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Parasitoid suppression and life-history modifications in a wolf spider following infection by larvae of an acrocerid fly

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Abstract. Flies of the family Acroceridae are specialized internal parasitoids of spiders. We infected hatchlings of wolf spiders *Pardosa prativaga* (L. Koch 1870) (Araneae: Lycosidae) with larvae of *Acrocera orbiculus* (Fabricius 1787); most hosts were infected by a single larva, but others endured multiple infections of up to eight larvae. The infected spiders and a group of uninfected control spiders were raised in the laboratory for up to 23 weeks. We found that most (81%) spiders infected by only one larva were able to suppress the infection, whereas most multiple infections (73%) were ''successful'' (i.e., a larva emerged or was recovered by dissection, perhaps from a prematurely dead spider). Infected spiders had their survival reduced in proportion to the infection load, but the reduction was not significant if the infection was suppressed. Infected spiders had higher growth rates than uninfected, and growth stimulation was proportional to the number of initially infecting larvae and independent of whether the larva was suppressed or not. Due to these patterns, we suggest that growth enhancement results from the spider's mobilization of extra resources for combating the infection rather than parasitoid manipulation of spider growth. Spiders with multiple infections took longer to mature than uninfected spiders, and the pattern of instar durations was changed compared with that of control and singly infected spiders. As multiple infections were important for the parasitoid's success, we suggest that the parasitoid fly's habit of laying eggs in large clumps may be an adaptation to increase the chance of success via multiple infections.

Keywords: Acroceridae, life history, Lycosidae

The interplay between a parasitoid and its host is intriguing. On one hand, a parasitoid's survival and growth depend entirely on the successful survival and growth of its host (Godfray 1994; Brodeur & Boivin 2004). On the other hand, a host in excellent condition may more easily avoid a parasitoid infection in the first place, but also be more effective at suppressing a current parasitoid infection. Since a successful parasitoid is deadly, it is always in the host's interest to get rid of it as early as possible. The parasitoid should be virulent enough to avoid suppression from the host, but at the same time minimize the physiological costs to the host and selectively manipulate specific aspects of host physiology and behavior (Godfray 1994; Brodeur & Boivin 2004). Though we expect a parasite infection to be costly to the host, there should be strong selection on the parasitoid to reduce the negative impact of the infection until it finally kills the host (Slansky 1986). If possible, it might even be advantageous for the parasitoid to stimulate the growth of the host. This would either provide more resources for the parasitoid that may obtain a larger body size, or reduce the time of development and thus enhance the chances of survival. Growth enhancement is common for hosts of gregarious parasitoids, whereas growth inhibition and reduced activity is the rule for hosts of solitary parasitoids (Slansky 1986; Harvey et al. 1999; Harvey 2000), reflecting a difference in nutritional demands of multiple versus single parasitoids, though this may also depend on the size of the host (Harvey et al. 2010).

Flies of the family Acroceridae are specialized parasitoids of spiders, mostly cursorial species (Schlinger 1987). Most published accounts of these animals are lists of parasite-host relationships and data on spider infection rates based on rearings of field-collected spiders. Information on the interactions between the spider host and the developing parasitoid is very limited (Schlinger 1987) because the adult flies are difficult to maintain in the laboratory. Chance events have allowed us to obtain eggs and first-instar larvae (planidia) from a few mated females of the species *Acrocera orbiculus* (Fabricius 1787) from the field. In the first place this allowed us to observe its unique mode of entrance into the spider host (Nielsen et al. 1999); subsequently, we infected a large number of wolf spider hatchlings and thus were able to get some information on the fate of the parasitoids after infection, as well as on how the life history parameters of the spider are modified by the infection. Since some spiders were singly and others multiply infected, we were also able to analyse how parasitoid success and spider life history depend on the infection load. We were interested in how effective the spider host may be in suppressing parasitoid infections, and whether the parasitoid is able to manipulate the spider's growth pattern for its own benefit. Considerable attention has been devoted to modifications of host behavior (Godfray 1994; Thomas et al. 2005), also in spiders (Eberhard 2000, 2010), but less attention has been paid to how spider life histories are molded by parasitoid infection and the relative role of parasitoid and host strategies in these modifications.

METHODS

The parasitoid.—*Acrocera orbiculus* has a wide distribution covering both the Palaearctic and the Nearctic (Nartshuk 2010). Adult females lay clumps of several hundred eggs in the vegetation, and the tiny, hatched planidia larvae (0.3–0.4 mm) move about actively searching for a potential spider host. Having found a potential host, the larva first attaches itself by the mouthparts, usually to a leg, to first become an ectoparasite. It then becomes an internal parasite after the first molt when the amoeboid second instar larva enters the spider's haemolymph via the tiny hole where the planidium larva's mouthparts are attached (Nielsen et al. 1999). This second instar larva enters the abdomen (via legs, prosoma and pedicel) and takes up a position near the booklungs. Here it

grows to its final size, mainly during the third and fourth instar. The fully grown larva exits the dying spider to pupate outside the carcass.

Procedure.—Two females of *A. orbiculus* were collected live at Mols, Denmark, and brought to the laboratory. During subsequent days they laid a large number of eggs in the collecting vials. After approximately two weeks at 21°C the eggs hatched into active planidia larvae. Approximately 100 hatchlings of the wolf spider *Pardosa prativaga* (L. Koch 1870) (Araneae: Lycosidae) were released into the vials and left there overnight to allow the planidia to infect the spiders. The spiderlings were inspected for attached larvae under a binocular microscope and transferred to individual breeding vials with a plaster bottom. The number of attached larvae varied between 0 and 8 per spider; thus the parasitoid load was not strictly controlled. We had 39 uninfected spiders, 37 infected with 1 larva, 7 with 2, 4 with 3, 1 with 4, 2 with 5, and 1 with 8 larvae. The spiderlings were raised in the vials at 25 °C and a 16L:8D photoperiod, being fed fruit flies ad libitum and watered 2–3 times per week. The non-infected spiderlings served as a control group. The fruit flies used were nutrient-enriched by being raised in a mixed medium of Carolina Biological Supply *Drosophila* medium (Formula 4–24) and crushed dog food. This mixed medium is nutritionally optimal for survival and growth of the spiders (Mayntz & Toft 2001). The spiders were weighed weekly from the start of the experiment and up to 23 weeks. At this time some spiders had died; acrocerid larvae had emerged from others in order to pupate; the remaining spiders were killed at this time. Spiders that died, as well as those that remained at the end, were preserved in alcohol and subsequently dissected to check for presence and number of acrocerid larvae in the abdomen.

Statistical analyses.—JMP 8 was used for the statistical analysis. We distinguished three groups of spiders according to the initial infection load (''# infecting larvae''): control (Acr = 0, non-infected), single infection (Acr = 1), and multiple infection (Acr = 2+, 2–8 larvae). Spiders were also grouped according to the success of the parasitoid, defined by the result of the dissections: no larva (L = 0) or one or more larvae present (L = 1+). Survivorship was analyzed by a Wilcoxon test. The growth curves were analyzed by repeated measures ANOVA of the weekly weight measurements. The time* parasite load (# initially infecting larvae or # surviving larvae) interaction terms were used to indicate significantly different growth patterns. Due to mortality, meaningful tests could only be made on data up to week 15. Developmental parameters were analysed by ANOVA and *t*-tests; the data were checked for homogeneity of variances (Levene's test); when this criterion could not be met, the Welch *t*-test was used.

RESULTS

Parasitoid suppression.—Only three fully developed acrocerid larvae emerged from their spider hosts. However, several spiders that died during the experiment or were killed when the experiment ended turned out by dissection to contain one or two acrocerid larvae of intermediate or large (i.e., close to fully grown) size in their abdomens. These are here considered successful. However, many of the originally infected spiders turned out to have no acrocerid larva inside, suggesting that they had been able to suppress the parasitoid infection. Most of the spiders originally infected with only one larva (30 out of 37 = 81%) got rid of it, while this was the case for only a minority (4 out of 15 = 27%) of multiply infected spiders ($\chi^2_1 = 13.96$, $P < 0.0002$). All four spiders originally infected with 4–8 planidia had larvae in their bodies when dissected; of those infected with 2–3 planidia, 4 out of 11 spiders had suppressed the infection. Of the three emerging larvae, two were from singly infected and one from a doubly infected spider.

Spider survival.—Survival of the spiderlings was higher in the control group than among infected spiders (Wilcoxon test, $\chi^2_2 = 23.0$, $P < 0.0001$; Fig. 1) and directly related to the number of infecting larvae (Acr = 0 vs. Acr = 1: $\chi^2_1 = 4.44$, $P = 0.0351$; Acr = 1 vs. Acr = 2+: $\chi^2_1 = 8.08$, $P = 0.0045$). Among the singly infected spiders, survival was much better in those that suppressed the infection than in those that did not ($\chi^2_1 = 7.7$, $P = 0.0054$), and the same was true for multiply infected spiders in spite of low sample size ($\chi^2_1 = 4.7$, $P = 0.0296$). Survival of singly infected spiders that suppressed the infection was intermediate between control spiders and all singly infected spiders and did not differ from that of control spiders ($\chi^2_1 = 1.0$, $P = 0.31$). Thus, survival seems to be determined by whether or not the larvae survive and grow in the spider's body. Therefore, overall survival of singly infected spiders was only slightly reduced because most spiders suppressed the infection, whereas multiply infected spiders (with 2–8 larvae initially), of which fewer succeeded in suppressing the infection, had more strongly reduced survival (Fig. 1).

Spider growth.—Spiders infected with acrocerid larvae had higher growth rates than control spiders, and the more larvae initially attached themselves, the higher the growth stimulation (repeated-measures ANOVA, Wilk's $\lambda = 0.3$, $P < 0.0001$; Fig. 2). Growth stimulation was significant in the spiders that suppressed the infection, both as regards the singly (contrast: $F = 2.39$, $P = 0.0126$) and the multiply infected (contrast: $F = 4.41$, $P < 0.0001$) spiders. There was no difference in growth stimulation, however, between the spiders that remained infected and those that suppressed the infection (singly infected spiders: $F = 1.59$, $P = 0.18$); multiply infected spiders could not be tested due to small sample sizes, but show a trend of reduced growth stimulation in spiders with a growing larva (Fig. 2).

Spider development.—For individuals that completed development to the adult stage, total development time was independent of the number of initially infecting acrocerid larvae (ANOVA, $F_{2,49} = 0.38$; $P = 0.69$) and of spider sex (Welch *t*-test, $t = 1.85$; $P = 0.0734$). Spiders with a successfully developing acrocerid larva took longer to reach maturity than uninfected spiders (142.0 vs. 122.8 days; *t*-test, $t = 4.82$, $P = 0.0019$, $n = 52$). The first three instars were rather short (1–2 weeks) in the normal pattern of juvenile development unaffected by parasitoids, and were followed by two very long (5–6 weeks) and an intermediate (ca. 3 weeks) instar. This pattern was repeated in singly infected spiders (most of which suppressed the parasitoid), but not in the multiply infected spiders (Fig. 3). In the latter, the fourth instar was as short as the three previous ones, and the fifth and sixth instars were prolonged. Spiders matured after 5–7 juvenile instars; the maturation instar was independent of

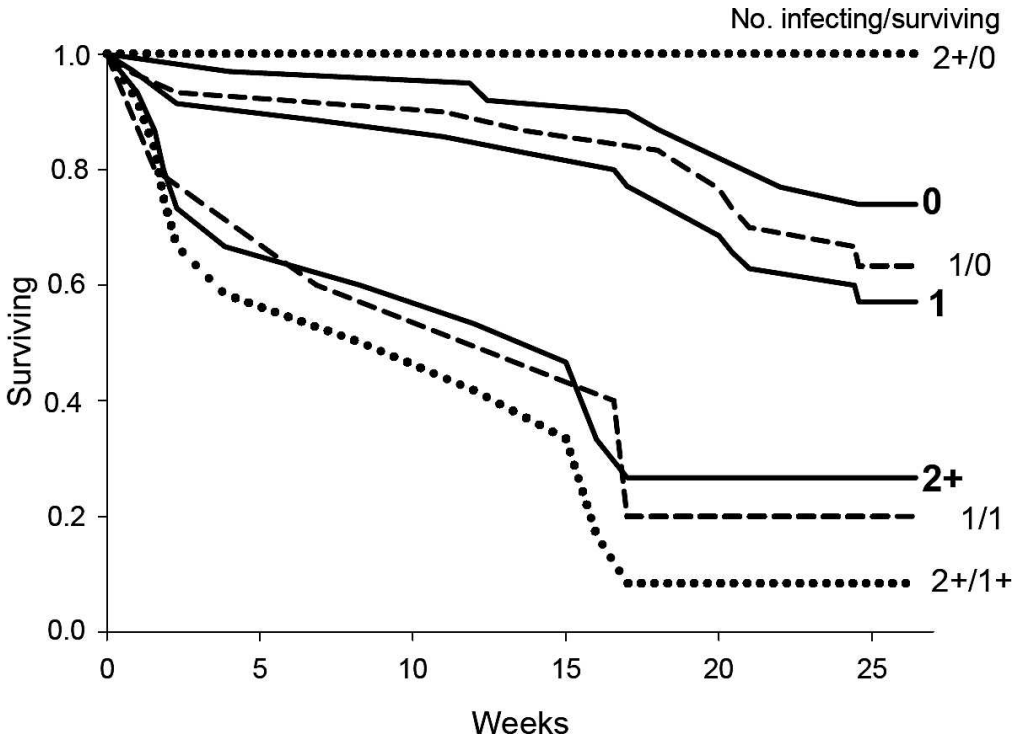

Figure 1.—Survivorship curves for groups of spiders subjected to infection by larvae of the acrocerid fly *Acrocera orbiculus*: uninfected (control) spiders (marked **0**); spiders originally infected with one (marked **1**), split into those that suppressed the infection (1/0) and those that did not (1/1); spiders originally infected with 2–8 (marked **2+**) larvae, split into those that suppressed the infection (2+/0) and those that did not (2+/1+).

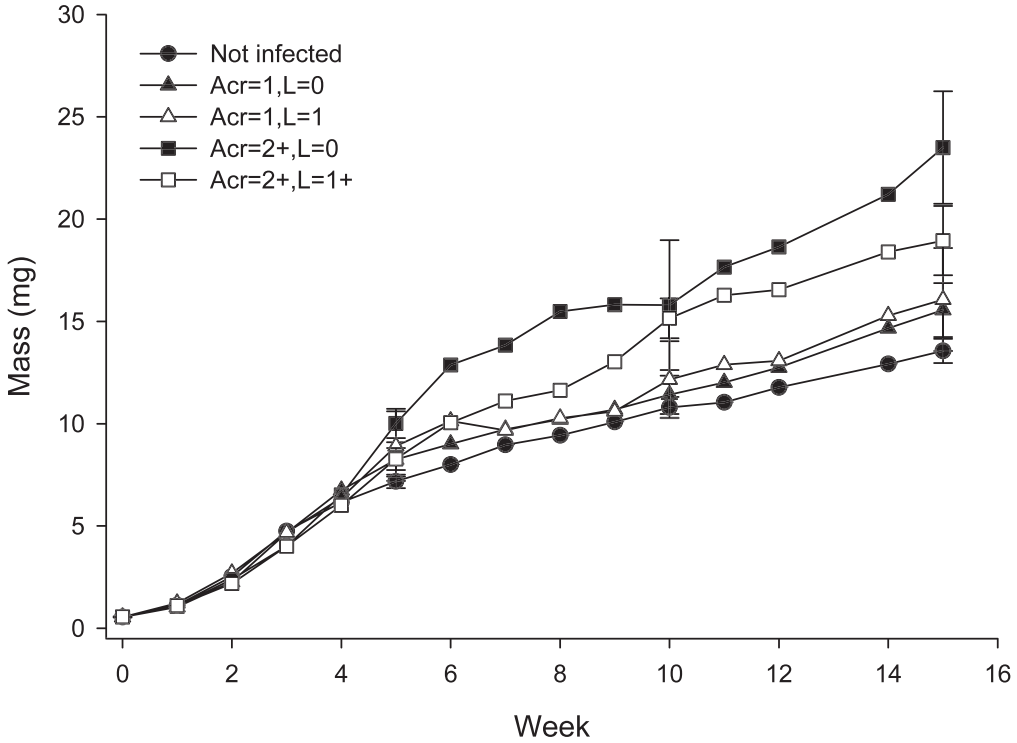

Figure 2.—Growth curves for control spiders and spiders infected with planidia larvae of an acrocerid fly. Acr: number of initially infecting larvae (0, 1 or ≥2); L: number of growing larvae in the spiders' body (0, 1 or ≥2). For clarity, error bars (SE) indicated only at weeks 0, 5, 10 and 15.

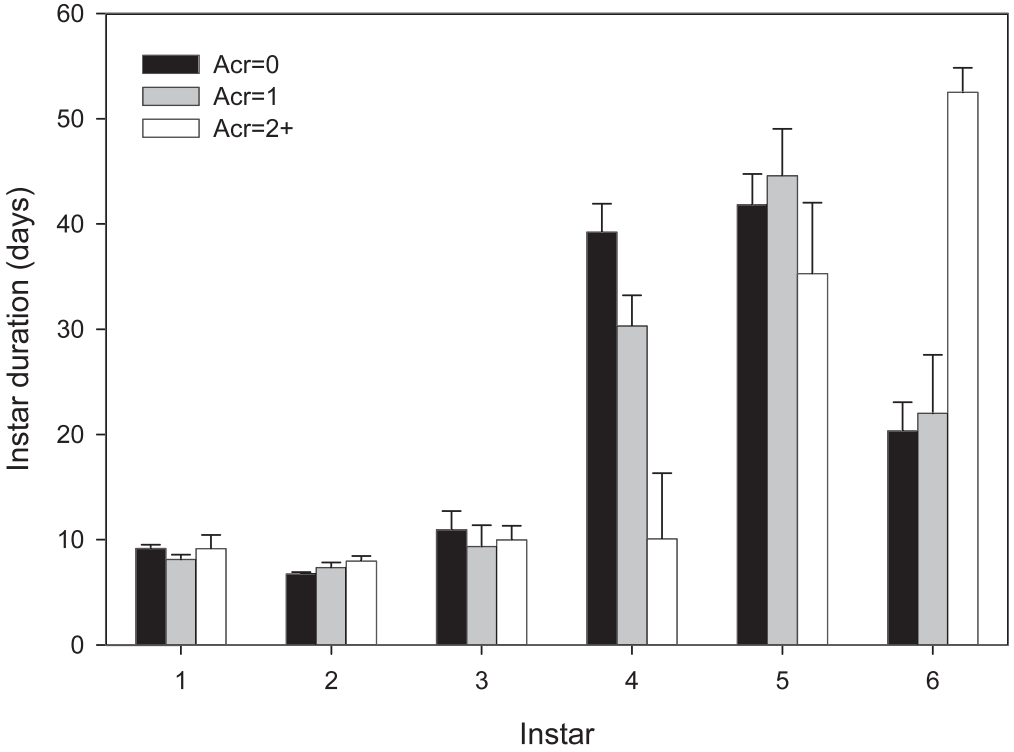

Figure 3.—Duration (mean + SE) of juvenile instars of the wolf spider *Pardosa prativaga* depending on initial infection load by planidia larvae of an acrocerid fly. Acr = 0: uninfected control spiders; Acr = 1: spiders infected with 1 larva; Acr = 2+: spiders infected with 2–8 larvae. Infection happened at the start of instar 1.

sex ($\chi^2_2 = 0.11$, $P = 0.95$), number of initially infecting larvae ($\chi^2_4 = 5.62$, $P = 0.23$) and whether there was a surviving larva ($\chi^2_2 = 0.96$, $P = 0.62$).

DISCUSSION

Most spiders infected with a single parasitoid succeeded in suppressing the infection and in obtaining a close to normal life in terms of survival and growth. It is unknown to what extent the spiders' high success of parasitoid suppression will apply also to natural conditions. The ad libitum feeding of the spiders with nutrient-enriched flies may have strengthened the spiders' immune system (Slansky 1986) to more effectively combat the infection. Several environmental conditions in the laboratory also differed from those in the field, but it cannot be decided whether these have benefitted the spider or the parasitoid. The wolf spider may also be an inferior host for *A. orbiculus*. Species of *Acrocera* have a wide host range, including seven spider families (Schlinger 1987), but the relative suitability of these potential hosts is unknown. The greatly increased mortality of successfully infected spiders compared with control spiders and unsuccessfully infected spiders may indicate a possibly low suitability of *P. prativaga*. The fact that we only allowed infection of spider hatchlings may also have been of importance, as the instar infected is known to affect the suitability of insect hosts of parasitoids (Harvey 2000; Harvey et al. 2010; Khafagi & Hegazi 2008). It prevented the acrocerid larva from immediate growth and development and enforced upon it a period of developmental arrest, waiting for the spider to grow sufficiently for the parasitoid to complete its development. We have no information on the optimal host size for infection, or how *A. orbiculus* synchronizes its life cycle with that of its spider hosts.

Multiple infections had higher costs for the spiders in terms of reduced survival, but were much more successful from the fly's point of view than single infections. More larvae developed from multiply than from singly infected spiders in spite of many fewer multiply infected spiders. Superparasitism thus seems advantageous, probably because it may help to break the host's resistance, even in a situation where only one parasitoid per host can complete development, as known also from insects (Blumberg & Luck 1990; Khafagi & Hegazi 2008). The adult fly is of approximately the same size as the adult spider, probably eliminating the possibility that more than one fly can successfully emerge. It may therefore be hypothesized that the females' habit of laying eggs in large clumps on the vegetation is an adaptation that increases the chance of multiple host infection. Multiple infections will lead to strong competition between the larvae with only one of them being successful. However, when they occur, multiple infections are likely to be by larvae from the same batch of eggs; i.e., they will be kin (at least half-sibs), and even the larvae that succumb will gain an inclusive fitness benefit. In some cases two acrocerid larvae were found in a spider's abdomen, but in no case were these of the full size ready for pupation, as was common with single larvae. It is therefore doubtful if enduring multiple infections ever lead to pupation. The benefit may be restricted to cases where competition between the larvae results in an early winner.

Contrary to most solitary hymenopterous parasitoids of insects (Slansky 1986; Harvey et al. 1999), acrocerid infection

caused a stimulation of spider growth and thus an increased rate of feeding. Multiply infected spiders also had enhanced growth compared with singly infected spiders; i.e., the stimulation depended on the infection load. Since the body mass measurements included the combined masses of the spider and the parasitoid larva, the enhanced total growth might result from simply adding the growth of the larva to that of the spider. Enhanced growth of infected hosts may be interpreted as a result of parasitoid manipulation through which the parasitoid adjusts the resources available from the host for optimizing its own growth. Thus, Harvey (2000) and Harvey et al. (2010) found that parasitoid infection reduced the growth of large, but stimulated that of small, host species. Stimulation of growth in *P. prativaga* might thus be due to infection of the hatchling instar. However, the data do not support this view: growth enhancement occurred as a result of the original infection, independently of the fate of the parasitoid; it was seen also in the singly infected spiders that suppressed the parasitoid, and parasitoid larvae successfully developing in the spider's body did not further increase the spider's growth rate. This indicates that growth enhancement might be part of the process by which the spider fights the parasite. Our experimental spider, *Pardosa prativaga*, is known to be able to quickly catch up with a setback in growth and development following periods of food stress (toxic or nutrient deficient food) by compensatory growth (Jespersen & Toft 2003). We suggest that a similar process of enhanced resource acquisition and mobilization is induced in response to parasitoid infection, perhaps induced already by the planidia larva. The enhanced growth is obvious already from Week 5 (Fig. 2), which is probably much earlier than the period of intense growth of the parasitoid larvae. Thus, it may not so much be a reduction in resources for its own growth that stimulates the spider, but rather the increased nutritional demands of the spider's immune system during encapsulation (i.e., resisting a parasitoid infection) of the parasitoid (Slansky 1986; Strand & Pech 1995). Since there may be a cost of increased growth per se (Higgins & Rankin 2001), the reduced survival of infected spiders may be explained not only by a negative ''health effect'' due to the infection but also by this growth cost, or both. This line of argument interprets the enhanced growth rate of the spiders as a costly response likely to be paid later in terms of reduced reproductive success (Metcalfe & Monaghan 2001). Encapsulation was also found to have costs in a pyralid moth (Harvey et al. 1996).

The study has revealed an impressive ability of the wolf spider to suppress an infection by the acrocerid fly. This comes on top of previously reported pre-infection attacks on the larvae as prey, and early post-infection ability to mechanically free itself from attached larvae (Nielsen et al. 1999). Physiological and life history costs to spiders that suppressed their infection seemed minimal. Unfortunately, we did not also have the opportunity to test whether parasitoid suppression modified the spider's subsequent reproductive life. At the same time the parasitoid had a heavy impact on the spiders in terms of higher mortality. It remains to be established whether *A. orbiculus* has more suitable hosts than *P. prativaga* available in the northern European part of its distribution. If not, this may explain its rarity here.

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