*, *C. roberti*, or both were attacked by solitary ectoparasitoids in the family Pteromalidae: *Trichomalus perfectus* (Walker), *T. lucidus* (Walker), and *Stenomalina gra-*

cilis (Walker). *Trichomalus perfectus* was reared only from hosts collected in the sympatric range, whereas the two other species occurred throughout their ranges.

In the sympatric range, the solitary, koinobiontic ichneumonid endoparasitoid *Tersilochus obscurator* Aubert, was reared from larvae of the shoot-miners. On rare occasions, endoparasitoid cocoons also were found from the range where only *C. alliariae* occurs, but we were unable to successfully rear out adults. No information on the larval stages attacked by *Tersilochus obscurator* is available from the literature. Because of observations of this species early in the season, we assume that all larval stages can harbor the parasitoid, and we therefore calculated parasitism rates as the number of parasitoid cocoons divided by the number of all larval instars found upon dissection.

Parasitism rates ranged from 0.4 to 14.0% (ectoparasitoids) and 0–0.9% (endoparasitoids) in the range where only *C. alliariae* occurs ($n = 3$ field sites), and from 0 to 51.6% (ectoparasitoids) and 0–15.3% (endoparasitoids) in the sympatric range ($n = 7$ field sites).

Discussion

For the niche dimensions investigated in this study, we only found small differences between the coexisting weevil species, *C. alliariae* and *C. roberti*. This is a rare finding in animals in general, and to our knowledge never has been reported for internally feeding

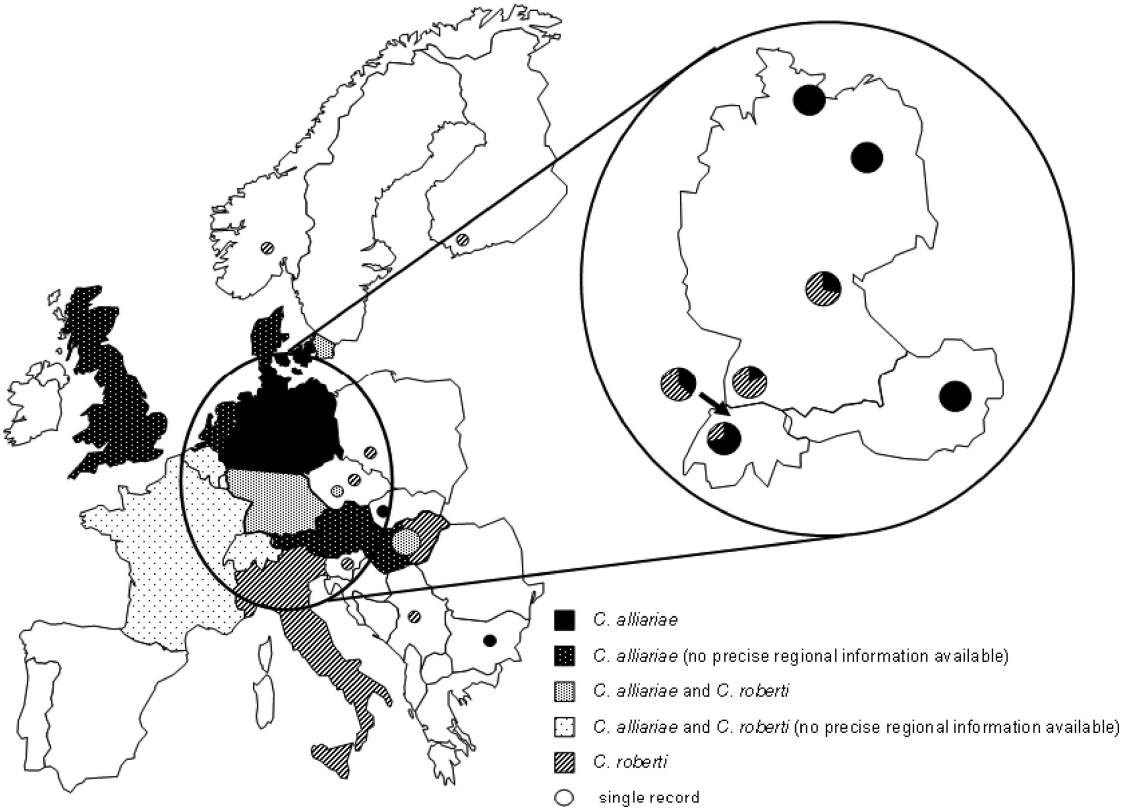


Fig. 3. Distribution of *C. alliariae* and *C. roberti* according to literature and personal communication (large scale) and based on field sampling (small scale). Pies: proportion of adult *C. alliariae* (black) and *C. roberti* (dashed) sampled in different regions in Switzerland, Germany, and Austria. Numbers next to pies indicate number of sites sampled.

phytophagous insects where competition can be expected to be strong (Denno et al. 1995).

According to the original biological species concept, species are groups of actually or potentially in-

breeding populations that are reproductively isolated from other such groups (Mayr 1942). In the case of *C. alliariae* and *C. roberti*, we found strongly reduced oviposition in mixed-species pairs. Only a small pro-

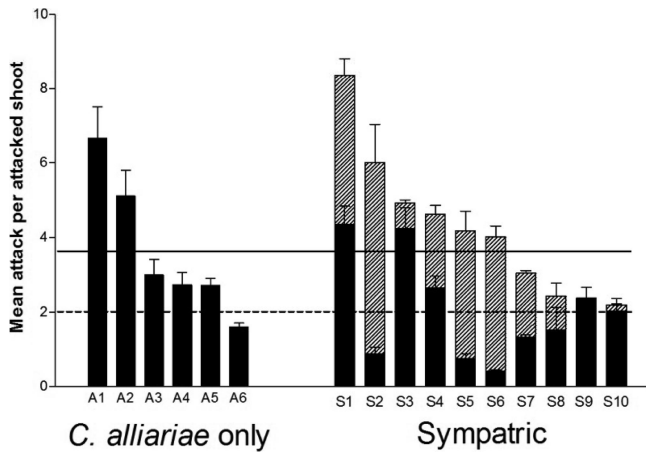


Fig. 4. Attack of garlic mustard by *C. alliariae* and *C. roberti* at different field sites sampled between 1998 and 2002. *C. alliariae* only: field sites in the area where only *C. alliariae* occurs; sympatric: field sites in area where both species occur; black bars: attack by *C. alliariae*, hatched bars: attack by *C. roberti*. The lines indicate mean attack attributed to *C. alliariae* in the sympatric range (dashed line) and in the range where only this species occurs (black line). Attack (mean \pm SE) corresponds to the sum of all larvae and exit holes found upon dissection.

portion of these eggs were fertile and only one plant offered to *C. alliariae* mixed-species pairs was attacked. In addition, it cannot be excluded that plant material used in tests was contaminated by incident adults of the two species occurring naturally in the Center's garden. Because of these experimental limitations, we therefore were unable to unambiguously confirm that *C. alliariae* and *C. roberti* are indeed reproductively isolated. However, our results nevertheless strongly support the opinion of Strejček (1969) that *C. alliariae* and *C. roberti* are true, reproductively isolated species. Results also confirm that *C. roberti* tends to be slightly larger than *C. alliariae* (Dieckmann 1972). However, the species are morphologically very similar and their larvae and eggs cannot be distinguished based on size.

Our field surveys and personal communications with European taxonomists confirmed the distribution reported in the literature. *Ceutorhynchus alliariae* and *C. roberti* partially differ in their geographical distribution in Europe; however, their distribution pattern cannot be linked to differences in climatic preferences between the species. Although in central Europe, *C. roberti* seems to have a northern distribution limit at 50° latitude, the species is again present in Scandinavia (Fig. 3). In addition, *C. roberti* is found in eastern Hungary, a region with a continental climate, and can therefore not be considered limited in its distribution range by low temperature. Alternative explanation might underlie the current distribution pattern of the two species in Europe. For instance, the two species might have resisted in different glaciation refugia and subsequently, recolonization histories after glaciation varied. Similarly, local differences in abundance between the two species might be linked to differences in the colonization history of individual garlic mustard patches. In contrast to literature records (Dieckmann 1972), our investigations revealed no differences in habitat preference between *C. alliariae* and *C. roberti*. *Ceutorhynchus alliariae* was not more frequent in shady, and *C. roberti* not more common in open, sunny habitats. In general, *C. alliariae* and *C. roberti* were frequently found throughout the range investigated and can reach high attack levels, both in the sympatric range and in the range where only *C. alliariae* occurs.

We found no major differences in the phenology between the species. After a short period of feeding on garlic mustard in summer, emerged weevils remain largely inactive until oviposition commences the next spring. Surprisingly, both species survived more than one season and had a second oviposition period. Although for other species within the family Curculionidae, life spans of two or more years have been recorded (McMullen and Condrashoff 1973, Dieckmann 1980, Purrini 1981, Cerezke 1994), to the best of our knowledge, this is the first record for species within the genus *Ceutorhynchus*.

Both species had identical oviposition periods, laid a similar number of eggs, and showed very similar spatial use of their common host plant. They clearly preferred the top part of shoots for oviposition, pre-

sumably because plant tissue is softer at the top and therefore more suitable for oviposition and development of first-instar larvae. In addition, female choice might be driven partially by the higher content of nitrogen in the top shoot parts (Gerber et al. 2007). The only clear difference in oviposition behavior observed was that females of *C. roberti* laid 40% of their eggs in clusters of up to eight eggs, whereas females of *C. alliariae* laid all of their eggs singly. Females that lay eggs in clusters potentially spend less time searching for suitable oviposition sites and for preparing oviposition holes (Stamp 1980, Weis et al. 1983, Freese and Zwölfer 1996, Hinz 1998). However, offspring mortality may increase because of intraspecific cannibalism (which was observed for first larval instars kept in petri dishes in the laboratory; E. G. and H.L.H., unpublished data) and egg parasitism (although rarely observed for *C. alliariae* and *C. roberti*; E. G. and H.L.H., unpublished data). In the common garden study (i.e., excluding parasitoids) the number of larvae mining in shoots did not differ between species, and neither of the two species appeared to be more common than the other in the sympatric range.

Another possibility of spatial niche separation between phytophagous insects is the use of different host plant species (Zwölfer 1979). Alternate host plant species can reduce competition (Gibson and Visser 1982). *Ceutorhynchus alliariae* and *C. roberti* both are described as monophagous on garlic mustard (Dieckmann 1972). Although ongoing host range tests have revealed that under lab and common garden conditions a limited number of other Brassicaceae can support larval development, so far, mainly the same plant species are attacked by both weevil species (i.e., their host range does not appear to differ substantially; E. G. and H.L.H., unpublished data).

Therefore, based on the niche dimensions examined in this study, we conclude that *C. alliariae* and *C. roberti*, unlike other congeneric stem-borers (Bacher 1994, Freese 1995), appear to have nearly identical fundamental niches. In addition, we found no indication for differential attack by natural enemies. All parasitoid species we reared have been recorded previously from other curculionid species (U. Kuhlmann, personal communication), which makes it unlikely that these parasitoids will distinguish between larvae of *C. alliariae* and *C. roberti*. Low discriminative behavior of parasitoids has little potential to influence species coexistence (McClure 1980).

Other investigations on stem-borers with highly overlapping resources either revealed on-going competitive displacement (Kfir 1997, Ofomata et al. 1999), or no effect of interspecific competition (Rathcke 1976, Stiling and Strong 1983), presumably because of lack of resource limitation. The latter generally has been assumed for phytophagous insects, and it was concluded that interspecific competition is therefore too infrequent or weak to play an important role in structuring these communities (Shorrocks et al. 1984, Strong et al. 1984). However, *C. alliariae* and *C. roberti* can reach high population levels in the field, and

heavily attacked plants of garlic mustard showed clear signs of damage, occasionally resulting in shoot or plant death (E. G. and H.L.H., unpublished data). *Ceutorhynchus alliariae* and *C. roberti* can therefore encounter resource limitation in the field, and it could be expected that the two species compete. In fact, attack levels of *C. alliariae* were reduced in areas where it co-occurred with *C. roberti* compared with areas where it occurred alone, suggesting interspecific competition. However, although the frequencies of *C. alliariae* and *C. roberti* in mixed populations fluctuated over time, there was no indication for a trend of one species to replace the other, which would be expected if one species would be a superior competitor (MacArthur and Levins 1967). Further, the near complete colonization of host plant populations by both species does not indicate that their coexistence is based on a competition-colonization trade-off (Wilson et al. 1999, Amarasekare and Nisbet 2001, Harrison et al. 2001). A competition-colonization trade-off is predicted to result in regional coexistence with the two species occupying mutually exclusive subsets of patches in the metapopulation (Amarasekare and Nisbet 2001), which is clearly not the case for *C. alliariae* and *C. roberti*. Contrary to the expectations of aggregated distributions as a mechanism to promote coexistence (Shorrocks and Sevenster 1995), the distributions of *C. alliariae* and *C. roberti* larvae were independent of each other at the majority of field sites investigated (i.e., individuals of the two species neither avoided nor searched out the presence of the other species). Only at two field sites with high weevil populations, mixed-species aggregations were found, potentially indicating that both species exploited high quality hosts and therefore aggregated on the same plants when resources were strongly limited. Finally, our study revealed no character displacement (Brown and Wilson 1956) in regard to adult size or shoot choice of *C. alliariae* between regions where it occurs sympatrically with *C. roberti* and where it occurs alone.

Overall, our results, based on investigations of a subset of niche dimensions known to be important in other systems, indicate that *C. alliariae* and *C. roberti* may present one of the rare cases, in which niche differentiation is not the main mechanism underlying coexistence. To ultimately clarify the potential competitive interactions between the two species, our observational studies would need to be supported by experimental investigations.

Acknowledgments

We thank Gislaine Cortat, Nathalia Guazzone, Leonore Lovis, Jessica McKenney, Stephanie Michler, Marjolein Schat, and Marion Zuefle for their assistance in the field and laboratory. We gratefully acknowledge the following people for providing detailed information on the geographic distribution of *C. alliariae* and *C. roberti*: Lutz Behne, Clemens Brandstetter, Stefan Björn, Alain Drumont, Wolfgang Gruschwitz, Christian Maus, Otto Merkl, Elvind Palm, Snezana Pesic, Claude Schott, Peter Sprick, Jan Stenlökk, Dré

Teunissen, and Jean-François Voisin. Ulrich Kuhlmann and his group at CABI Europe-Switzerland, in particular, Manfred Grossrieder, Beate Klander, and Franck Muller kindly provided additional data on parasitism and attack levels of *C. alliariae* and *C. roberti* and provided technical support in rearing parasitoids. Hannes Baur (University of Berne, Switzerland) identified the ectoparasitoids and Klaus Horstmann (University of Würzburg, Germany) the endoparasitoids of *C. alliariae* and *C. roberti*. We thank Urs Schaffner (CABI, Switzerland), the editor and three anonymous reviewers for helpful comments on the manuscript. Funding for this work was provided through the Strategic Environmental Research and Development Program, U.S. Department of Defense, Project CS 1146, through Cornell University.

References Cited

- Amarasekare, P., and R. M. Nisbet. 2001. Spatial heterogeneity, source-sink dynamics, and the local coexistence of competing species. *Am. Nat.* 158: 572–583.
- Atkinson, W. D., and B. Shorrocks. 1981. Competition on a divided and ephemeral resource: a simulation model. *J. Anim. Ecol.* 50: 461–471.
- Bacher, S. 1993. Vergleichende ökologische Studien über zwei sympatrische Rüsselkäfer-Arten an der Geruchlosen Kamille (*Tripleurospermum perforatum*). Diploma thesis. Christian Albrechts Universität Kiel, Germany.
- Bacher, S. 1994. Die Strategie einer biologischen Bekämpfung der Geruchlosen Kamille in Kanada. *Z. Pflanzenkr. Pflanzenschutz, Sonderheft* 14: 221–230.
- Begon, M., J. L. Harper, and C. R. Townsend. 1990. *Ecology: Individuals, populations and communities*, 2nd ed. Blackwell Scientific Publications, Oxford, United Kingdom.
- Blossey, B., V. Nuzzo, H. Hinz, and E. Gerber. 2001. Developing biological control of *Alliaria petiolata* (M. Bieb.) Cavara and Grande (garlic mustard). *Nat. Areas J.* 21: 357–367.
- Brown, W. L., and E. O. Wilson. 1956. Character displacement. *Syst. Zool.* 5: 49–64.
- Cerezke, H. F. 1994. Warren rootcollar weevil, *Hylobius warreni* Wood (Coleoptera: Curculionidae), in Canada: ecology, behavior, damage relationships, and management. *Can. Entomol.* 126: 1383–1442.
- Cripps, M. G., H. L. Hinz, J. L. McKenney, B. L. Harmon, F. W. Merickel, and M. Schwarzlaender. 2006. Comparative survey of the phytophagous arthropod faunas associated with *Lepidium draba* in Europe and the western United States, and the potential for biological weed control. *Biocontrol Sci. Technol.* 16: 1007–1030.
- Denno, R. F., M. S. McClure, and J. R. Ott. 1995. Interspecific interactions in phytophagous insects: competition re-examined and resurrected. *Annu. Rev. Entomol.* 40: 297–331.
- Dieckmann, L. 1972. Beiträge zur Insektenfauna der DDR: Coleoptera-Curculionidae: Ceutorhynchinae. *Beitr. Entomol.* 22: 3–128.
- Dieckmann, L. 1980. Beiträge zur Insektenfauna der DDR: Coleoptera-Curculionidae (Brachycerinae, Otiorhynchinae, Brachyderinae). *Beitr. Entomol.* 30: 145–310.
- Forrester, G. J. 1993. Resource partitioning between two species of *Ceutorhynchus* (Coleoptera: Curculionidae) on *Echium plantagineum* in a Mediterranean habitat. *Bull. Entomol. Res.* 83: 345–351.
- Fowler, W. W. 1891. *The Coleoptera of the British Islands*. L. Reeve and Co., London, United Kingdom.
- Freese, G. 1995. Structural refuges in two stem-boring weevils on *Rumex crispus*. *Ecol. Entomol.* 20: 351–358.

- Freese, G., and H. Zwölfer. 1996. The problem of optimal clutch size in a trophic system: the oviposition strategy of the thistle gallfly *Urophora cardui* (Diptera, Tephritidae). *Oecologia* 108: 293–302.
- Gerber, E., H. L. Hinz, and B. Blossey. 2007. Interaction of specialist root and shoot herbivores of *Alliaria petiolata* and their impact on plant performance and reproduction. *Ecol. Entomol.* 32: 357–365.
- Gianoli, E. 2000. Competition in cereal aphids (Homoptera: Aphididae) on wheat plants. *Entomol. Soc. Am.* 29: 213–219.
- Gibson, C., and M. Visser. 1982. Interspecific competition between two field populations of grass-feeding bugs. *Ecol. Entomol.* 7: 61–67.
- Hanski, I. 1981. Coexistence of competitors in patchy environment with and without predation. *Oikos* 37: 306–312.
- Hardin, G. 1960. The competitive exclusion principle. *Science* 131: 1292–1297.
- Harrison, M. A., Y.-C. Lai, and R. D. Holt. 2001. Dynamical mechanism for coexistence of dispersing species. *J. Theor. Biol.* 213: 53–72.
- Hegi, G. 1986. *Illustrierte Flora von Mitteleuropa: Pterophyta, Spermatophyta, Band IV, Angiospermae Dicotyledones 2, Teil 1* Verlag Paul Parey, Berlin, Hamburg, Germany.
- Heijerman, T. 1993. Naamlijst van de snuitkevers van Nederland en het omliggend gebied (Curculionoidea: Curculionidae, Apionidae, Attelabidae, Urodoontidae, Anthribidae en Nemomychidae). *Nederlandse faunistische Mededelingen* 5: 19–46.
- Hinz, H. L. 1998. Life history and host specificity of *Rhopalomyia* n. sp. (Diptera: Cecidomyiidae), a potential biological control agent of scentless chamomile. *Environ. Entomol.* 27: 1537–1547.
- Hinz, H. L., and H. Müller-Schärer. 2000. Suitability of two root-mining weevils for the biological control of scentless chamomile, *Tripleurospermum perforatum*, with special regard to potential non-target effects. *Bull. Entomol. Res.* 90: 497–508.
- Hoffmann, A. 1954. Faune de France 59, Coléoptères Curculionides. Edition Paul Lechevalier, Paris, France.
- Hutchings, G. E. 1957. Concluding remarks. *Cold Spring Harb. Symp. Quant. Biol.* 22: 415–427.
- Kfir, R. 1997. Competitive displacement of *Busseola fusca* (Lepidoptera: Noctuidae) by *Chilo partellus* (Lepidoptera: Pyralidae). *Entomol. Soc. Am.* 90: 617–624.
- Kuhlmann, U., P. G. Mason, H. L. Hinz, B. Blossey, R. A. De Clerck-Floate, L. Dosdall, J. P. McCaffrey, M. Schwarzaender, O. Olfert, J. Brodeur, et al. 2006. Avoiding conflicts between insect and weed biological control: selection of nontarget species for test list to assess host specificity of cabbage seedpod weevil parasitoids. *J. Appl. Entomol.* 130: 129–141.
- MacArthur, R., and R. Levins. 1967. The limiting similarity, convergence, and divergence of coexisting species. *Am. Nat.* 101: 377–385.
- Mayr, E. 1942. Systematics and the origin of species from the viewpoint of a zoologist. Columbia University Press, New York.
- McClure, M. S. 1980. Competition between exotic species: scale insects on hemlock. *Ecology* 61: 1391–1401.
- McMullen, L. H., and S. F. Condrashoff. 1973. Notes on dispersal, longevity and overwintering of adult *Pissodes strobi* (Peck) (Coleoptera: Curculionidae) on Vancouver Island. *J. Entomol. Soc. B. C.* 70: 22–26.
- Ofomata, V. C., W. A. Overholt, A. van Huis, R. I. Egwuatu, and A. J. Ngi-Song. 1999. Niche overlap and interspecific association between *Chilo partellus* and *Chilo orichalcociliellus* on the Kenya coast. *Entomol. Exp. Appl.* 93: 141–148.
- Purrini, K. 1981. *Nosema hylobii* n. sp. (Nosematidae, Microsporidia), a new microsporidian parasite of *Hylobius abietis* L. (Coleoptera, Curculionidae). *Z. Angew. Entomol.* 92: 1–8.
- Ratke, B. J. 1976. Competition and coexistence within a guild of herbivorous insects. *Ecology* 57: 76–87.
- Rheinheimer, J., and M. Hassler (eds.) 2010. *Die Rüsselkäfer Baden-Württembergs*, pp. 944. Verlag Regionalkultur, Heidelberg, Germany.
- Schoener, T. W. 1974. Resource partitioning in ecological communities. *Science* 185: 27–39.
- Schott, C. 2000. Catalogue et atlas des Coléoptères d'Alsace Musée Zoologique de l'Université et de la Ville de Strasbourg, Strasbourg, France.
- Shorrocks, B., W. D. Atkinson, and P. Charlesworth. 1979. Competition on a divided and ephemeral resource. *J. Anim. Ecol.* 48: 899–908.
- Shorrocks, B., and J. G. Sevenster. 1995. Explaining local diversity. *Proc. R. Entomol. Soc. Lond.* 260: 305–309.
- Shorrocks, B., J. Rosewell, K. Edwards, and W. Atkinson. 1984. Interspecific competition is not a major force in many insect communities. *Nature* 310: 310–312.
- Stamp, N. E. 1980. Egg deposition pattern in butterflies: why do some species cluster their eggs rather than deposit them singly? *Am. Nat.* 115: 367–380.
- Stiling, P. D., and D. R. Strong. 1983. Weak competition among *Spartina* stem borers, by means of murder. *Ecology* 64: 770–778.
- Strejček, J. 1969. Zur Taxonomie einiger Mitteleuropäischen Curculionidae-Arten. *Entomol. Blätter* 65: 160–162.
- Strejček, J. 1996. Coleoptera: Curculionidae, pp. 577–599. In R. Rozkosny and J. Vanhara (eds.), *Coleoptera: Curculionidae*, vol. 94. Masaryk University, Brno, Czech Republic.
- Strong, D. R., J. H. Lawton, and R. Southwood. 1984. *Insects on plants*. Blackwell, Oxford, United Kingdom.
- Tschamke, T. 1993. Connection of insect population dynamics with community structure in *Phragmites*-habitats, pp. 37–44. In P. J. den Boer, P. J. M. Mols, and J. Szyszko (eds.), *Dynamics of populations: proceedings of the meeting on population problems*. Agricultural University, 9–15 September 1992, Warsaw, Smolarnia, Poland.
- Tóth, P., and L. Cagán. 2005. Organisms associated with the family Convolvulaceae and their potential for biological control of *Convolvulus arvensis*. *Biocontrol News Inf.* 26: 17N–40N.
- Tutin, 1964. *Flora Europaea*, vol. I. Book *Flora Europaea Vol I*. Cambridge University Press, Cambridge, United Kingdom.
- Vayssières, J. F., and A. J. Wapshere. 1983. Life-histories and host specificities of *Ceutorhynchus geographicus* (Goeze) and *C. larvatus* Schultze (Col.: Curculionidae), potential biological control agents for *Echium*. *Bull. Entomol. Res.* 73: 431–440.
- Weis, A. E., P. W. Price, and M. Lynch. 1983. Selective pressure on clutch size in the gall maker *Asteromyia carbonifera*. *Ecology* 64: 688–695.
- West, C. 1985. Factors underlying the late seasonal appearance of lepidopterous leaf-mining guild on oak. *Ecol. Entomol.* 10: 111–120.
- Wilson, C. G., G. J. Flanagan, and J. D. Gillett. 1990. The phytophagous insect fauna of the introduced shrub *Mimosa pigra* in northern Australia and its relevance to biological control. *Environ. Entomol.* 19: 776–784.

- Wilson, W. G., C. W. Osenberg, R. J. Schmitt, and R. M. Nisbet. 1999. Complementary foraging behaviors allow coexistence of two consumers. *Ecology* 80: 2353–2372.
- Yu, D. S. 2009. Taxapad: scientific names for information management [online]. ([http:// www.taxapad.com/taxapadmain.php](http://www.taxapad.com/taxapadmain.php)).
- Zwölfer, H. 1979. Strategies and counterstrategies in insect population systems competing for space and food in flower heads and plant galls. *Fortschr. Zool.* 25: 331–353.
- Zwölfer, H. 1980. Distelblütenköpfe als ökologische Kleinsysteme: Konkurrenz und Koexistenz in Phytophagenkomplexen. *Mitt. Dtsch. Ges. Allg. Angew. Entomol.* 2: 21–37.

Received 23 December 2011; accepted 6 July 2012.
