Forms of skeleton weed (*Chondrilla juncea* L.) in Western Australia

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Summary

Plants collected from eight sites within the Western Australian wheatbelt were positively identified as belonging to the narrow-leafed (A) form and broad-leafed (C) form of skeleton weed (*Chondrilla juncea* L.). The intermediate-leafed (B) form has yet to be found. Electrophoretic separation of plant enzymes is considered a useful tool to distinguish between forms within a variable weed species such as skeleton weed.

Introduction

Skeleton weed (*Chondrilla juncea* L.) is a perennial herbaceous weed of European origin which was probably introduced into eastern Australia about 1910 (Wells, 1971). It is thought to have spread from eastern areas to Western Australia, where it was first found at Ballidu (Figure 1) in 1963 (Meadley, 1965).

Because of its weediness in cropping systems in other parts of Australia, it has been the subject of an increasingly active eradication campaign in Western Australia (Figure 2). The species is apomictic and exists in three distinct and stable forms in eastern Australia (Hull and Groves, 1973) — the narrow-leafed or A form, the intermediate-leafed or B form and the broad-leafed or C form.

Surveys of the distribution of skeleton weed in Western Australia show it to be present in four discrete areas of the State (Figure 1), each of which has predominantly sandy soils. Skeleton weed appears to be most abundant in the central portion of the wheatbelt, due east of Perth.

Despite the existence of distinct differences in behaviour between the three Australian forms of this apomictic species, for example in response to disturbance of the root system (Hull and Groves, 1973) and in susceptibility to agents of biological control (Burdon *et al.*, 1981), the forms of skeleton weed present in Western Australia have not been identified except for some preliminary observations reported in Cullen and Groves (1977). The aims of the present study were to identify the

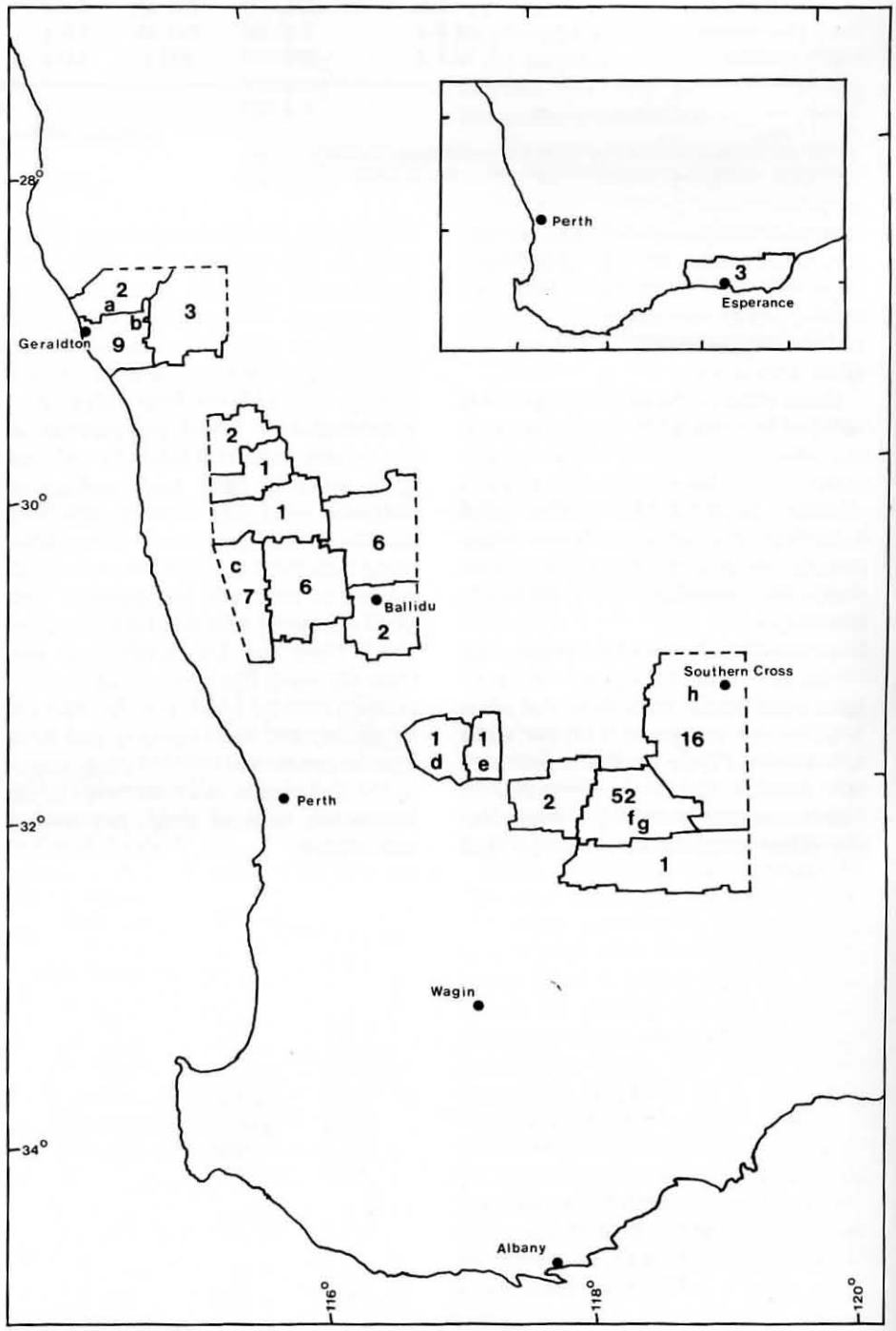

Figure 1 The number of properties per non-metropolitan shire or portion thereof on which skeleton weed has been found in Western Australia. Sites for the collection of plant materials used in the present study are a — Chapman Valley, b — Eradu, c — Badgingarra, d — Cunderdin, e — Tammin, f and g — Narembeen Shire and h — Moorine Rock. (Compiled from records of the Agriculture Protection Board of Western Australia.)

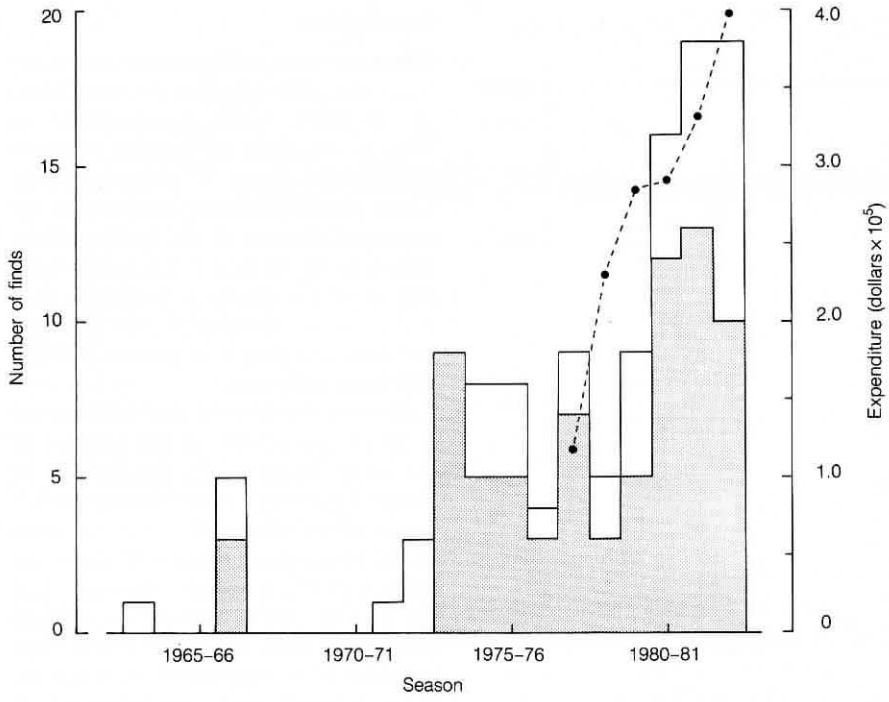

Figure 2 The relationship between the number of new finds of skeleton weed and expenditure on the eradication campaign initiated in 1977-78. Open bars represent infestations located in the areas north of Perth. Stippled bars represent infestations located ot the east and south-east of Perth. (Compiled from records of the Agriculture Protection Board of Western Australia.)

Table 1 Buffer solutions used to separate enzymes by starch gel electrophoresis

System	Buffers	
	Tray	Gel
Borate	192 mM Borate 30.0 mM LiOH	19.2 mM Borate 3.0 mM LiOH 7.2 mM Citrate 45.0 mM Tris
Citrate	86.0 mM Citrate 233 mM Tris 10 mM $MgCl_2$ (adjusted to pH 7.0 with NaOH)	1:25 dilution of tray buffer

forms occurring in Western Australia from a more comprehensive sampling and to map their distributions.

Methods

Morphological variation

Achenes (hereafter referred to as seed) of skeleton weed were obtained from plants growing at eight sites in the Western Australian wheatbelt (Figure 1), and sown on 29 December 1982 on moist filter paper which was maintained at 25°C. Ten days later the seedlings were planted into 15 cm pots, in a mixture of 75% sandy loam and 25% sawdust. There were five pots for each collection and plants were thinned to one per pot 10 days after transplanting. The plants were grown under natural light in a phytotron at South Perth and were subjected to a 12 hour thermoperiod regime of 15/20°C, which was similar to the regime established in previous work (Hull and Groves, 1973). The pots were kept moist with tap water and were flushed with a complete nutrient solution at approximately weekly intervals.

Rosette leaf numbers 10, 15, 20, 25 and 30 were detached from plants when fully expanded and were photocopied for later measurement. The leaf length, minimum leaf breadth and maximum leaf breadth as defined by Hull and Groves (1973) were recorded for each of the leaves and quantitative indices derived from them for the ratios of maximum to minimum leaf breadth and leaf length to minimum leaf breadth.

Gel electrophoresis

Seeds of the forms of skeleton weed in Western Australia were obtained as above and seeds of Forms A, B and C were obtained from the CSIRO Division of Entomology in Canberra to serve as standards. Samples of each form were prepared for electrophoretic analysis by germinating seed on moist filter paper for 96 hours at 25°C.

Individual seedlings were ground in 5 μL of buffer solution (0.16 M phosphate buffer pH 7.0, containing 2 mg dithiothreitol per mL) and the crude extract absorbed by paper chromatography wicks. The wicks were then inserted into a slot in a horizontal starch gel made from 11% Starch-Hydrolysed (Connaught).

Electrophoresis was carried out in both a continuous (citrate) and discontinuous (borate) system (Table 1). The peak currents run through the two gels were 70 mA and 80 mA respectively. In both systems electrophoresis was conducted until the fronts had migrated 8 to 9 cm from the sample slot. Each gel was then cut horizontally into four slices and the cathodal portions were assayed for a range of enzymes. The staining procedures were generally similar to those described by Shaw and Prasad (1970), with the exception of menadione reductase, the assay for which followed that developed by Burdon *et al.* (1980).

Results

Morphological variation

The temporal trends for the quantitative indices of leaf shape are presented in Figure 3. Data for Forms A, B and C from Hull and Groves (1973) have been included for comparison. When logarithmically transformed values of the leaf shape indices for all collections were subjected to analysis of variance, the effect of collection site was found to be highly significant ($P < 0.001$) for all leaf numbers. The major part of this effect was due to differences between collections from Chapman Valley, Eradu, Badgingarra and Moorine Rock (Group 1), and those from Cunderdin, Tammin and Narembeen (Group 2).

Individual site means were then compared using Least Significant Differences. Within Group 2 there were no significant differences except at leaf 30 when the ratio of leaf length to minimum leaf breadth for one of the Narembeen collections was signifi cantly higher ($P < 0.05$) than that for plants from Tammin. By contrast, within Group 1 there were a number of

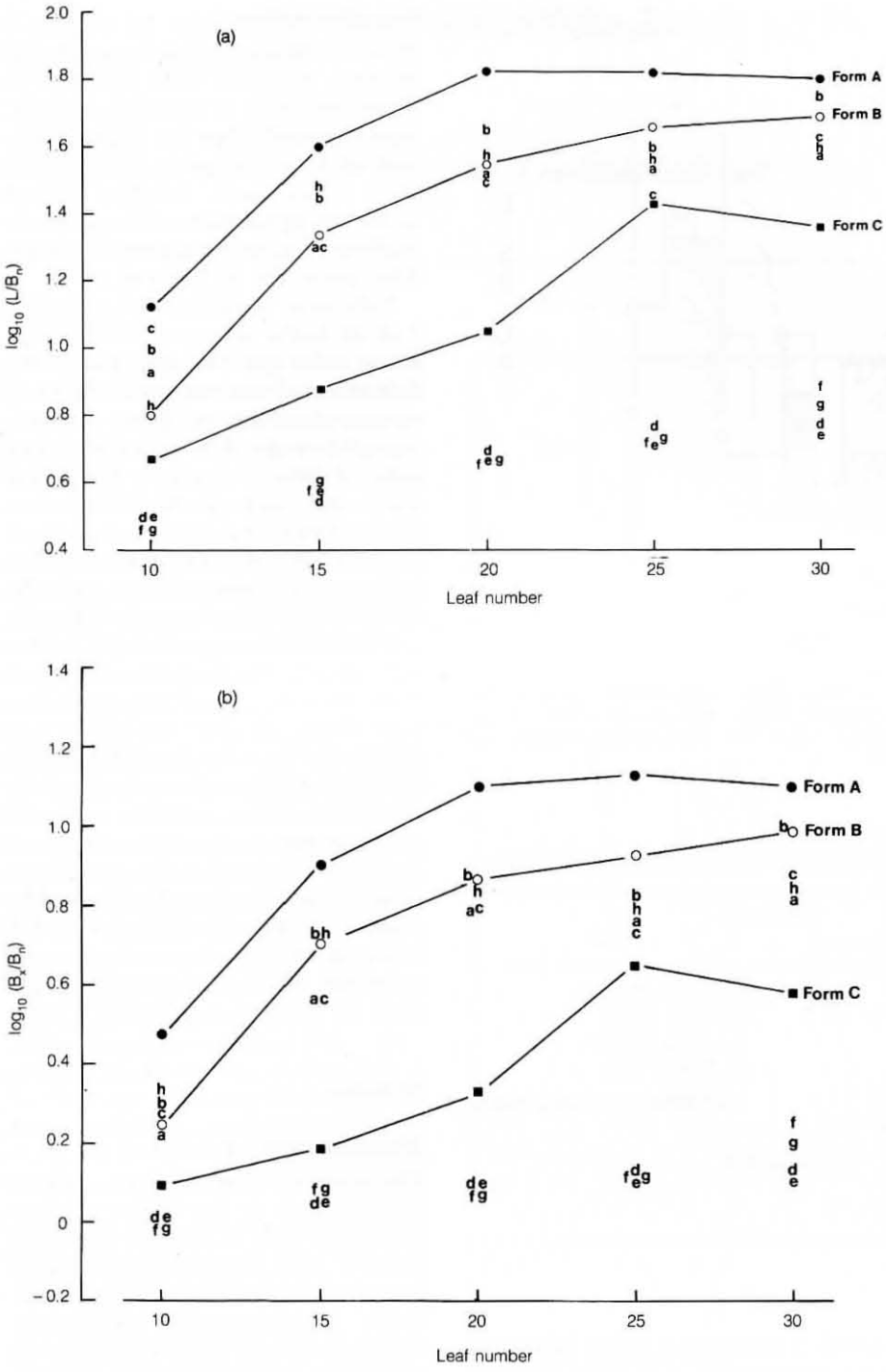

Figure 3 Changes in leaf shape with time as expressed by (a) the ratio of leaf length to minimum breadth, and (b) the ration of maximum to minimum leaf breadth of plants from Western Australian collections of skeleton weed grown in a phytotron at South Perth. Symbols for collections as in Figure 1; data for Forms A, B and C from Hull and Groves (1973).

significant differences between collections from different sites with regard to both indices, although the order of the differences changed with different leaf numbers. However, the division between Groups 1 and 2 was clear at all leaf numbers, as collections comprising Group 1 always demonstrated signifi cantly higher values ($P < 0.01$) than all collections within Group 2.

If the assumption were made that the Western Australian forms of skeleton weed did not differ from those occurring in eastern Australia, then Group 2 could be ascribed only to Form C of Hull and Groves (1973). It was not possible to equate Group 1 with either of the other two forms.

Biochemical variation

When results from both gel buffer systems were considered, assays for four enzymes enabled Forms A, B and C to be distinguished with confidence (Table 2 and Figure 4). Groups 1 and 2 corresponded to Forms A and C respectively.

Discussion

The examination of enzyme characteristics demonstrates that the two forms of skeleton weed encountered in Western Australia are Form A (narrow-leafed) and Form C (broad-leafed). Three possibilities may account for the apparent absence of the intermediate-leafed Form B: that it has never been introduced to Western Australia, that it has been introduced but has not persisted, or that it is present but has not been collected.

The results indicate that both Forms A and C are present in the portion of the weed's range which lies due east of Perth (Figure 1), confirming an earlier report of the occurrence of both forms in the Narembeen district (Cullen and Groves, 1977). It appears, however, that Form C has yet to become established in the two areas north of Perth. This hypothesis is supported by observations of local weeds inspectors (R. F. Martin, personal communication). Form C appears to be the more abundant of the two where it does occur. This has important implications for management of the species, as plants of Form C both regenerate more vigorously from root fragments than those of Form A (Hull and Groves, 1973) and are generally resistant to agents which exercise biological control over Forms A and B (Burdon *et al.*, 1981). Forms B and C have increased in frequency in eastern Australia as the numbers of Form A declined following the introduction of the rust fungus *Puccinia chondrillina* and the mite *Aceria chondrillae* (Burdon *et al.*, 1981; Cullen *et al.*, 1982). There is, however, reason to doubt the potential of *P. chondrillina* to control Form A in Western Australia, as this rust is not known to reproduce sexually and its ability to persist and spread may be reduced during the very dry summers typical of the Western Australian wheatbelt (A. J. Wapshere, personal communication).

Whilst the two forms of skeleton weed which have been collected in Western Australia are distinguishable by morphological and biochemical traits, there are significant discrepancies between data collected in eastern Australia and those presented in Figure 3 and Table 2. Hull and Groves (1973) found no significant direct response of leaf shape to a range of temperatures, day lengths and light intensities, but it is possible that the leaf shape in plants grown at South Perth may have altered due to first or second order interactions between such factors, as these plants were established at different times of the year (January versus August in the

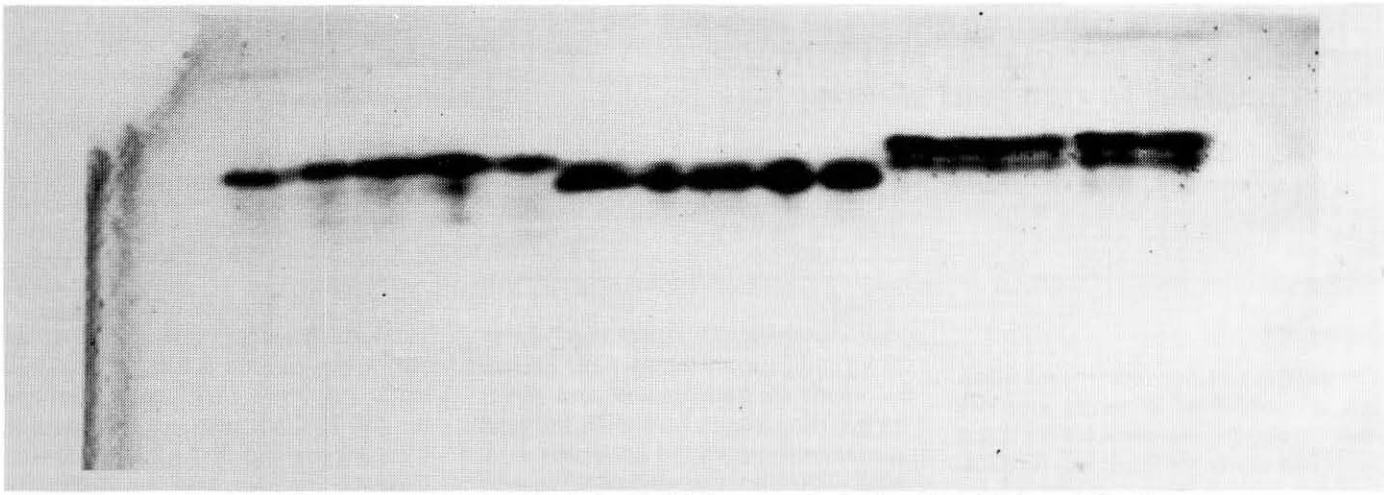

Figure 4 Esterase isozyme patterns on the borate gel buffer system showing, from left to right, Forms A, B and C. With the exception of Form B, for which all plants are of eastern origin, only the first plant in each group is from a non-Western Australian collection.

Table 2 The effectiveness of methods of distinguishing between the three forms of skeleton weed. The morphological forms distinguished are separated by solidi.

Enzyme assayed	Gel buffer system	
	Borate	Citrate
Esterase (EST)	A/B/C	A/B/C
Acid phosphatase (ACPH)	B/A C	A/B/C
Phosphoglucomutase (PGM)	A B C	A B C
Alcohol dehydrogenase (ADH)	A/B/C	poor resolution
Leucine amino peptidase (LAP)	A/B/C	not attempted
Phosphoglucose isomerase (PGI)	A B C	not attempted
Glutamate oxalate transaminase (GOT)	poor resolution	poor resolution
Menadione reductase (MDR)	no activity	no activity

earlier study). Whatever the cause(s) of differences in leaf shape, plants of the Western Australian collections of Form C demonstrated considerably more plasticity with regard to this attribute than those of Form A (Figure 3).

Although variation in characteristics of the enzyme menadione reductase provided Burdon *et al.* (1980) with a means to distinguish between Forms A, B and C, assays for this enzyme were unsuccessful in the present study, due, presumably, to differences in the gel buffer systems employed. The Western Australian collections of skeleton weed demonstrated polymorphisms for the enzymes acid phosphatase, alcohol dehydrogenase and leucine amino peptidase, particularly in seedlings belonging to Form A (F. D. Panetta, unpublished results). This variation at the molecular level of organization was paralleled by a greater number of differences in leaf shapes demonstrated between collections of this form (Figure 3).

Use of electrophoresis in weed research

In his review, Baker (1974) showed that weed species demonstrate highly variable responses to environmental factors. Such variability is partly due to the developmental plasticity demonstrated by individual plants. Another basis for inconsistent behaviour of different populations of particular species concerns traits which are under relatively strict genetic control. The expression of such traits is relatively constant in spite of large variations in growing conditions.

In contrast to morphological traits, whose expression may vary in response to growing conditions, enzyme characteristics appear to be rigidly determined (Gottlieb, 1977), and hence provide a reliable means of identification (Burdon *et al.*, 1980). Therefore, starch gel electrophoresis constitutes a very powerful tool both in weed taxonomy and in attempts to monitor the responses of weed populations to biological and chemical control methods, particularly when populations contain forms of varying degrees of susceptibility.

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