Distribution of skeleton weed (*Chondrilla juncea* L) biotypes in Victoria

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Summary

A survey of three regions in Victoria, the Wimmera, Mallee and Goulburn Valley, was carried out during the summers of 1982 and 1986 to determine the presence and density of the three biotypes of Skeleton Weed, designated Form A, Form B and Form C by Hull and Groves (1973).

The biotypes were identified visually in 1982 and some plants were verified later using polyacrylamide gel electrophoresis of leaf and/or stem material. All material was identified using electrophoresis in 1986. Three distinct biotypes, narrow-leaved or form A, broad-leaved or form C and intermediate-leaved or form B (Hull and Groves 1973) were identified or confirmed using electrophoretic methods. These three biotypes are referred to as types rather than forms, which is used here to refer to the morphological variations or forms of the plant. Three morphological forms of narrow-leaved plants and two morphological forms of broad-leaved plants were observed in the field and confirmed as narrow- or broad-leaved plants using electrophoretic methods. Narrow- and broad-leaved plants were found in all regions surveyed. The intermediate-leaved plant was present only in low numbers and limited in distribution to the central Mallee and one site in the western Mallee in 1986. Only in five sites, mainly in the central Mallee, were all three biotypes found. Narrow-leaved plants remained the predominant biotype and significant differences in plant density were found within a region, between years and between regions. Broad-leaved plants were commonly found in the Mallee, with significantly more plants present in the eastern than western section during 1986.

Introduction

Skeleton weed, *Chondrilla juncea* L., is still a serious weed in some cropping areas of Victoria, particularly the Mallee and north Wimmera regions of the north-west and central-west of the state, and in the Goulburn Valley. It is an apomictic perennial herbaceous weed of European origin that can very occasionally reproduce sexually (P. Charboudez personal communications). Skeleton weed is thought to have been introduced into Australia with vine stock prior to 1910 (Wells 1971), and is now present throughout the wheat belt of south-eastern Australia (McVean 1966, Hull and Groves 1973) and also in Western Australia (Cullen and Groves 1977, Panetta 1984).

In eastern Australia *C. juncea* exists as three biotypes or forms, the narrow-leaved or form A, the broad-leaved or form C and the intermediate-leaved or form B (Hull and Groves 1973). All three biotypes, designated forms by Hull and Groves (1973), can be distinguished visually using leaf shapes and the angle of the lateral branches to the main flowering stem (Hull and Groves 1973).

Until the initiation of the biological control program for skeleton weed in Australia in 1966, the existence and distribution of each biotype was unknown. One broad-leaved plant was found near Ouyen, north-western Victoria, in 1968 (Hull and Groves 1973). This plant was well outside the then known distribution of broad-leaved plants, which was central New South Wales (Hull and Groves 1973). Elsewhere in the Mallee large and broader-leaved plants were observed occasionally but these plants were not considered to be a separate plant biotype (T. Donaldson personal communication). When seed of these plants were grown in the glasshouse they resembled morphologically the narrow-leaved plants (R. Groves personal communication). After the releases of biological control agents in the early 1970s broad- and intermediate-leaved plants began to be found in north-western Victoria and in the South Australian Mallee (Burdon *et al.* 1981), but their distribution in northern Victoria was not surveyed in detail in the 1980 survey. Narrow-leaved plants were considered the main biotype present in Victoria at the time.

As two biological control agents, the rust fungus, *Puccinia chondrillina* Bubak & Syd., and the mite, *Aceria chondrillinae* (G. Can.), only attack narrow-leaved plants (Burdon *et al.* 1981), these agents can be used for identification purposes. A third biological control agent, the midge, *Cystiphora schmidti* (Rubsaamon), attacks all biotypes and is not a useful diagnostic tool. Because visual means of identification are not always accurate, especially for field material, a starch gel electrophoretic method was developed to identify 14 enzymes of *C. juncea* (Burdon *et al.* 1980). No differences were found between the three biotypes for 6 of the enzymes but major differences were found for some enzymes using three different buffer systems. Esterase, alcohol dehydrogenase and phosphatase were identified from seeds germinated for up to 96 hr and these systems were used to identify the three biotypes or forms of skeleton weed (Burdon *et al.* 1980). Panetta (1984) also used starch gel electrophoresis to identify *C. juncea* biotypes in Western Australia. A polyacrylamide gel electrophoretic method was developed to give improved resolution of the esterase isozyme in the closely related plant forms (Reinganum 1986). This method did not rely on seed germination as had the earlier method, it used rosette and stem leaves, stems or root material. Polyacrylamide gel electrophoresis can be used throughout the year, whenever green plant material is available, whereas starch gel electrophoresis uses seeds only.

A survey was carried out in the summer of 1982 and repeated in 1986 to determine the relative abundance and distribution of the biotypes in Victoria and whether they changed with time.

Materials and methods

Distribution and abundance of C. juncea

In both years the major north-south and east-west roads of the Mallee and northern Wimmera, and the northern section of the Goulburn Valley were sampled for *C. juncea* (Figure 1). Paddocks abutting these roads and occasionally roadsides were surveyed. Sites were approximately 10 km apart. At each site, counts of each plant biotype were made in 10×1 m^2 quadrats along a transect, each quadrat was 10 m apart. A total of 117 sites was examined, 90 in the Mallee-Wimmera region and 27 in the Goulburn Valley. In the Mallee, 60 sites were in stubble paddocks, 13 in fallow paddocks, two in pasture, three in legume crops and 12 on roadsides. In the Goulburn Valley, 10 were in stubble paddocks, seven in fallow, five in pasture and five on roadsides. The same paddocks were examined in both surveys.

All data were transferred to square root (density) and analyzed using General Linear Models, type III SS and tested for significance ($P = 0.05$) and interaction (Little 1981). Re-transformed data show the actual mean number of plants present per m^2 in all areas (Tables 1-3).

Identification of C. juncea

Field identifications depend on leaf shape and branch angles (Hull and Groves 1973). In 1982 seed or root stock of unidentifiable plants were collected and returned to the laboratory. These were grown in the glasshouse, with standard seed of the three biotypes supplied by the

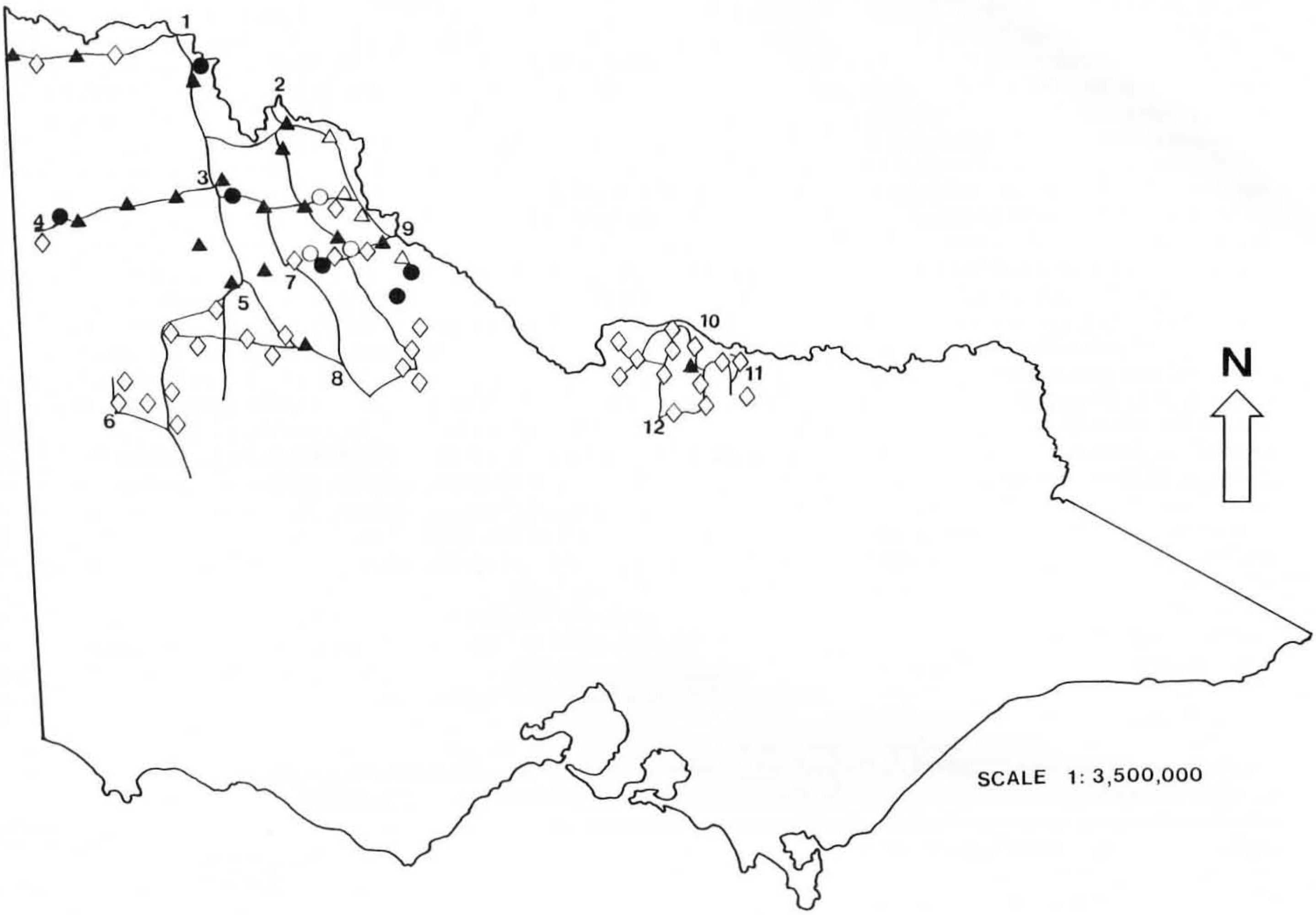

Figure 1. Major road systems in Victoria which were surveyed for the presence of *Chondrilla juncea* L. and the distribution of each biotype in 1986. Numbers indicate towns :- 1. Mildura; 2. Robinvale; 3. Ouyen; 4. Murrayville; 5. Hopetoun; 6. Nhill; 7. Sea Lake; 8. Wycheproof; 9. Swan Hill; 10. Cobram; 11. Yarrawonga; 12. Shepparton. Towns 1-5 and 7-9 are in the Mallee; 6 in the Wimmera; 10-12 in the Goulburn Valley. Distribution of biotypes:- narrow-leaved plants only ( ); broad-leaved plants only ( ); narrow- and broad-leaved plants ( ); narrow- and intermediate-leaved plants ( ); all biotypes ( ) (1986).

Commonwealth Scientific Industrial and Research Organization (CSIRO), Canberra. The leaf and plant shapes of field collected and glasshouse grown material were compared.

Plants not positively identified in the field and plants collected in 1986 were tested for esterases using polyacrylamide gel electrophoresis in the presence of Triton-X (Reinganum 1986). This method allows the three biotypes to be distinguished by the presence of one or more bands in the lower portion of the gel. These bands were compared to bands obtained from the CSIRO plants.

A 25 mg sample of actively growing leaf or stem tissue was ground and extracted in carbon tetrachloride and Tris-HCl buffer. Fifteen plants were identified per gel. Each gel was run at 15 mA for approximately 3 hrs and the gels stained in 0.1% Fast Blue RR for 10 mins to develop the esterase bands. The clearest bands were obtained using 12% gels (Figure 2). Field material morphologically different from the three standard biotypes were tested to determine whether they were new biotypes or unusual morphological forms.

Results

Abundance and distribution of C. juncea

The 1982 survey indicated that narrow-leaved plants were the commonest biotype present in the three regions studied, with

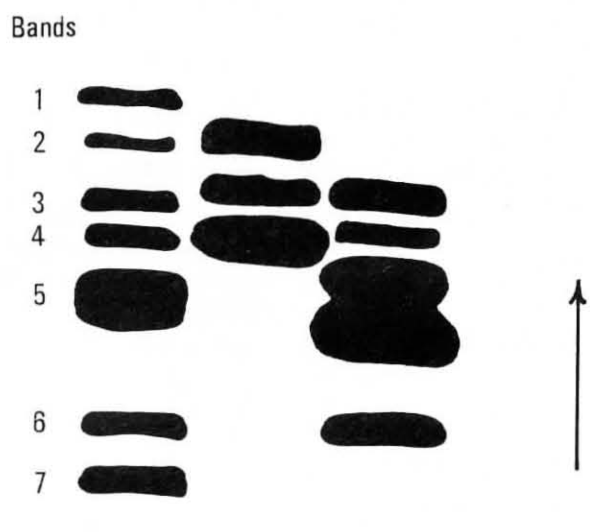

Figure 2. Banding of standard *Chondrilla juncea* L., A. Narrow-leaved plants (Form A), B. Intermediate-leaved plants (Form B), C. Broad-leaved plants (Form C).

the highest average density of 23.2 plants m^{-2} found at Yarrawonga in the Goulburn Valley. Broad-leaved plants were commonly found in the Mallee, with a maximum average density of 16.8 plants m^{-2} at Piangil, and infrequently found in the Wimmera, where the maximum average density was 1.1 plants m^{-2} at Horsham; in the Goulburn Valley the maximum average density was 2.3 plants m^{-2} at Numurkah. Intermediate-leaved plants were found only occasionally in the Mallee and not in the Wimmera or the Goulburn Valley (Table 1).

The 1986 survey indicated that narrow-leaved plants were still the dominant biotype present, with the highest average density of 14.7 plants m^{-2} found at Meringur in the Mallee. The maximum average density of broad-leaved plants was 12.3 plants m^{-2} at Robinvale in the Mallee (Table 1). There were significant differences in density for narrow-leaved plants between the years and between the regions and in broad-leaved plants between regions but not between years (Table 2).

Narrow-leaved and broad-leaved plants were frequently observed in the same paddock, however narrow-leaved plants only were found in paddocks in the Wimmera, western Mallee and Goulburn Valley during 1982. By 1986 some broad-leaved plants were found in the western Mallee and the Goulburn Valley. Broad-leaved plants only were occasionally found in the eastern Mallee; the number of these plants increased between surveys, especially in the Red Cliffs and St. Arnaud areas. In the years between surveys, intermediate-leaved plants spread into the Goulburn Valley and the western Mallee, near Meringur and were found in the central Mallee; however they were present only in very small numbers and no significant differences were found between year, region and year × region (Table 2). All biotypes were found at five sites, four in the central Mallee and one in the western Mallee, near Meringur.

There was no interaction between the year and region for *C. juncea* density (all biotypes), ($P>0.05$). However the Goulburn Valley and Mallee had significantly higher densities in 1982 than the Wimmera ($P<0.05$), and the Goulburn Valley than the Mallee, and the Mallee than the Wimmera for 1986 ($P<0.05$). For narrow-leaved plants there was no interaction between region and year, but it was only in 1982 that differences in density between the Goulburn Valley and Mallee or Wimmera were significant ($P<0.05$). With broad-leaved plants, there was no interaction between regions and year, and in both years densities in the Mallee were significantly higher ($P<0.05$) than in the Goulburn Valley or Wimmera (Table 2).

The mean density for each biotype in the Mallee was analyzed according to location, east or west of the Sunraysia Highway between Ouyen and Mildura (Figure 3). For narrow-leaved plants there were significant differences between year and location ($P<0.05$) and no interaction between year and location. Between 1982 and 1986 the means east of the highway fell from 4.4 m^{-2} to 2.6 m^{-2} and west of the highway from 7.1 m^{-2} to 4.6 m^{-2}. Broad-leaved plants showed significant differences for location only ($P<0.05$), and no

Table 1. The mean and standard deviation[A] of *C. juncea* plants m^{-2} recorded during the surveys of 1982 and 1986 grouped according to districts within the three regions, Mallee, Wimmera and Goulburn Valley.

District	Narrow-leaved Plants		Intermediate-leaved Plants		Broad-leaved Plants		Total	
	1982	1986	1982	1986	1982	1986	1982	1986
MALLEE								
Birchip	14.2	6.6	[B]	0.2		0.5	14.2	7.3
Boort	5.4 ± 3.6	4.5 ± 3.5				0.2 ±0.4	5.4	4.7
Lake Boga	8.0 ± 2.0	3.1 ± 2.8	0.02 ± 0.04	0.1 ± 0.2	0.08 ± 0.08	4.1 ±5.8	8.2	7.3
Manangatang	5.4 ± 7.4	0.5 ± 0.4			3.6 ± 4.4	9.5 ±7.9	9.0	10.0
Meringur	23.0	14.7				0.22	3.0	14.9
Murrayville	13.2 ± 9.0	5.5 ± 1.2		0.1 ± 0.1	3.6 ± 3.4	0.6 ±1.1	15.8	6.2
Ouyen	9.0 ± 8.6	6.4 ± 6.1	0.02 ± 0.04	0.1 ± 0.3	7.6 ± 7.8	5.4 ±8.1	16.6	11.9
Piangil	0.3 ± 0.4	1.0 ± 0.9			16.8 ± 2.9	10.7 ±3.2	17.2	11.7
Rainbow	9.5 ± 8.2	3.7 ± 2.5				0.2 ±0.5	9.5	3.9
Red Cliffs 1	9.8	1.9			6.8	2.9	16.6	4.8
Red Cliffs 2	4.6 ± 1.3	2.9 ± 1.1			0.9 ± 0.8	5.9 ±5.2	5.5	8.8
Robinvale	0.6 ± 0.7	1.8 ± 2.0			15.5 ± 4.5	12.3 ±6.0	16.1	14.1
Sea Lake	5.2 ± 3.1	7.5 ± 3.9			4.5 ± 5.2	0.7 ±0.5	9.7	8.2
Speed	5.8 ± 4.2	4.7 ± 3.0			2.9 ± 7.5	1.9 ±3.6	8.7	6.6
Swan Hill	5.7 ± 6.9	6.4 ± 9.2		0.3 ± 0.6	8.0 ± 5.3	4.5 ±7.4	13.7	11.2
Underbool	6.4 ± 4.1	2.6 ± 1.4		0.05 ± 0.1	4.5 ± 3.4	3.3 ±2.1	10.9	6.0
WIMMERA								
Warracknabeal	7.5 ± 5.0	3.1 ± 1.1			0.05 ± 0.07		7.6	3.1
Werrimull	5.4 ± 1.3	5.7 ± 2.0			0.9 ± 0.3	0.2 ±0.2	6.3	5.9
Wycheproof	16.6 ± 2.4	4.7 ± 6.9				0.4 ±0.6	16.6	5.1
Dimboola	3.9 ± 5.5	1.5 ± 1.3					3.9	1.5
Horsham	14.2 ± 2.1	4.8 ± 5.2			1.1 ± 2.0	0.5 ±1.1	15.3	5.3
Nhill	17.1 ± 26.1	12.6 ± 20.2			0.03 ± 0.06	0.03 ±0.06	17.1	12.6
Woomelang	10.0 ± 9.1	5.4 ± 3.5			2.5 ± 2.7	1.1 ±1.8	12.5	6.5
GOULBURN VALLEY								
Nathalia	20.6 ± 12.1	2.7 ± 1.2			0.7 ± 1.3	0.2 ±0.4	21.3	2.9
Numurkah	16.8 ± 16.1	2.5 ± 1.6			2.3 ± 5.2	0.6 ±1.3	19.1	3.1
Wangaratta	10.6	5.1					10.6	5.1
Yarrawonga	23.2 ± 18.6	2.5 ± 1.5		0.03 ± 0.08	0.09 ± 0.2		23.3	2.5

[A] From transformed data. [B] Gaps indicate 0 rater than not determined.

Table 2. The mean number m^{-2} and standard deviation* of *C. juncea* plants in each regions, the Mallee, Wimmera and Goulburn Valley, and their statistical comparisons.

Year Region	All Forms	Narrow -leaved Plants	Intermediate -leaved Plants	Broad -leaved Plants
1982 Mallee	18.7 ± 1.6	6.3 ± 0.1	0.03 ± 0.05	3.9 ± 0.1
Wimmera	9.0 ± 3.0	8.8 ± 0.2	0	0.04 ± 0.2
Goulburn Valley	20.3 ± 3.3	17.5 ± 0.2	0	0.2 ± 0.2
1986 Mallee	14.9 ± 0.1	4.0 ± 0.1	0.05 ± 0.14	3.7 ± 0.9
Wimmera	5.1 ± 0.2	4.0 ± 0.2	0.03 ± 0.06	0.2 ± 0.2
Goulburn Valley	7.6 ± 0.2	6.0 ± 0.2	0.03 ± 0.06	0.2 ± 0.2
Statistical Comparisons				
1982 Goulburn Valley: Mallee	NS	P<0.05	NS	P<0.05
Goulburn Valley: Wimmera	P<0.05	P<0.05	NS	NS
Mallee:Wimmera	P<0.05	NS	NS	P<0.05
1986 Goulburn Valley:Mallee	P<0.05	NS	NS	P<0.05
Goulburn Valley:Wimmera	NS	NS	NS	NS
Mallee:Wimmera	P<0.05	NS	NS	P<0.05

* Converted from square root (density), General Linear Models Procedure.

interaction between year and location. In 1982 there was no significant difference between the mean number of plants m^{-2} (3.9 m^{-2} for east and 2.6 m^{-2} for west), but for 1986 the difference between the means (4.3 m^{-2} and 2.0 m^{-2} respectively) was significant (P<0.10) (Table 3).

Biotypes of C. juncea

Morphologically there seems to be several variants or morphotypes of narrow and broad-leaved plants. The rust fungus, *P. chondrillina*, attacks all morphotypes of narrow-leaved plants although not all of them are equally susceptible to the fungus (Shepherd and Bruzzese unpublished data). Some narrow-leaved plants have characteristics described by Hull and Groves (1973), 'normal' narrow-leaved plants which do not have an upright growth habit, while other plants grow as tall, sturdy and upright as broad-leaved plants; they are vigorous in growth with branch angles at 90° to the main stem. Other narrow-leaved plants are also vigorous in growth and have lower branches at angles of 60° to the main stem and top branches at 90°. These plants were designated AC plants. All forms occurred throughout the skeleton weed areas and were not restricted to one site, one soil type or one crop type.

Two morphotypes of broad-leaved biotypes were present; large and vigorous plants with numerous stem leaves and less vigorous plants with few stem leaves. Again, these types did not seem to be related to different soil, site or crop types.

Results of the electrophoresis showed that narrow-leaved plants exhibit seven bands, six light and one darker band (Figure 2). Variations in the number of bands in these plants were occasionally found, as either two to four faint bands present well above the normal bands (plants collected from three sites in the Goulburn Valley) or as a faint band below the fifth or seventh band. Electrophoresis of all morphological types showed, basically, typical A banding. Vigorously growing types of narrow-leaved plants frequently had an eighth band, whereas AC plants had only seven bands. Plants from Rainbow, near the southern limit of Mallee distribution, seemed to have slightly different banding from that of other narrow-leaved plants; the bands were at a slightly different position and were not shadows. One plant showed a combination of banding, with bands 1 to 4 and 6 to 7 of narrow-leaved plants and bands 3 to 4 of broad-leaved plants. This plant was collected as a broad-leaved plant not as a narrow- or immediate-leaved plant.

Typically broad-leaved biotypes have five bands, three light plus two heavy bands (Figure 2); however some plants had four bands only. Electrophoretically, the different morphotypes were not readily distinguishable, although the bands in position 3 and 4 were more commonly found in the vigorous type than in the other plants. At eight sites in the Mallee, broad-leaved plants with more than the normal number of flowers per branch were collected. The banding of these plants appeared different from standard broad-leaved plants. Bands 1, 2, 4 and 5 were present as well as a band in position 7 of narrow-leaved plants. All bands were of equal intensity, with no heavy banding.

Intermediate-leaved plants had no bands below band 4 of narrow-leaved plants and band 2 of broad-leaved plants (Figure 2).

Discussion

Despite the introduction of biological control agents, *C. juncea* is still considered a serious weed in Victorian cropping areas. Many farmers consider that it is increasing and the aim of the original survey was to estimate the distribution and density of each biotype and then to determine whether plant densities had changed over time. Distribution of skeleton weed and its biotypes is probably wider than this sur-

Table 3. The mean and standard deviation[A] for narrow-leaved and broad-leaved *C. juncea* plants m^{-2} recorded east and west of the Sunraysia highway between Ouyen and Mildura[B] in north-western Victoria

	Narrow-leaved Plants			Broad-leaved Plants		
	East	West	Significance	East	West	Significance
1982	4.4 ± 0.4	7.1 ± 0.5	Year P<0.05	0.9 ± 0.6	2.6 ± 0.7	Year NS
1986	2.6 ± 0.4	4.6 ± 0.5	Location P<0.05	4.3 ± 0.6	2.0 ± 0.7	Location P<0.05
			Year & Location NS			Year & Location NS

Comparison	Narrow-leaved Plants	Broad-leaved Plants
1982 East : West	NS	NS
1982 East : 1986 East	NS	NS
1982 West : 1986 West	NS	NS
1986 East : West	NS	P<0.10

A Converted from square root (density), General Linear Model Procedure.

B Sites 1 and 3 (Figure 1).

vey indicates, as the 1986 portion only recounted the 1982 sites and aimed to find changes in density rather than increases in distribution.

Significant differences in densities were found between all regions and between years for narrow-leaved plants but not for broad-leaved plants. Although distribution changes between 1982 and 1986 were not monitored, reports by Land Management Officers of the Department of Conservation and Environment and personal observations indicated that there had been an increase in range since 1982, especially of broad-leaved and intermediate-leaved plants. Decreases in narrow-leaved plants were not as great as expected after 10 years of biological control, maybe because susceptible plants have disappeared and the plants left are not as susceptible to *Puccinia*, and because the mites are not wide-spread across the Mallee, they are only common in areas where the plant is relatively undisturbed. Better seasons have been experienced since the drought years of 1983, and have probably contributed to this increased distribution.

It appears that broad-leaved plants are spreading west from New South Wales across Victoria and into the South Australian Mallee, as the heaviest infestations of these plants (Table 1) were found in Piangil and Robinvale, adjacent to the Murray River and therefore near to New South Wales. Farmers in Victoria, especially along the Murray River, consider their plants originated in New South Wales. Few broad-leaved plants were found in western Victoria, Meringur 0.02 m^{-2}, Murrayville 0.6 m^{-2}, Nhill 0.02 m^{-2} and Rainbow 0.2 m^{-2}, and significantly more ($P<0.05$) were found in the east than in western Victoria. It is assumed that this biotype originated in the *C. juncea* areas of central New South Wales and has spread from there (Hull and Groves 1973).

The presence of three basic biotypes was confirmed using polyacrylamide electrophoresis, despite the existence of several morphotypes of narrow- and broad-leaved biotypes in Victoria. Although it appeared that there were detectable differences the method used, the identification of esterase only, was not sufficiently sensitive to distinguish between the morphotypes. Samples of *C. juncea* were therefore sent to CSIRO, Montpellier, France for confirmation of the biotype banding of the morphotypes, using polyacrylamide gel electrophoresis of 22 enzyme systems (P. Chaboudez personal communication). These analyses confirmed that there was a different narrow-leaved *C. juncea* present near Rainbow, possibly the result of sexual reproduction (J. Cullen personal communication). Whether these plants have a different genetic constitution because they are a marginal population influenced by environment or whether they are a different biotype is unclear. Morphologically they were not as distinct as their electrophoretic pattern showed. The electrophoresis carried out in France also showed that the intermediate-leaved biotype was probably more widely distributed in the Mallee than was originally thought (J. Cullen personal communication), thus efforts to find effective biological control agents for all plant biotypes is necessary.

It is possible that there are definite morphological forms of *C. juncea* present in Victoria. Some morphotypes, grown in the glasshouse from field collected root stock to observe whether morphological variation was maintained, did show this was not just an environmental phenomenon. However, these morphological variations were not as distinct as in the field. Thus these plants would appear to be stable morphological variations rather than environmentally induced variants. It therefore does appear that there is genetic variation within biotypes, confirmed by the isozyme data, but whether these are the result of infrequent sexual reproduction is not known.

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