Interspecific competition between *Rhinocyllus conicus* and *Urophora solstitialis*, two biocontrol agents released in Australia against *Carduus nutans*

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Abstract. The univoltine receptacle-weevil, *Rhinocyllus conicus*, introduced into Australia in 1988, has a major impact on seed-set in capitula that are produced early in the flowering season of nodding thistle, *Carduus nutans*. Due to the extended flowering season, compared to Europe or North America, this weevil has very little impact on total seed-set. The seed-fly, *Urophora solstitialis*, which has a partial second generation, was introduced in 1991 to complement the affects of the weevil. This fly attacks capitula produced throughout the flowering season, and in Australia is more effective on its own in reducing total seed-set than the weevil. Field results from one site show that in the first part of the season, contrary to expectations from the literature, *R. conicus* outcompeted *U. solstitialis*. Competition was less severe at a neighbouring site where the density of weevil eggs per capitulum was much lower. A native parasitoid, *Anaphes* sp. is increasingly utilizing *R. conicus* eggs as a host, and the implications of this for biological control of nodding thistle are discussed.

Introduction

Rhinocyllus conicus was chosen as the first agent to be introduced to control nodding thistle, *Carduus nutans*, in Australia (Woodburn and Cullen 1995). This weevil overwinters as an adult, and it commences to feed on the rosette leaves in spring. Ovarian development has occurred by the time that the first green capitula appear, and oviposition begins. The eggs are laid on the involucre bracts, and are covered with a frass cap. Upon hatching, the larvae eat their way down the bracts and end up in the receptacle tissue, which they then proceed to mine. Pupariation occurs within the capitula. The life-cycle takes about six weeks from egg to adult and for the most part there is only one generation a year (Zwölfer and Harris 1984).

Rhinocyllus conicus was selected as the first agent because of its success in controlling the weed in Virginia (Kok and Surles 1975) and in Canada (Harris 1984). It has also been rated fourth as one of the most successful cases of biocontrol of weeds (Crawley 1990). However, this weevil has not been successful in controlling populations of the weed in the southern hemisphere, either in New Zealand (Kelly and McCallum 1995) or in Australia (Woodburn and Cullen 1993).

Ecological studies in the thistle's home range in Europe have identified the tephritid seed-fly, *Urophora solstitialis*, as a herbivore with potential as a biocontrol agent (Sheppard *et al.* 1994), due in part to its ability to undergo a second generation (Möller-Joop and Schroeder 1986). This insect overwinters inside woody galls as fully-fed third-instar larvae and emerges over a six-week period, commencing in early spring (Woodburn 1996). Ovarian development occurs during the pupal stage, and the females are capable of mating and ovipositing soon after eclosion. The female drills her ovipositor through the involucre bracts of green capitula (also the preferred stage for oviposition by *R. conicus*), and lays an egg into a developing floral tube. Upon hatching, the larva eats its way down through the developing ovule and into the receptacle tissue. Here it induces the plant to form a gall, which can coalesce with neighbouring galls to become a multi-locular, lignified structure. Progeny from the first eggs laid develop into adults, and hence undergo a second generation (Möller-Joop and Schroeder 1986), whilst progeny from eggs laid after the summer solstice enter diapause (Woodburn unpublished). The gall structure caused by tephritids acts as a powerful metabolic sink, utilizing resources that would otherwise be used for seed production (Harris 1980).

Urophora solstitialis was released in Australia as a second biocontrol agent for *C. nutans* (Woodburn 1993).

There are conflicting reports in the literature about the competitiveness of these two capitulum-feeding insects. Zwölfer (1973) showed that *U. solstitialis* was largely displaced by *R. conicus*. Nonetheless he believed that *R. conicus* would be the more effective agent because of its high oviposition potential and propensity to spread its eggs over many capitula. Also, he predicted that the presence of the weevil should not prejudice any later introduction of the seed-fly which tends to oviposit many eggs on fewer capitula than the weevil (Zwölfer 1973). Another field study showed that, although the fly tended to avoid capitula that had been attacked by *R. conicus*, fly-survival in heads with and without weevils was similar (Woodburn and Sheppard 1995). In a laboratory study, Möller-Joop and Schroeder (1986) demonstrated that survival of *R. conicus* larvae was slightly higher in heads where the fly was absent, than in heads where both insects were present. Harris (1989a) hypothesized that a woody-gall-former would be his first choice as a biocontrol agent. He cautioned against introducing a receptacle-feeder that hatched at the same time as a gall-producer, as he predicted that the receptacle-feeder would displace the gall-former.

This paper reports on some preliminary results of interspecific competition between these two biocontrol agents in the field in Australia.

Materials and methods

The receptacle-weevil, *R. conicus*, was released in the Yaouk Valley, in the southern tablelands of New South Wales (NSW) in 1988 (Woodburn and Cullen 1995). By the spring of 1994, this weevil had spread to all infestations in the valley and travelled at least 15 km in one direction. The seed fly, *U. solstitialis*, was released in the same area (but at two sites about 1 km apart) in autumn and spring of 1992 (site B and A respectively), and by the spring of 1994 had spread only a limited distance from each release site. From spring through autumn, random samples of mature capitula were collected each fortnight from both sites for later dissection in the laboratory. The samples were of all available mature capitula, or up to a maximum of 50 per sample, with the exception of the first sample at site A which consisted of a collection of aborted capitula. Data gained from these samples included

attack-rates of both biocontrol agents, and their impact on seed production.

At site A, measurements were taken each fortnight of the diameters of all the flowering capitula found on the plants in a known area, which allowed a calculation of the theoretical seed-rain per m^2 to be estimated. Quadrats (each of 0.25 m^2) were thrown 15 times before there were sufficient plants included to be representative of the thistle population at the site. It was assumed that the phenology of flowering was similar at both sites and at the end of the flowering season at site B, a count was made of the number of flowering plants over a randomly selected transect of 8x1 m which provided a scaling factor for the theoretical seed-rain at this site. The influence of the biocontrol agents on seed-rain was calculated by assuming that each final-instar *R. conicus* larva destroyed 29 seeds (Woodburn unpublished). This figure was the same as Popay *et al.* (1984) and similar to those of Kelly *et al.* (1990) and Sheppard *et al.* (1994) who recorded 27.7 and 26 seeds respectively. The number used for seed-loss attributed to each *U. solstitialis* final-instar larva was 6.9 (Woodburn 1996), which was similar to the number, seven, obtained by Sheppard *et al.* (1994). For further details about sampling see Woodburn and Cullen (1993). Sampling commenced at site A (the primary research site) on 18 December 1994 and a fortnight later at site B.

Results

Phenology of flowering
Plants on the designated seed-rain quadrats at site A (3.75 m^2), which were chosen since they reflected the thistle population, did not commence flowering until early in January (Table 1). Samples of mature capitula, however, were collected from mid-December, initially over an area of about 0.5 ha from those few plants which commenced flowering early in the season. The number of capitula sampled at both sites throughout the season is also presented in Table 1. These early capitula, although they are the largest produced and therefore contain the most seed (Woodburn and Cullen 1993), have minimal input to the total seed-rain. The densities of flowering plants at sites A and B were 3.7 and 9.9 plants per m^2, respectively.

Attack by agents at site A
All of the capitula from the first two samples were heavily attacked by *R. conicus*, with a mean of 197 and

Table 1. Phenology of flowering, and the number of flowering capitula dissected, from *C. nutans*, from two sites in the Yaouk Valley, NSW.

Date	Flower production/ m²		No. of capitula dissected	
	site A	site B	site A	site B
18/12/94	0	0	28	0
4/01/95	1.3	3.3	6	24
18/01/95	1.3	3.3	44	50
1/02/95	1.7	4.4	51	49
15/02/95	9.8	25.4	49	50
2/03/95	17.0	44.3	50	50
15/03/95	1.7	4.4	50	51
30/03/95	0.4	1.1	50	50
13/04/95	0	0	22	10
Totals	33.2	86.3	350	334

68 eggs per capitulum, respectively (Fig. 1a). From the start, both the proportion of capitula attacked and the number of eggs per attacked capitula declined. Estimates of survival were calculated from egg to final-instar larvae because, in many samples, development did not proceed beyond this stage, due to the mature capitula being sampled too soon or due to the fact that dissection in the laboratory did not allow sufficient time for pupation and or eclosion to occur. Survival of *R. conicus* was initially poor (2.2%, Fig. 2a), due to intense intra-specific competition (Sheppard and Woodburn in press). The proportion surviving increased as the season progressed, which corresponded with the decline in the mean number of eggs per capitulum.

The successful attack rate by *U. solstitialis*, as measured by gall establishment, is shown in Fig. 3a. There was no evidence of this agent in the capitula that were formed during December, with the first small galls appearing in January and which averaged five insects per gall. The proportion of capitula attacked

was not very high (0.33, two out of six capitula, with 70 and 78 weevil eggs, recorded for early January and which should be treated with caution because the measurement was derived from a small sample; Table 1). The second generation of *U. solstitialis* was more successful, and from late February through March both the incidence of attack and the number of insects per gall increased (Fig. 3a). Unlike the situation with *R. conicus*, estimates of survival from egg to diapausing larvae or adults could not be made because there was no way of determining the initial number of eggs laid, since these were deposited inside the florets of the developing flowers. The combined, estimated proportion of seeds destroyed by both agents is presented in Fig. 4. There were no seeds set in the first two samples due to the fact that all heads were heavily attacked by the weevil. The proportion destroyed in each randomly-collected sample fell in January, and remained between 10 and 20% for the rest of the season.

Seed-rain m⁻² is presented in Fig. 5a. The theoretical total seed-rain, calculated using the capitula diameters measured in the field each fortnight, was estimated to be 3696 m⁻². The observed seed-rain, which is the theoretical estimate modified to account for seed-destruction attributable to the biocontrol agents (provided from the dissections) was calculated as 3083 m⁻², giving a total reduction of 17%.

Attack by agents at site B

The pattern of attack of *R. conicus* was similar to that seen at site A, albeit at lower levels, i.e. initial egg numbers and attack rates were high, both of which declined with time (Fig. 1b). The survival rates from egg to final instar larva were also higher than at site A (Fig. 2b). *Urophora solstitialis* was found to be present in every sample collected from site B (Fig. 3b). The
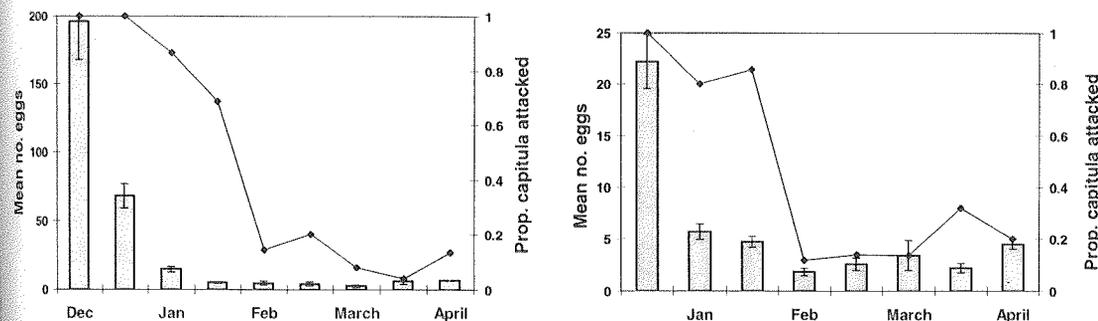
Fig. 1. Proportion of capitula attacked by *R. conicus* (graph line) and the mean (± SE) number of eggs per attacked-capitulum (histogram bars), at site A (Fig. on left) and site B (Fig. on right).
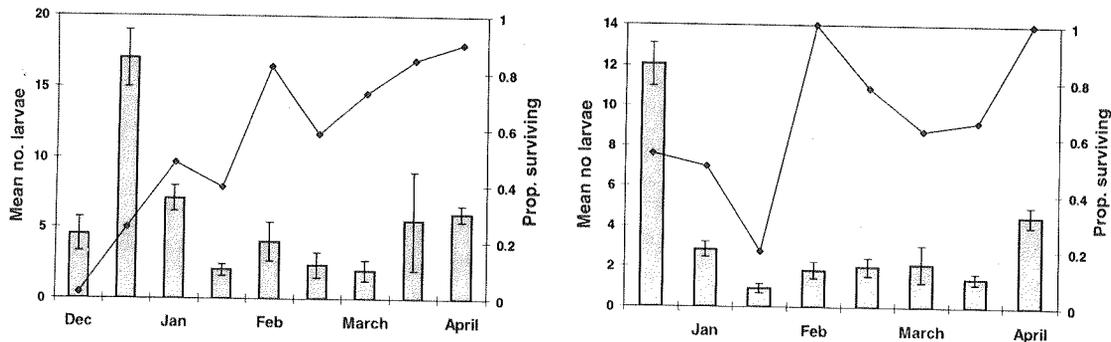
Fig. 2. Survival, from the egg stage, of final-instar larvae of *R. conicus* (graph line) and the mean (±SE) number of larvae per attacked-capitulum (histogram bars), at site A (Fig. on left) and site B (Fig. on right).

early-January sample (when *R. conicus* averaged 22.2 eggs, with a range of 6-60, in capitula where *U. solstitialis* larvae were also present) had 62.5% of the capitula attacked, with each of these averaging 7.9 insects. Both the mean number of insects and the proportion of heads attacked declined in the next two samples, which was reversed by the second week in February. All the capitula in the first two samples in March were attacked by the seed-fly, with means of 25 and 33 insects per capitulum, respectively. There was a decline in both insect numbers and proportion of capitula attacked in the last two samples (Fig. 3b). The proportion of seed destroyed at each sampling date is shown in Fig. 4. Initially, the majority of the seeds was destroyed, but fewer were destroyed in the January and early-February samples. The proportion of destroyed seeds then rose again and declined in the late-March and April samples. When these results are corrected to account for the density of capitula produced m^{-2} (Fig. 5b) (see above), the theoretical and observed totals for seed-rain were calculated as 9777 and 2865 seeds m^{-2}, respectively, which was a reduction of 71%.

Discussion

Plant density at site B was 2.7-fold higher than at Site A. The dissection results for the samples on 4 January 1995 showed a 3.1-fold higher attack-rate by *R. conicus* at Site A (Fig. 1a), which would indicate that the populations of the weevil at both sites were at similar densities. The pattern of attack at both sites was also similar, with all capitula heavily attacked at the start of the season, which then lessened in both the proportion and the intensity of attack. There was an increase in weevil activity in autumn at both sites, which was the result of a cohort of weevils undergoing a second generation (Zwölfer and Harris 1984). The higher proportion of capitula attacked by *R. conicus* at site B towards the end of the season is consistent with the higher survival-rate of the larvae produced early in the season at that site (Fig. 2b).

Such inferences on population sizes could not be made for *U. solstitialis* as survival could not be estimated. However, at site A the overwintering population (estimated at 7.7 diapausing larvae m^{-2};
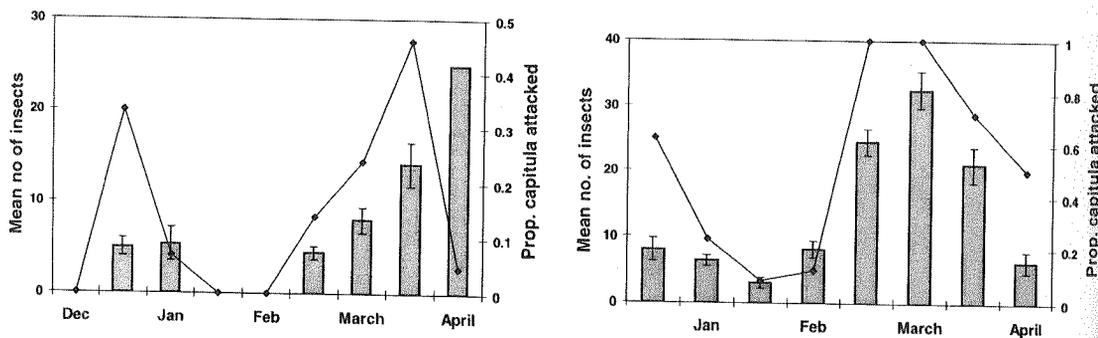

Fig 3. Proportion of capitula attacked by *U. solstitialis* (graph line) and the mean (±SE) number of insects per attacked-capitulum (histogram bars), at site A (Fig. on left) and site B (Fig. on right).

seed-fly averaging between 21 and 35 insects per attacked capitulum (Woodburn 1996). There is then a slight decline in attack-rate and number of insects per capitulum, whereupon both attack-rates and intensity of attack increase.

The results from site A are in line with Harris' prediction (1989a) that a receptacle feeder that lays large numbers of eggs would outcompete a woody gall-former that lays its eggs at the same time as the receptacle feeder. However, the findings are in contrast to those of Zwölfer (1979), who reported a decline in breeding success of *R. conicus* in the presence of *U. solstitialis* larvae. In his study, the mean number of weevil eggs was <8 per capitulum, and the number of fly larvae was <20 per capitulum. The most likely explanation for these differences is that the grazing pressure exerted by *R. conicus* is much greater in the introduced ranges than it is in the home range. In its introduced ranges, the numbers of eggs per capitulum in the first part of the season are high, e.g. in Montana (Rees 1977) but not in Virginia (Smith *et al.* 1984; Kok and Pienkowski 1985), in Canada (Harris 1984), in New Zealand (T. Jessep personal communication) and in Australia (Woodburn and Cullen 1993, in press). In Sheppard's three-year study of *C. nutans* in France, the maximum number of eggs observed early in the season was 120, but the mean at this time was 40 per capitulum (A.W. Sheppard personal communication). Although these figures from Europe are low compared to the Australian results, the number of first-instar larvae is even lower due to egg parasitism (59%, 34% and 26% at three sites in France; Sheppard and Woodburn in press). The overall result of lower egg numbers and high rates of parasitism, is lower numbers of weevil larvae attacking the capitula in Europe, compared to Australia.

Reducing the grazing pressure of *R. conicus* early in the season was achieved at site B as a direct result of the higher population density of the thistles there. Another means of achieving reduced grazing pressure is to reduce egg to first-instar survival of larvae, which is achieved in Europe by parasitoids. In Australia, *Anaphes* sp. (Hymenoptera: Mymaridae), a native parasitoid of a cosmopolitan, generalist genus that attacks the eggs of certain Coleoptera, Hemiptera and Diptera (I D Naumann personal communication), is increasingly attacking the eggs of *R. conicus* in recent years and attacking them progressively earlier in each season (Woodburn unpublished). Its impact in the present study has been minimal. For example in the

first sample at site B, two eggs were parasitized out of 533 examined, and the seasonal rate of parasitism was 3.2%. Levels of parasitism between 0 and 19% have so far been recorded for this parasitoid at other sites (Sheppard and Woodburn in press). *Anaphes* sp. could therefore become very important in the dynamics of this thistle-capitulum system, if increased parasitism of *R. conicus* eggs leads to increased survival of *U. solstitialis*, through lower interspecific competition in capitula produced early each season.

Acknowledgments

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