New method for identifying forms of skeleton weed (*Chondrilla juncea* L.)

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Summary

Extracts of the three forms of skeleton weed, *Chondrilla juncea* L., were analysed by polyacrylamide gel electrophoresis in the presence of Triton X-100. Each form was distinguished by the presence of one or more esterase isozyme bands. Using the broad-leaved form (*C*), it was shown that seedlings, roots, rosette leaves and stem leaves all produce the same pattern of major esterase bands. The technique could be relied upon for the identification of specimens collected in the field.

Introduction

In Australia, skeleton weed (*Chondrilla juncea* L.) exists in three forms – the narrow-leaved or A form, the intermediate-leaved or B form and the broad-leaved or C form (Hull and Groves 1973). The A and C forms can usually be distinguished reliably on morphological characters, unlike the B form which may resemble the C form in leaf shape. This prompted Burdon *et al.* (1980) to use starch gel electrophoresis to analyse a range of isozymes of the three forms. Depending on the buffer system used, they were able to distinguish between the three forms on the basis of five out of 14 enzyme systems examined. Panetta (1984) who also used starch gel electrophoresis, distinguished the three forms on the basis of four out of eight enzyme systems examined.

It is generally recognized that electrophoresis of proteins in acrylamide rather than in starch gels results in greatly improved resolution between proteins which differ little in electrophoretic mobility. This is particularly true when a discontinuous buffer system is used in combination with a stacking gel, in which the proteins concentrate as a sharp band, and a resolving gel, where the proteins then migrate according to their net charge and/or size.

Non-specific esterases are among the most complex of isozyme systems, and they would seem an obvious choice of a particular isozyme system with which to differentiate between closely related plant forms.

Several workers (e.g. Loxdale *et al.* 1983) have found that the inclusion of Triton X-100 in the sample extraction medium greatly improves the solubility of membrane-bound esterases. This approach therefore has been adopted in the present work.

To date, germinated seedlings of *C. juncea*, up to 96 h old, have been used as the starting point for isozyme analyses (Burdon *et al.* 1980; Panetta 1984). The purpose of this approach was to eliminate the need to grow the seedlings to a stage when the plants could be identified on the basis of rosette leaf morphology – an unreliable guide for distinguishing between the three forms. Seeds are available only at certain times of the year, and it seemed reasonable to determine whether rosette leaves could not, instead, be used to identify reliably the isozymes of the three forms. This paper indicates that this is indeed the case and, furthermore, that not only the rosette leaves but also other parts of the plant will yield reliable esterase isozyme patterns.

Methods

Plants of the three forms were grown under glasshouse conditions from seeds obtained from the Division of Entomology, CSIRO, Canberra. Plant material (1 g) was ground in a pestle and mortar in 2 ml of 0.125 M Tris-HCl buffer (pH 6.8) containing 0.5% Triton X-100, 0.5% diethyldithiocarbamate, 0.2% 2-mercaptoethanol, 1 mM phenylmethanesulfonyl fluoride, 10% sucrose and 0.005% bromophenol blue. Carbon tetrachloride (1 ml) was also added and, after thorough grinding, the homogenate was centrifuged at 27 000 g for 10 min. The clear aqueous layer was withdrawn with a Pasteur pipette and added to the wells in the stacking gel.

Slab gels (1.5 mm thick) were prepared essentially according to the method of Laemmli (1970), except that the 5% stacking gel contained 0.2% Triton X-100 and the separating gel

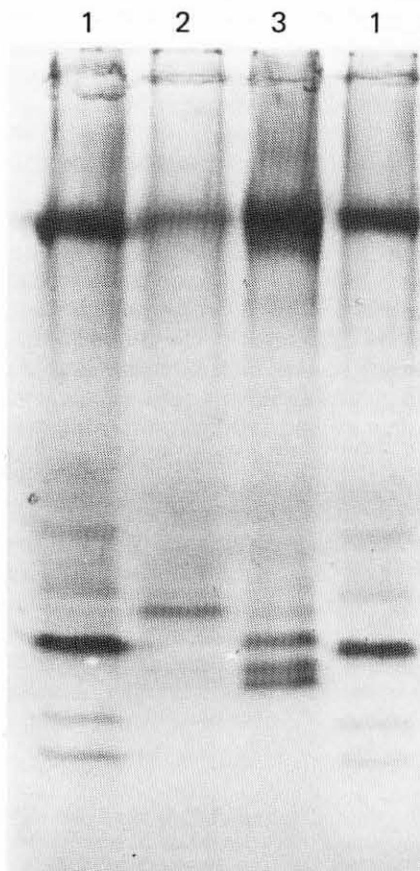

Figure 1 Esterase isozyme patterns of form A (1), forms B (2) and form C (3).

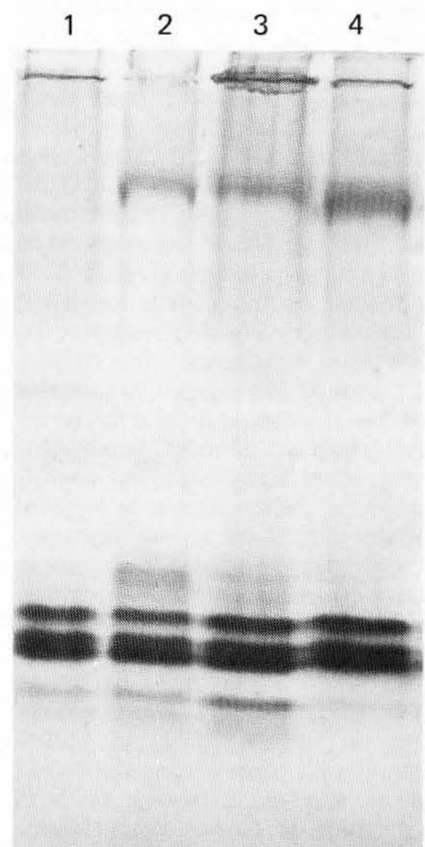

Figure 2 Esterase isozyme patterns derived from the seedlings (1), roots (2), rosette leaves (3) and stem leaves (4) of form C.

0.1% Triton X-100. The gels were run at 25 mA constant current at 4°C until the pigmented buffer front had migrated close to the bottom of the gel. For esterase detection, the gels were immersed in 200 ml 50 mM sodium phosphate buffer (pH 7.0) containing 0.1% Fast Blue RR Salt and 6 ml of a 1% solution of 1-naphthol acetate in acetone. Although a precipitate developed in the staining mixture, it was unnecessary to filter the solution before use. When the esterase bands had developed to their full intensity (after *c.* 10 min), the gels were rinsed in distilled water and fixed in 5% acetic acid.

Results and discussion

Multiple isozymes were detected when extracts of the three forms were subjected to electrophoresis and stained for esterases. The forms could be distinguished clearly by the appearance of one or more intensely staining bands in the lower half of the gel (Figure 1). The distinction between the B and C forms was quantitative in that the three major bands produced by the latter were present as minor bands in the former, whereas the major band of form B was also present as a faint band in form C. On the other hand, the major isozyme present in form A was unique since it migrated to a position intermediate between the first and second major bands of form C. A number of minor bands were also detected in the three forms. In the upper half of the gel, a dense and rather diffuse staining band appeared in about the same position in all three forms. Clearly, this was of no diagnostic value.

When fully grown plants of form C were separated into roots, rosette leaves and stem leaves and their isozyme patterns compared with those derived from 4-day-old seedlings, all produced essentially the same pattern (Figure 2). Minor differences appeared in some of the more faintly staining bands. The forms can therefore be identified on one or more parts of field-collected plants without the need to collect and germinate seed for analysis.

Since the introduction and release of the rust, *Puccinia chondrillina*, for the control of skeleton weed, the abundance of form A in New South Wales has declined, with a concomitant increase in the resistant forms B and C (Burdon *et al.* 1981). This difference in susceptibility to the fungus appears to be mirrored in the esterase isozyme patterns of the three forms, since the distinction between forms B and C is quantitative, whereas distinction between both these forms and form A is both quantitative and qualitative.

References

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