

Release and Establishment of the Thistle-Head Weevil, *Rhinocyllus conicus*, in Australia

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Three strains of thistle-head weevil, *Rhinocyllus conicus*, have been released in Australia for biological control of nodding thistle, *Carduus nutans*. Two of these strains, from southern France and central Italy, were heavily contaminated with *Nosema*. Frass was examined microscopically and any diseased insects were discarded. The third strain, from New Zealand (originally from northeast France), was disease-free. Each strain has been introduced into the northern, central and southern tablelands of New South Wales. The strain from New Zealand has established at, and is dispersing away from, each release site. The French strain has established at all sites, but is only building in numbers and dispersing at 1 site. The Italian strain, released several years after the others, has successfully overwintered at least at 1 site. Plant numbers, the timing of flowering and grazing by sheep appear to be the factors responsible for establishment success, rather than different climatic tolerances of the strains.

Introduction

Nodding thistle, *Carduus nutans* (L.) (Asteraceae), was first noticed in 1950 after it had been accidentally introduced to Australia from New Zealand as a seed contaminant. There were many introductions over the next several years until it was declared a prohibited contaminant in the mid sixties. It is now a serious pasture weed in the tableland regions of New South Wales (NSW), and is presently contained to a few regions of Victoria and Tasmania. However, it has the potential to spread over a much wider area than it currently occupies (Medd and Smith 1978). A recent review by Popay and Medd (1990) provides an excellent overview of the weed's biology.

Flowering plant densities in southern France are $<2 \text{ m}^{-2}$, producing an estimated 217 seeds m^{-2} and resulting in soil seed banks of fewer than 60 m^{-2} (Sheppard *et al.* 1990). This markedly contrasts with the situation in Australia, where flowering plant densities have been recorded as high as m^{-2} , which were calculated to produce 38,000 seed m^{-2} , while soil seed banks exceeding 10,000 m^{-2} are

common (Woodburn & Cullen, unpublished data). From this it is easy to see why nodding thistle has been declared a noxious weed in Australia.

CSIRO commenced the Australian part of its biological control programme on nodding thistle in 1986 and this paper documents the release and establishment of the seed-head weevil, *Rhinocyllus conicus* Froel. (Coleoptera: Curculionidae), an agent that has been successfully introduced into North America and New Zealand for biological control of *C. nutans* (Julien 1987).

Origins of the Strains

One of the aims of this phase of the biological control of nodding thistle was to compare the performance of weevils collected from different climatic regions of Europe in the different climatic zones of Australia.

Weevils newly collected from 2 regions of Europe have been introduced: from the Larzac Plateau (Aveyron) in southern France, and from around Scritto in central Italy, climatically similar to the southern and northern tablelands of NSW

respectively. The strain that had been released in New Zealand via Canada (originally from Mulhouse (Alsace), northern France) has also been released.

Screening of the Strains for *Nosema*

The southern French weevils were the first arrivals into quarantine in Australia. These weevils proved difficult to rear in large numbers (Walker, A., personal communication), which subsequently resulted in the identification of *Nosema*, a microsporidian disease, in the culture (Milner, R.J., personal communication). A second shipment of 914 overwintered but unfed weevils was received from France, and was transferred to a laboratory at 27°C 14/10 light/dark (l/d) cycle. Each weevil was kept and fed separately. Subsequently, frass was examined for the presence of *Nosema* using a phase contrast microscope. When the first lot of frass was examined, 46% of the insects were found to be infected. An insect was not considered to be potentially disease free unless the frass remained free of disease for at least 14 d after the first check. This time allowed *Nosema* sufficient time to complete 1 life cycle and hence build up a detectable number of spores. Eighty-nine pairs of weevils were eventually found to be disease free; i.e., an infection level of 78.6%.

Single mated pairs of disease-free weevils were then caged on thistle heads and allowed to oviposit. Each pair was changed to a new flower head every day. The frass from each pair was checked periodically and, as a result, 1 pair was found to be infected. A preparation was made from every individual adult after death and was examined microscopically. None was found to be infected. The progeny of each line was kept separately through the overwintering period (3 months at 5-6°C, 8/16 l/d cycle). The lines were then transferred to 15°C, 14/10 l/d for a week and finally to 27°C, 14/10 l/d. As a final precaution, the insects were then transferred to individual pill boxes, fed and examined as above for the presence of *Nosema* before field release. This procedure resulted in one line being discarded as a precaution, although it was probably infected with a yeast that looked similar to *Nosema*.

All weevils from the first shipment collected in Italy were also infected with *Nosema*, and had to be destroyed. A second importation of overwintering unfed adults from the same area was screened as above and provided 21 disease-free pairs from a total of 151 insects received; an infection rate of 72%. The insects imported from New Zealand were *Nosema* free, and were reared in quarantine for 1 generation before field release.

Infested and disease-free insects from France were compared for longevity and fecundity. The disease-free insects laid an average of 97.9 ± 13.9 eggs over 21.8 ± 3.7 d whilst the infected ones laid 21.6 ± 3.7 eggs over 10.4 ± 1.8 d.

Release Procedures

Each weevil strain was released at well separated sites (at least 15 km apart) in 3 regions of NSW: the northern tablelands north of Armidale, the central tablelands west of Goulburn, and on the southern tablelands centred around Cooma. The releases were made both over caged plants (cages 0.5 x 1.7 m with 2 pairs/cage) and as free ranging adults (see Table 1). As flower heads from the cages matured, they were sprayed with adhesive to prevent dehiscence, and subsequently collected (a week or so after spraying) and dissected in the laboratory, where the parameters: number of eggs hatched, pupae, adults and seed set, were scored. Any adult weevils found in the heads were kept over winter in the laboratory and released back in the field in the following early summer.

Establishment of the Strains

Each weevil strain successfully completed a generation and overwintered at least at 1 site. The New Zealand strain established at all 3 sites (Table 2). For example, regular dissection of 50 random heads from the northern area in the 1990-1 season revealed that 80% of heads were attacked with an average of 18.6 ± 2.1 eggs/head in late November. This attack level fell to about 5% of heads throughout summer with 3.6 ± 1.5 eggs/head, rising in autumn when

April samples had 22% of heads attacked with 7.0 ± 1.2 eggs/head.

The French strain also established at each site, but is only increasing in numbers in the southern tablelands. In the 1990-1 season from this area, dissection of randomly selected heads in early December showed that 86% of heads were attacked with an average of 23.3 ± 3.6 eggs/head. The last sample to have *R. conicus* attack was in the first week of January, when the level recorded was 33% with 5.4 ± 1.8 eggs/head. Samples until late March revealed no further weevil activity, in marked contrast to the New Zealand strain.

Table 1. Details of total numbers of the 3 strains of *Rhinocyllus conicus* released in Australia.

Origin	Founding Number	Number Released
La Cavalerie (French)	178	420
New Zealand (French)	220	420 ¹
Scritto (Italian)	42	680

¹ 1,800 insects were reared but the same number of both New Zealand and French weevils were released at each site.

In the northern tablelands, the French strain died out, or is present at undetectable levels. This seemed to be due to a combination over 2 seasons of late flowering of the plants (beginning of January instead of start of November) and sheep eating the mature heads (that contain the developing weevils) when there was a shortage of pasture. In the central region, numbers built up well, but again due to lack of pasture all heads were eaten by sheep. At present, at this site, there is an established population, albeit at a low level.

Evidence for the establishment of the Italian strain, which was not released in all 3 areas until the 1990-1 season, has only been gathered at the southern region site, where eggs from overwintering field adults were first seen in the first week of January 1992. This is despite the fact that the thistles had been in flower at the site since at least mid November, and both the French and New Zealand weevils had been reproductive in this region since the end of November. This retarded development, compared with its host plant, will have to be examined experimentally and it raises some questions regarding its supposed climatic suitability.

Table 2. Establishment of 3 strains of *Rhinocyllus conicus* with an estimate of the distance moved.

Strain	Area ¹	Year Released	Status	Distance Moved (km)
French	NT	1988	Low levels 2 yrs, now ?	—
	CT	1988	Population buildup, grazing, now low	0
	ST	1988	Population buildup, spreading	1
New Zealand	NT	1988	Population buildup, spreading	4
	CT	1988	Population buildup, spreading	2
	ST	1988	Population buildup, spreading	3
Italian	NT	1990	?	—
	CT	1989	?	—
	ST	1990	Overwintered	0

¹ N = northern, C = central, S = southern, T = tablelands

Dispersal of the strains

Although nodding thistle is a serious weed on the tablelands of NSW, its distribution is very patchy and not continuous over large areas. This distribution makes it impossible to carry out

fixed point sampling at set distances from the release areas to measure dispersal of the weevil.

Given this limitation, estimates were made at the start of the 1991 season of movement from the release sites (Table 2). The New Zealand

strain is dispersing faster than the French strain at all 3 sites, although there is only 1 French-strain site where weevil numbers have been at similar levels to the New Zealand sites for a similar period. Plant quality and numbers have been good at this French-strain site over the years since release and, as yet, the insects have not colonised plants within 2 km of the release area, so it would be unlikely that availability of the host plants was responsible for the slower spread. It is more likely to be due primarily to lower fecundity of the French strain; a mean of 75.3 ± 14.2 eggs *cf.* 149.2 ± 18.9 for the New Zealand strain. (These data were collected from field cages that housed single pairs of weevils, and are likely to be underestimates of fecundity since some insects may have escaped from the cages either when heads were collected or through holes caused by the wind. The estimate of mean fecundity obtained in the laboratory for disease-free French weevils, for example, was 99.7.) Of lesser importance, perhaps, is the suggestion in our data that a small percentage of the New Zealand population undergoes a second generation, whilst the French strain does not.

During this establishment phase of the programme, most strain differences (apart from fecundity) that may have allowed a comparison of their relative performance, have been masked by phenological and demographic variability in the populations of the host plant.

This work with *Rhinocyllus* is part of a broader study of the biological control and population dynamics of nodding thistle that involves detailed monitoring of seed production, soil seed-banks and germination and subsequent survival of the seedlings at the release sites plus control sites set up in each region. Numbers of *Rhinocyllus* are now approaching levels where we can measure an impact on seed production under natural conditions. Early indications are that there is substantial reduction in seed production in the first part of the season, but that late summer/autumn production is only marginally lowered, or not reduced at all. This is the part of the season where our second agent, *Urophora solstitialis* (L.) (Diptera: Tephritidae) should have a major impact.

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