

# **Specificity, and bionomics of south-western Palaeartic biotypes of *Rhinocyllus conicus* Frölich (Col., Curculionidae), a biological control agent of Palaeartic thistles (Asteraceae) accidentally introduced to Australia**

Autor(en): **Aeschlimann, Jean-Paul**

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Specificity, and bionomics of south-western Palaearctic biotypes  
of *Rhinocyllus conicus* FRÖLICH (Col., Curculionidae),  
a biological control agent of Palaearctic thistles (Asteraceae)  
accidentally introduced to Australia

JEAN-PAUL AESCHLIMANN

AGROPOLIS, Avenue Agropolis, F-34394 Montpellier CEDEX 5 (France)  
Present address: 66 allée MacLaren, F-34090 Montpellier (France)

The host plant relationships, the phenological occurrence, and the various mortality factors operating on the egg stage of a range of *R. conicus* biotypes were investigated from 1985 to 1995 at over 130 sites in 5 countries of south-western Europe. Several discrete weevil biotypes could be recognized based on their preferred thistle host species, geographic and altitudinal distribution ranges, as well as timing of seasonal appearance, all these differentiations being further confirmed by the concordant findings obtained by KLEIN & SEITZ (1994) from allozyme and morphometric analyses of the same weevil material.

Based on these findings, 5 of the most promising *R. conicus* biotypes could be selected for shipment to Australia, each of them matching a well-defined host-plant target within a particular set of environmental conditions. Additional specificity testing could be restricted to cultivated artichoke, and its results showed that in the area of origin each of the 5 biotypes was perfectly safe with regard to this crop. As a consequence, shipments of French and Italian biotypes to Australian quarantines were organized with a view to biologically control weed populations of the accidentally introduced thistles *Carduus nutans*, *C. pycnocephalus*, *C. tenuiflorus*, *Cirsium vulgare*, and *Silybum marianum* in Australia. The drift of such biotypes onto non-target host plant species is being discussed in the light of a recent controversy on the environmental risks associated with the deliberate introduction of *R. conicus* into North America.

Keywords: Biological control, host plants, natural enemies, *Rhinocyllus conicus*, specificity, thistles, weeds.

#### INTRODUCTION

A good dozen of different Carduinae (true thistles) species from half a dozen genera of Western Palaearctic origin have been accidentally introduced to Australia where they are now being considered economically important weeds (WAPSHERE, 1984). In 1985 the CSIRO Division of Entomology headquartered at Canberra (ACT, Australia) initiated long term research programmes on the potential of natural enemies to biologically control introduced European thistles, based at its Montpellier (Southern France) Biological Control Unit.

Over the years, these efforts have resulted in an in-depth understanding of the life cycle, of the relative importance of each recruitment form, and of the factors of mortality operating on the main thistle genera involved in their area of origin, including *Carduus* (SHEPPARD *et al.*, 1991; 1994), *Onopordum* (BRIESE *et al.*, 1994), *Silybum* (AESCHLIMANN, unpubl.), and the centaureine *Carthamus* (AESCHLIMANN, 1997).

During the whole 1985–1995 period of investigation the weevil *Rhinocyllus conicus* FRÖLICH (Coleoptera, Curculionidae) was observed as an ubiquitous phytophage throughout all the countries surveyed along the northern Mediterranean basin.

Discrete biotypes (or «populations»<sup>[1]</sup>) of *R. conicus* were in particular recorded to occur as distinct entities in relation to each single weedy thistle host considered. A clarification of the status of these various biotypes was therefore needed in terms of host plant preferences, phenology, degree of biochemical differentiation, as well as sexual compatibility with a view to select the most appropriate weevil biotypes for deliberate introduction in an attempt to biologically control individual thistle species in Australia.

The present paper summarises the main results obtained in the course of this study. The most promising biotypes of *R. conicus* were collected, mass-reared, compared, and tested with regard to their specificity towards cultivated artichoke both in the field and in the laboratory ; the principal mortality factors of *R. conicus* eggs were determined, and their relative incidence estimated. As a result, several south-western European weevil biotypes could be selected for shipment to Australian units, subsequent quarantining, release and distribution against 5 introduced thistle species. The origin of the biotypes is recorded and their distinguishing characteristics analysed and discussed in the light of a recent controversy on environmental risks arising from classical biological control operations.

#### MATERIAL AND METHODS

Surveys covered areas in Spain, France, Italy, the former Yugoslavia, and Greece situated under predominantly Mediterranean, or temperate climatic conditions. The criteria used for selecting sites, the various destructive and non-destructive sampling techniques developed for assessing the occurrence of both the host plants and their natural enemies have been already described in detail (AESCHLIMANN, 1997). Sites chosen were in general sheep pastures on which one or more of the 6 following host thistles of *R. conicus* were present: *Carduus nigrescens* VILL., *C. nutans* L., *C. pycnocephalus* L., and *C. tenuiflorus* CURTIS, *Cirsium vulgare* (SAVI) TENORE, and *Silybum marianum* GAERTNER.

Sampling comprised either maturing flowerheads to assess the relative abundance and impact of the guild of phytophages, or adult weevils ready to oviposit after overwintering which were collected directly from the host plant by means of a mouth aspirator. In each biogeographic area, the presence of *R. conicus* was only studied in detail after a conspicuous occurrence of the weevil in association with one at least of its 6 main weedy carduine host plants had been detected.

Rearing occurred either in well ventilated plastic boxes (15 x 15 x 20 cm) for samples comprising 20–25 flowerheads, or in 50 l plastic containers tightly covered with a fine insect proof mesh for mass-producing adult *R. conicus* from large quantities of plant material, any emerging new adults being collected, identified, separated into sexes, and recorded twice a week. At the end of the emergence period, all capitula in each sample were dissected in the laboratory to determine the total number of galls produced, and the fate of every *R. conicus* individual in each cohort considered.

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<sup>[1]</sup> Alternatively referred to as «ecotypes» by ZWÖLFER & PREISS (1983), «faces» by HARRIS (1984), UNRUH & GOEDEN (1987), and STRONG (1997), «populations» by KLEIN (1991), or «strains» by WOODBURN & CULLEN (1993). As proposed by GONZALEZ *et al.* (1979), the term of «biotypes» [cf. also GOEDEN *et al.*, (1885) and KLEIN (1985)] seems, however, to be the most appropriate with regard to *R. conicus* and will hence be used throughout the present paper.

Specificity testing was based on the use of individual pairs of *R. conicus* once mating had been observed. Each mated female was placed in a single organdie bag (5 x 10 cm), and left at least 3 days on its original host plant. It was then transferred for another 3 days onto artichoke (*Cynara scolymus* L.), cv. Violet de Provence, a very close cultivated relative to carduine thistles, and back to its original host plant again for a final 3 days. Only such tests were considered successful in which female weevils survived for the complete testing period of 9 days, and laid eggs on their original host plant both before and after exposure to artichoke.

Shipping adult weevils either for further rearing or for biochemical analysis was made by packing lots of up to 200 individuals in well ventilated plastic boxes (5 x 10 x 3 cm) filled with several layers of slightly humid absorbing paper. Several boxes were dispatched at the same time in a polystyrene cool box maintained at about 10°C.

## RESULTS

### *Distribution and bionomics*

Over the whole 1985–1995 period a total of 132 sites were selected in the 5 countries surveyed, *i.e.* 10 in Spain, 54 in France, 35 in Italy, 22 in Yugoslavia (ending in 1991), and 11 in Greece. Those situated within 50 km distance of Montpellier were visited every fortnight on average, the others once a year, but in some instances up to 10 times in succession.

At every study site considered so far the egg-laying activity of *R. conicus* occurred each year during the same period, irrespective of the prevailing seasonal climatic conditions. The various biotypes examined could clearly be attributed to two distinct groups separated by an interval of about 2 months on average with regard to their seasonal field occurrence. The first group comprised “Mediterranean” biotypes<sup>[2]</sup> of *R. conicus*, *i.e.* weevils that were typically active during spring (*e.g.* La Crau and St-Gély in Tab. 1); the progeny of such biotypes all emerged as adults at the beginning of the summer (*cf.* Fig. 1). The second group of *R. conicus* was constituted by “Temperate” to continental biotypes that mainly oviposited during early summer (*e.g.* Larzac, Umbria, and Dordogne in Tab. 1); most adults of the subsequent generation emerged during August from flowerheads in which they developed, irrespective of their host plant and altitude, *i.e.* usually about 40 days later on average than those of the Mediterranean group. Also, it is worth emphasizing that all southern European biotypes in both groups observed so far went through one generation only per year, and had no partial second generation as described in particular by ZWÖLFER & HARRIS (1984) from Central European segments of the weevil’s distribution range.

Large batches of the 5 *R. conicus* biotypes selected for detailed investigations and subsequently for shipment to Australia (*cf.* Tab. 3) were collected each year in the course of several research projects. Tab. 1 and 2 exemplify the most striking biological differences observed between biotypes during the present study, and summarise all the data obtained from 1985 to 1995 based on 1–4 batches of at least 500 individuals each that were field sampled every year.

<sup>[2]</sup> As discussed below, these 2 groups of biotypes would have to be considered as 2 distinct subspecies of *R. conicus* according to KLEIN & SEITZ (1994).

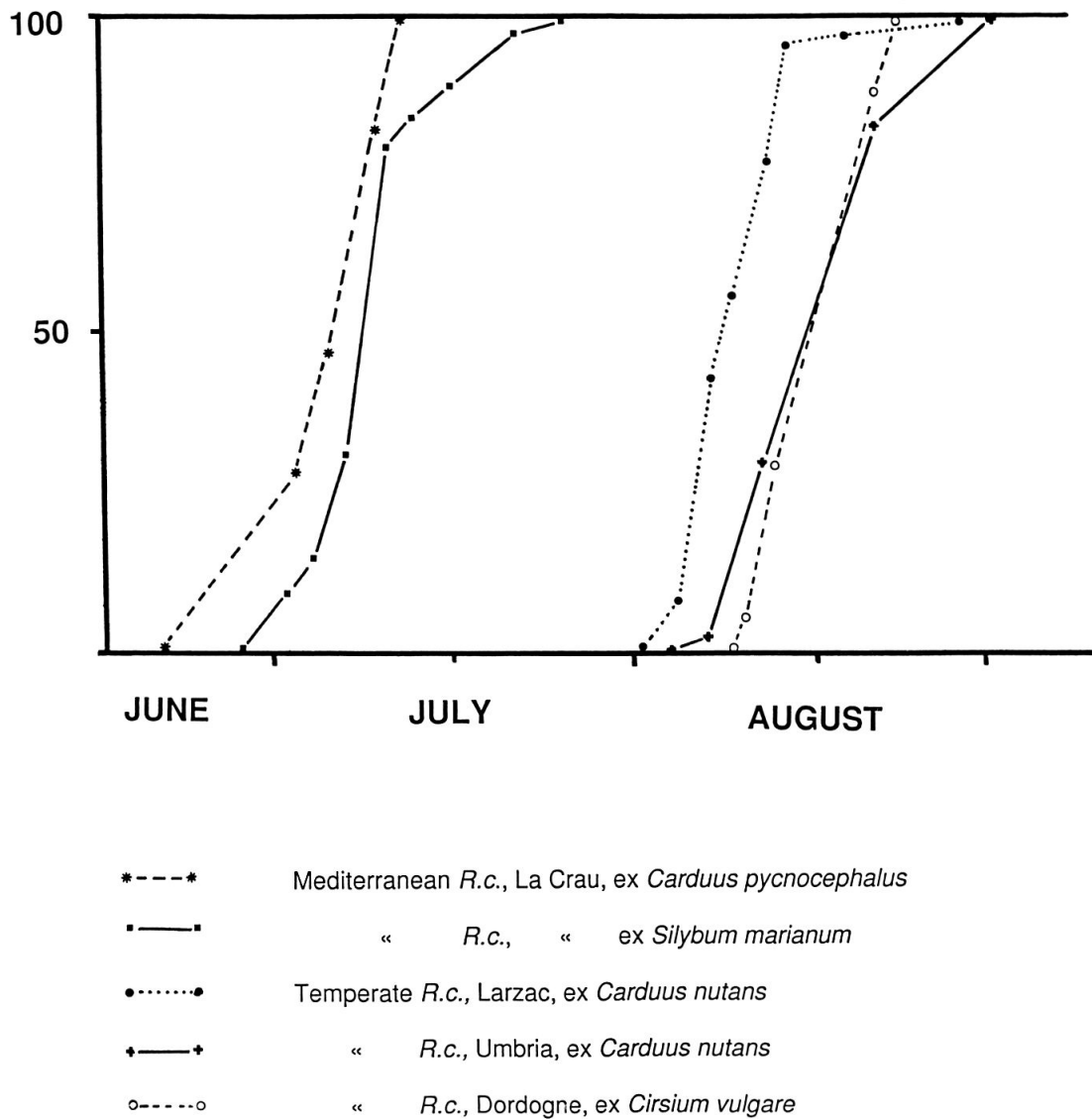


Fig. 1. Adult emergence (%) of 5 different biotypes of south-western European *Rhinocyllus conicus* (cumulated over the years 1985–95).

The occurrence of any single biotype peaked year after year during exactly the same time (Fig. 1), and was mainly correlated with the flowering period of the preferred thistle host, and the altitude of the location considered. Furthermore, the sequence of relative occurrence of various biotypes as presented in Fig. 1 strictly followed the same pattern each year. Even at one and the same site, as La Crau for instance (left part of Fig. 1), adult weevils regularly emerged from *C. pycnocephalus* heads consistently earlier than those developing in *S. marianum*. From that point of view the most striking phenological difference within the temperate group of biotypes was noted for adults of *R. conicus* emerging from capitula of *C. nutans*. At the Larzac (France), adult weevils regularly appeared about 2 weeks earlier than

those occurring in Umbria (Italy) in spite of the considerably higher average altitude of the Larzac site (1000 vs. 500 m a.s.l.; cf. right part of Fig. 1). As a consequence, the Umbrian biotype of *R. conicus* seemed able to occupy a particularly interesting niche on *C. nutans*, i.e. significantly later towards the end of summer than the Larzac weevils, and at a time when the seed production still appeared to be significant in terms of population dynamics, but when the effect of most seed predators has already seriously decreased (cf. SHEPPARD *et al.*, 1994). Fig. 1 finally also demonstrates that the Umbrian and Dordogne biotypes (extreme right), which are widely separated geographically nevertheless emerged almost synchronically from their respective host plants.

As already discussed by ZWÖLFER & HARRIS (1984), *R. conicus* has a large spectrum of potential host plants within the true thistles, with a very strong preference, however, for a particular species in any given area. This is best documented by the example of La Crau (Bouches-du-Rhône département in southern France, cf. Tab. 1) where the bulk of the population was associated with *S. marianum* (variegated thistle) for phenological reasons, while its early fringe attacked slender thistles (*C. pycnocephalus* and *C. tenuiflorus*) between late April and early May, whereas females reproducing very late in the season had to switch to *C. nigrescens* for oviposition towards the beginning of June. From that point of view, the number of eggs laid on average per mm receptacle diameter of each host plant available (cf. last column of Tab. 1) represented a convenient way to indirectly measure and compare the preference of any *R. conicus* biotype in the field for a particular thistle species (in fact the latter's attractiveness/acceptability).

Tab. 1. Characteristic distribution of the oviposition of various *Rhinocyllus conicus* biotypes in relation to various southern European thistle host populations. Overall average from all data collected 1985–1995. <sup>a, b, c</sup> = average values not significantly different when followed by the same letter ( $p > 0.05$ ).

Location /country	Peak of egg-laying period	Host plant species	Flowerhead mean diam. (mm)	Average egg no./mm
La Crau, F	May	<i>S. marianum</i>	32.6 - 43.0	0.59 - 4.29 <sup>a</sup>
«	«	<i>C. nigrescens</i>	16.2	0.05 <sup>b</sup>
«	«	<i>C. pycnocephalus</i> (& <i>C. tenuiflorus</i> )	8.8	0.16 <sup>c</sup>
St-Gély, F	«	<i>S. marianum</i>	38.3	0.42 <sup>a</sup>
Larzac, F	July	<i>C. nutans</i>	15.1	0.52 <sup>a</sup>
Umbria, I	«	<i>C. nutans</i>	17.5 - 22.9	0.07 - 0.19 <sup>c</sup>
Dordogne, F	«	<i>C. vulgare</i>	12.9 - 16.4	0.81 - 1.45 <sup>a</sup>

After having laid an egg on the most adequate flowerhead substrate, each female covers it with a special, hard, protective cap made out of plant particles glued together. Sometimes, however, such caps are being produced by a female that actually has not laid any egg. In the course of the present investigations, this unusual behaviour was detected in particular at such sites where several sympatrically occurring thistle species flowered at about the same time. It is being interpreted here as an «ab-reaction» of the female against host plants considered as unsuitable and therefore represented another indicator for the acceptability of potential thistle hosts. At La Crau for instance (cf. Table 2), the relatively high proportion of eggs «absent» under protective caps produced on *C. nigrescens* (20.0%) certainly represented a good indication that this host thistle was not the plant species preferred by the local weevils. Based on the same indicator, the Umbrian biotype may be perceived as switching to *C. nutans* from another, usually more appropriate thistle (most likely *C. vulgare*). On the other hand, *C. nutans* may be recognized as the most adequate and preferred host plant for the Larzac, and *S. marianum* for the La Crau and the St-Gély biotypes respectively.

#### Mortality of *R. conicus* eggs

Apart from the very concise observations reported by ZWÖLFER & HARRIS (1984), the survival of *R. conicus* egg populations has hitherto been poorly documented, both in the weevil's Palaearctic region of origin (ZWÖLFER, 1967), and in the areas of deliberate, recent introduction (GOEDEN & RICKER, 1980; DOWD & KOK, 1982; LITTLEFIELD, 1991). During the investigations reported here, the fate of all weevil eggs present in a sample was determined in the laboratory at the end of the rearing period. Each individual egg was assigned to one of the following 5 categories, based on examination under the microscope (cf. Tab. 2):

Tab. 2. Relative impact of some factors of mortality on different egg cohorts of various *Rhinocyllus conicus* biotypes in southern Europe. Overall average from all data collected 1985–1995. <sup>(2)</sup> = proportion (%) of normally hatched L<sub>1</sub> = [100-(col. 3+4+5+6)]. <sup>(3)</sup> = egg «cap» deposited normally, but empty, i.e. without any actual egg. <sup>a, b, c, d</sup> = average values in the same column not significantly different ( $p > 0.05$ ) when followed by the same letter.

Location /country	Host plant species	Proportion (%) of the egg population affected <sup>(2)</sup> by			
		'Absence' <sup>(3)</sup>	No developm.	Predators	Parasitoids
La Crau, F	<i>S. marianum</i>	0.9 - 6.9 <sup>b</sup>	0.0 - 1.6 <sup>a</sup>	4.4 - 27.0 <sup>b</sup>	0.0 - 0.9 <sup>a</sup>
	« <i>C. nigrescens</i>	20.0 <sup>d</sup>	1.6 <sup>a</sup>	4.4 <sup>a</sup>	0.9 <sup>a</sup>
	« <i>C. pycnocephalus</i>	0.0 <sup>a</sup>	12.0 <sup>b</sup>	16.0 <sup>b</sup>	0.0 <sup>a</sup>
St-Gély, F	<i>S. marianum</i>	0.0 <sup>a</sup>	1.2 <sup>a</sup>	2.5 <sup>a</sup>	0.8 <sup>a</sup>
Larzac, F	<i>C. nutans</i>	0.0 <sup>a</sup>	1.7 <sup>a</sup>	4.2 <sup>a</sup>	80.8 <sup>d</sup>
Umbria, I	<i>C. nutans</i>	5.7 - 16.4 <sup>c</sup>	3.2 - 13.8 <sup>b</sup>	7.9 - 14.6 <sup>b</sup>	44.8 - 62.5 <sup>c</sup>
Dordogne, F	<i>C. vulgare</i>	0.6 - 2.6 <sup>b</sup>	0.0 - 3.1 <sup>a</sup>	5.3 - 10.1 <sup>b</sup>	8.4 - 28.8 <sup>b</sup>

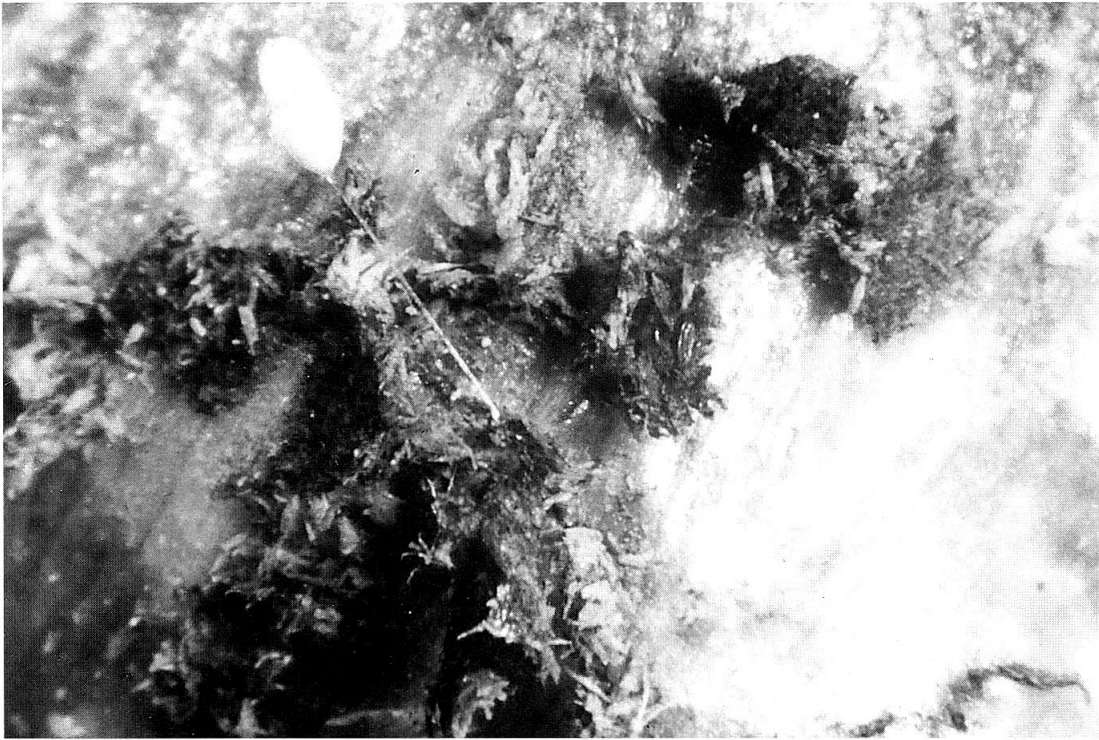


Fig. 2. Characteristic egg of chrysopid (centre to left) laid amidst a batch of empty *Rhinocyllus conicus* caps, the eggs of which have been destroyed by larval chrysopids underneath a flowerhead of *Silybum marianum*.

1. Absence of egg underneath the cap as described above (strictly spoken no «mortality» but a measure of the actual suitability of the host plant);
2. Unsuccessful development (egg either unfertilized, embryo dead for undetectable reasons, or unemerged, dead L<sub>1</sub>);
3. Destruction of egg by generalist predators (predominantly chrysopids, some of which typically laid their own eggs close to egg caps [cf. Fig. 2], but also heteropterans, and coccinellids);
4. Parasitisation by *Pterandrophysalis levantina* NOWICKI (Hym.: Trichogrammatidae; nec Pteromalidae cf. ZWÖLFER & HARRIS, 1984), a very peculiar ooparasitoid specialised in the detection of eggs under protective caps with a curious, strong sexual dimorphism easily observed at the wing venation;
5. Viable L<sub>1</sub>, that obviously hatched normally.

Most of the average numbers of eggs unable to complete normal development for various reasons (Tab. 2, col. 4) did not differ significantly between areas considered, nor between years at the same sites. Similarly, the overall proportion of eggs destroyed by insect predators remained fairly constant between years over the whole period of study and fluctuated within the same limits between different sites. In terms of rates of parasitisation by contrast, considerable differences were observed between sites. *P. levantina* was present under the Mediterranean climatic conditions that prevailed at sites like La Crau or St-Gély, but had almost no measurable impact there on *R. conicus* egg populations. Exactly the reverse was true at more temperate sites (e.g. in particular Larzac or Umbria), where several successive generations of the parasitoid affected from 50 to over 80 % of the eggs throughout the egg-laying season.



Tab. 3. Origin of the 5 *Rhinocyllus conicus* biotypes forwarded 1987–95 from southwestern European sites to Australia.

No.	Group <sup>(1)</sup>	Geographic origin of the biotype selected	Target plant
1.	T	La Cavalerie (Aveyron, F, 1000 m a.s.l.)	<i>C. nutans</i>
2.	T	Scritto (Perugia, I, 500 m a.s.l.)	«
3.	M	St-Martin-de-Crau (Bouches-du-Rhône, F, 50 m a.s.l.)	<i>C. pycnocephalus</i> & <i>C. tenuiflorus</i>
4.	T	St-Aquilin (Dordogne, F, 400 m a.s.l.)	<i>C. vulgare</i>
5.	M	St-Martin-de-Crau (Bouches-du-Rhône, F, 50 m a.s.l.)	<i>S. marianum</i>

<sup>(1)</sup> T = Temperate ; M = Mediterranean groups of biotypes

#### *Additional specificity testing*

The present investigations demonstrated that several, discrete biotypes of *R. conicus* were worth considering for introduction into Australia against various thistle hosts. In each instance, the only requirement posed by the Australian Plant Quarantine in addition to results already obtained elsewhere was to have every biotype tested on its specificity with regard to cultivated artichoke, *C. scolymus*.

As a consequence, before despatching any material to Australia, weevil pairs of the 5 biotypes recorded in Tab. 3, which represented both the Mediterranean (M) and the Temperate (T) groups were submitted to a reduced specificity testing programme in Montpellier (France). It was carried out from 1987 to 1990 and included a total of 15 to over 30 pairs, i.e. complete, validated replicates per biotype. The evaluation showed that for each biotype some adult nibbling limited to a few mm<sup>2</sup> foliage surface only occurred on some of the artichoke test plants. Whereas females of biotypes (2) and (4) did not lay at all on artichoke during the whole testing period, one single egg only on total was deposited by each of the other 3 biotypes on artichoke flowerheads. By contrast, 2–4 eggs on average per female and per day were laid in each instance on the preferred host plant both before and after exposure to artichoke. It is worth emphasizing that the single egg laid by each of the 3 biotypes on artichoke did not develop as it had not been covered by the usual protective cap.

#### DISCUSSION

Evidence summarised here demonstrates the existence of a whole range of well-defined *R. conicus* biotypes within their south-western Palaearctic region of origin. As for other curculionids subjected to similar, in-depth investigations (cf. the bionomics of the *Sitona humeralis* STEPHENS group of species described by AESCHLIMANN [1984]), two main reproduction patterns have been recognised amongst the various biotypes, i.e. (1) a Mediterranean, essentially *S. marianum*-related mode, and (2) a Temperate one, primarily associated with *C. vulgare*. As a consequence,

KLEIN & SEITZ (1994) have recently suggested to consider biotypes following the former pattern as representing *R. conicus* ssp. *oblongus* CAPIOMONT, those developing according to the second mode as *R. conicus* ssp. *conicus*.

All the biotypes studied in the Mediterranean countries surveyed so far were strictly monovoltine. Some of them differed noticeably in terms of area of occurrence, host plant preference, peak of egg-laying activity, main period of emergence, and reproductive biology. The incidence of various mortality factors observed on *R. conicus* eggs also differed significantly between weevil biotypes, including the proportion of protective caps deposited without any eggs. Finally, results obtained by KLEIN & SEITZ (1994) using both electrophoretic and morphometric analysis allowed for genetic distances between biotypes to be measured that again entirely matched the grouping made according to biological characters.

A review of pertinent literature shows that most of the *R. conicus* liberation programmes undertaken so far have experienced difficulties during the early stages of the weevil establishment process. This is true in particular of the U.S.A. (GOEDEN *et al.*, 1984); Canada (HARRIS, 1984); South Africa (ZIMMERMANN, 1990); New Zealand (KELLY & MCCALLUM, 1995); and Australia (WOODBURN & CULLEN, 1995). Most of these difficulties, however, are herewith considered as being the result of deliberate introductions based on the use of *R. conicus* biotypes that were not fully appropriate for the targeted thistle. These classical biological control programmes undoubtedly occurred under the misleading general feeling that *R. conicus* was an "easy-going, straightforward natural enemy, able to fit anyway into every new environment". Furthermore, a certain drift of exotic *R. conicus* onto non-target, possibly rare and endangered native host plant species has recently been measured in North America by STRONG (1997), and LOUDA *et al.* (1997), who therefore seriously questioned the adequacy of similar operations.

The results reported here demonstrate that in addition to its preferred host plant, every *R. conicus* biotype clearly retained the ability to also exploit other, sympatric thistle host species that flower locally according to an allochrone pattern of sequence (cf. KLEIN, 1986). In each new area of distribution, some degree of *R. conicus* incidence on endemic thistles closely related to the main weedy host plant was therefore obviously to be expected. It is nevertheless suggested here that a more careful selection of the candidate biotype in terms of its host plant, climatic, altitudinal and phenological preferenda in relation with the conditions prevailing in the target area, to be confirmed by an adequate biochemical determination (cf. UNRUH & GOEDEN, 1987) would have been likely to avoid part at least of the problems. On the other hand, the various parties involved in the potential conflict of interest should have been invited by the relevant authorities to discuss its various aspects before any introduction was even considered.

Each of the 5 *R. conicus* biotypes screened in the course of the research programmes described here proved to be safe with regard to artichoke both in cage trials and field experiments. This safety was further attested by the observation made at one site in the Dordogne-département (south-western France) where the weevil heavily infested *C. vulgare* in a sheep pasture, but was totally absent on a large number of artichokes planted in a home garden adjacent to the pasture.

From 1987 to 1995, up to 4 shipments per year, each comprising some 500 *R. conicus* could be forwarded to Australia for mass-rearing, and subsequent release in the frame of classical biological control programmes directed against 5 different introduced thistle species. To minimise problems arising from the switch of hemispheres in terms of synchronisation between host plant development and the most

favourable periods of the weevils' reproductive activity, different life stages of *R. conicus* were alternatively considered (developing larvae and pupae, either freshly emerged, or field-collected egg-laying adults, or even artificially hibernated weevils). Ongoing Australian rearings associated with several weed species were regenerated once a year through the incorporation of freshly field collected material.

Thorough examination of the material received in the Australian quarantines (Frankston, VIC and Canberra, ACT) revealed that virtually every biotype of *R. conicus* shipped from south-western Europe was contaminated to some degree by an as yet undescribed *Nosema* sp. (Protozoa: Sporozoa). Diseased adult weevils had a mean life expectancy reduced by about 50 %, the reproductive potential of contaminated females being decreased to some 20 % on average as compared to unaffected, healthy individuals. A rigid procedure was developed in the facilities to circumvent the strong, negative impact of the disease, whereby single *Nosema*-free males and females only were selected for further rearing.

Pending final assessment of each specific biological control programme, initial monitoring has detected signs of early establishment (WOODBURN & CULLEN, 1995) in Australia. Should the final impact of the phytophage remain low, the field synchronisation between the development of each weevil's biotype and that of its host plant would need to be examined with a view to possibly introduce another, better suited biotype, active at a more favourable time point during the season. In addition, a series of rust fungi accessions have been collected during the present surveys, including *Puccinia cardui-pycnocephali* SYDOW from *C. pycnocephalus* and/or *C. tenuiflorus* in north-eastern Spain, and *P. mariana* SACCARDO from *S. marianum* in south-eastern Greece (Basidiomycetes: Pucciniaceae). Some virulent isolates are hence available now for mass-propagation and further laboratory screening with a view to possibly complement the impact exerted by *R. conicus* on these thistle species in Australia.

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#### RESUME

*Spécificité et biologie des biotypes du charançon ouest-paléarctique *Rhinocyllus conicus* FRÖLICH (Col., Curculionidae) utilisés dans le cadre de la lutte biologique contre des chardons (Asteraceae) accidentellement introduits en Australie.*— Les relations entre plantes-hôtes et insectes, la phénologie et les facteurs de mortalité opérant sur les populations d'oeufs de plusieurs biotypes de *R. conicus* ont pu être précisés au cours d'une étude d'une dizaine d'années portant sur plus de 130 sites d'Europe méridionale. Plusieurs biotypes distincts ont pu être caractérisés en fonction de l'altitude de leur aire géographique de distribution, de leur plante-hôte préférée et de leur pic d'activité saisonnière. Toutes ces différences se trouvent en outre confirmées par les résultats concordants obtenus par KLEIN & SEITZ (1994) et portant aussi bien sur la morphométrie que sur l'analyse biochimique des allozymes du même matériel.

En conséquence, 5 biotypes prometteurs de *R. conicus* ont pu être sélectionnés en France et en Italie pour introduction en Australie dans le cadre de programmes de lutte biologique classique. Après que des tests de spécificité aient démontré que ces 5 biotypes étaient inoffensifs pour l'artichaut cultivé

dans leur région d'origine, ils ont fait l'objet de plus de 20 envois entre 1987 et 1995. Chacun de ces 5 biotypes correspondait préférentiellement à un ensemble bien défini de conditions environnementales dans l'aire d'introduction et à l'une des 5 espèces-cibles suivantes de chardons accidentellement introduits: *Carduus nutans*, *C. pycnocephalus*, *C. tenuiflorus*, *Cirsium vulgare* ou *Silybum marianum*. L'avenir de tels projets de lutte biologique est discuté à la lumière d'une controverse récente liée aux risques d'une certaine dérive récemment constatée en Amérique du Nord chez *R. conicus* dont une fraction des populations semble apte à passer sur des plantes-hôtes endémiques botaniquement proches des mauvaises herbes cibles.

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