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Distribution and levels of phytoecdysteroids within individual plants of species of the Chenopodiaceae

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***Atriplex*, *Axyris*, *Beta*, *Chenopodium*, *Rhagodia*, *Spinacia*, *Kochia*, Chenopodiaceae, phytoecdysteroid, radioimmunoassay**

Abstract. Radioimmunoassay was used to quantify the levels of ecdysteroids in extracts of portions of single plants of several members of the Chenopodiaceae. Species were chosen because previous studies had shown that seeds of these species were ecdysteroid-containing and because they represent several tribes within the Chenopodiaceae. Total ecdysteroid levels varied considerably between species and between different parts of the same plant. Phytoecdysteroid-containing members of the genus *Chenopodium* appear to possess the same distribution as previously found for *C. album*, as does *Spinacia oleracea*. *Rhagodia candolleana* is characterized by very high levels of ecdysteroids, with the highest levels being associated with newly developing side-shoots. Preliminary data for *Beta patellaris* reveal high levels associated with the lower portions of the plant and rapidly increasing levels associated with the reproductive tissues during flowering and fruiting, to give the high levels previously found to be associated with the seed. Taken together these data tend to support a role for phytoecdysteroids in insect deterrence, but the situation is complex, probably reflecting the subtle interplay between the plant and detrimental/beneficial insects which has occurred during evolution.

INTRODUCTION

There is a significant body of knowledge about the chemistry of phytoecdysteroids, but far less is known about the biology/biochemistry of these compounds (Bergamasco & Horn, 1983). The biosynthetic/metabolic routes are largely unknown. The function(s) of these compounds remain to be conclusively demonstrated (Lafont et al., 1991). Understanding of the distribution within the plant kingdom is fragmentary and the significance of the vast array of different phytoecdysteroid structures is obscure. To begin to answer some of these questions we are focussing on a systematic study of one plant family, the Chenopodiaceae. Previous studies had revealed distinct patterns of phytoecdysteroid distribution in *Chenopodium album* (Dinan, 1992a,b). In pre-flowering plants there is a concentration gradient of phytoecdysteroids within the aerial portions of the plant such that the highest concentrations are found in the growing tips and uppermost (youngest) leaves. High concentrations are also present in the roots (Dinan, 1992a). Even higher levels of phytoecdysteroid are associated with flowering and analysis of the distribution within the flowers reveals the highest concentration to be present in the anthers, while low concentrations are present in the pollen (Dinan, 1992b). It was hypothesized that the phytoecdysteroids present in the growing tips and youngest leaves may help to protect these tender organs from insect predation in the period before quantitative defences are developed. Equally, the flower tissues would be attractive to insect predators and would need to be

protected. In wind-pollinated species such as *C. album*, the production of pollen represents a significant investment of resources, but each pollen grain has only a low probability of fertilizing another flower once it is released. Thus, protection the developing pollen within phytoecdysteroid-containing anthers without the pollen itself containing significant levels of phytoecdysteroid might make ecological sense.

Many other members of the Chenopodiaceae contain phytoecdysteroids (Báthory et al., 1982, 1987; Gebenok et al., 1991; Grebenok & Adler, 1991; Dinan et al., 1991; Báthori, pers. comm.; Dinan, unpublished). It is therefore of interest to determine if the phytoecdysteroid distribution characteristic of *C. album* prevails in other chenopods and to determine whether any differences might correlate with features of the biology of the plant or with any known interactions between the plant and insects. Here I report some recent results concerning the distribution of phytoecdysteroids within single plants of a number of species of chenopod. The biological significance of these distributions will be considered.

MATERIAL AND METHODS

Plant material

Seeds were either purchased from commercial suppliers or provided by botanical gardens. The species used and the sources of the seed were as follows: *Atriplex oblongifolia* W & K (Botanical Garden, Universität Halle, FRG), *Axyris amaranthoides* L. (University Botanical Garden, Oxford, UK; National Botanical Garden, Meise, Belgium), *Beta patellaris* Moq. (University Botanical Garden, Copenhagen, Denmark), *Beta procumbens* Chr. Sm. (CPRO-DLO, Wageningen, Netherlands), *Beta webbiana* Moq. (CPRO-DLO, Wageningen, Netherlands), *Chenopodium amaranticolor* Coste & Reyn. (National Botanical Garden, Meise, Belgium), *Chenopodium ficifolium* Sm. (University Botanical Garden, Copenhagen, Denmark; National Botanical Garden, Meise, Belgium; Botanical Garden, Universität Basel, Switzerland), *Chenopodium oahuense* (Meyen) Aellen (University Botanical Garden, Copenhagen, Denmark), *Kochia scoparia* (L.) Schrad. (Mr. Fothergill's Seeds, Kentford, UK), *Rhagodia candolleana* Moq. [= *R. baccata* (Labill.) Moq.] (Parks and Gardens, Barcelona, Spain) and *Spinacia oleracea* L. (Chiltern Seeds, Ulverson, UK; Mr Fothergill's Seeds, Kentford, UK; Botanical Garden, Universität Bayreuth, FRG). Plants were grown in a domestic greenhouse in coir-based compost. Growing plants were removed from the pots and the compost washed from the roots under running water. Non-flowering plant specimens were dismembered with a sharp scalpel. Stems and larger root systems were cut into one centimetre sections and these are numbered from the root/stem junction. Leaves and side-shoots are numbered from the bottom of the plant. Flowers and fruits were collected from older specimens grown from the same batches of seed.

Extraction

Plant portions were weighed, freeze-dried for 4 days and then extracted three times with methanol (1 ml). The extracts for each sample were pooled and 1.3 ml water and 2 ml hexane were added. After mixing and centrifugation, the hexane phase (containing pigments and apolar lipids) was discarded. Quadruplicate aliquots of the aqueous methanol phase were used for ecdysteroid quantification.

Radioimmunoassay

The radioimmunoassay procedure has been described previously (Dinan, 1992a). The antisera used were DBL-1 and White (both generously provided by Prof. J. Koolman, Universität Marburg). The DBL-1 antiserum was raised against a 20-hydroxyecdysone oxime-BSA conjugate while the White antiserum was raised against an ecdysone oxime-thyroglobulin conjugate. Ecdysone (kindly provided by Prof. R. Lafont, ENS, Paris) was used as the reference ecdysteroid. The cross-reactivity ratios for 20-hydroxyecdysone and polypodene B (the major phytoecdysteroids in most ecdysteroid-containing chenopods) relative to ecdysone (= 1) are 1.3 and 10.8 for the DBL-1 antiserum and 252 and 328 for the White antiserum, respectively (Dinan, 1995). Results are expressed in ng ecdysone equivalents/mg fresh mass of plant material. Mean values are presented in Figs 1–8. Standard deviations were generally < 15% of the mean value.

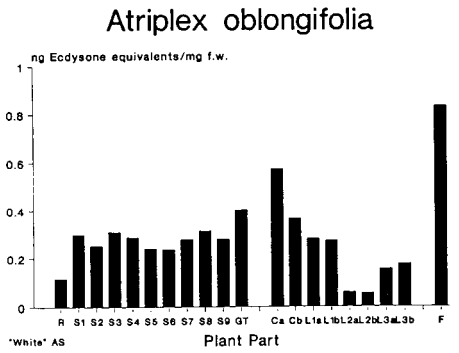

Fig 1. Distribution of phytoecdysteroids in *Atriplex oblongifolia*. Plant sections are abbreviated as follows: R – root, S1–S9 – 1cm sections of the stem (numbered from the root/stem junction), GT – growing tip, Ca/b – cotyledons, L1a/b – 1st pair of true leaves, L2a/b – 2nd pair of true leaves, L3a/b – 3rd pair of true leaves and F – flowers. Ecdysteroids quantified with the "White" antiserum.

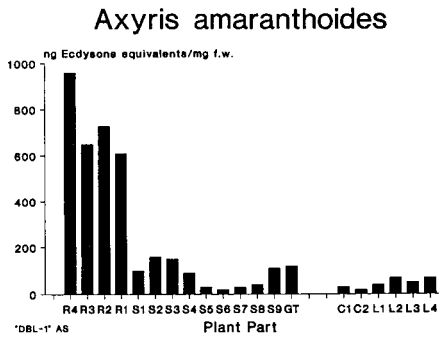

Fig. 2. Distribution of phytoecdysteroids in *Axyris amaranthoides*. Leaves on this species are alternate and are numbered sequentially up the plant. Other plant sections are designated as in Fig. 1, except R1–R4 – 1cm sections of the root (numbered from the root/stem junction). Ecdysteroids quantified with the "DBL-1" antiserum.

RESULTS AND DISCUSSION

Species were chosen for consideration on whether extracts of seed were previously found to contain phytoecdysteroids and on the phylogenetic status of the species within the Chenopodiaceae. The Chenopodiaceae may be viewed (Ulbrich, 1934; Blackwell, 1977; A.J. Scott, pers. comm.) as consisting of two sub-families: the Chenopodioideae and the Salsoloideae. No seed extract of any species in the Salsoloideae has yet been found to be ecdysteroid-positive. Of the five tribes in the Chenopodioideae, the Salicorniae is ecdysteroid-negative, while representatives of the Camphorosmae and Corispermae have been

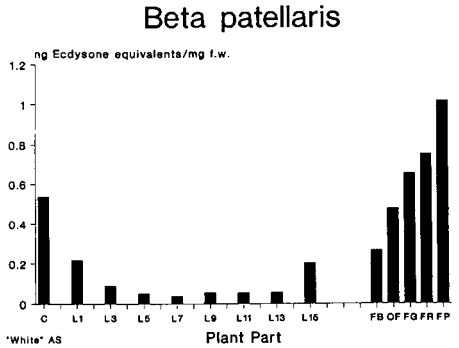

Fig. 3. Distribution of phytoecdysteroids in *Beta patellaris*. Plant sections designated as in previous figures with the addition of the following: FB – flower buds, OF – open flowers, FG – green fruits, FR – red fruits and FP – purple fruits. Ecdysteroids quantified with the "White" antiserum.

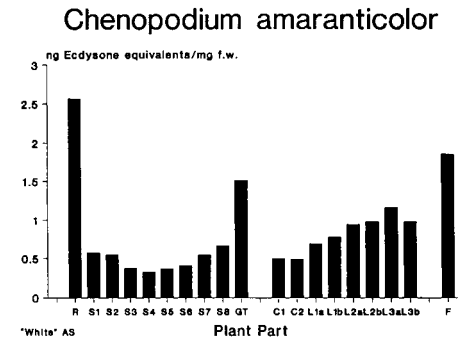

Fig. 4. Distribution of phytoecdysteroids in *Chenopodium amaranticolor*. Plant sections designated as in Fig. 1. Ecdysteroids quantified with the "White" antiserum.

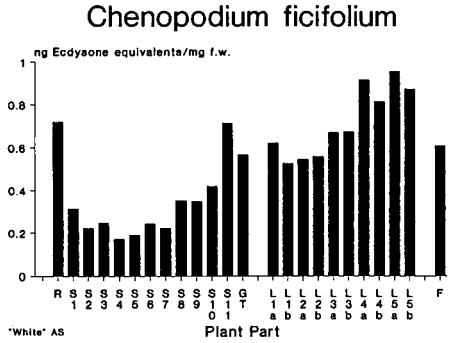

Fig. 5. Distribution of phytoecdysteroids in *Chenopodium ficifolium*. Plant sections designated as in Fig. 1. Ecdysteroids quantified with the "White" antiserum.

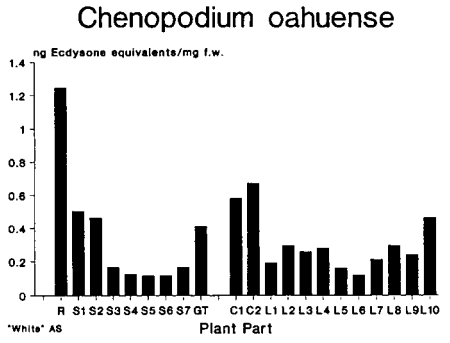

Fig. 6. Distribution of phytoecdysteroids in *Chenopodium oahuense*. Leaves on this species are alternate. Designations as in Fig. 2. Ecdysteroids quantified with the "White" antiserum.

found which contain only relatively low levels of ecdysteroids. Most species containing significant levels of phytoecdysteroids are found in the Chenopodieae and the Atripliceae. In the tribe Beteae, two species in the section Procumbentes of the genus *Beta* contain ecdysteroids, the rest being ecdysteroid-negative. Thus, the selected species in the genera *Atriplex*, *Spinacia* and *Axyris* represent the Atripliceae, and *Chenopodium* and *Rhagodia* represent the Chenopodieae. *Kochia scoparia* (Camphorosmeae) was investigated previously (Dinan, 1994).

TABLE 1. Phytoecdysteroid levels in seeds of the species considered, arranged by Tribe. Ecdysteroid levels were quantified with the DBL-1 antiserum.

	μg Ecdysone Equivalents/g seed
Atriplicaeae	
Atriplex oblongifolia	1463
Axyris amaranthoides	1311, 1286*
Spinacia oleracea	2546, 1204, 1104, 1087, 1033*
Beteae	
Beta patellaris	1234
Beta procumbens	0
Beta webbiana	448
Camphorosmae	
Kochia scoparia	30
Chenopodieae	
Chenopodium amaranticolor	1362
Chenopodium ficifolium	033, 975, 481*
Chenopodium oahuense	1382
Rhagodia candolleana	8892

* Samples from different souces or representing different varieties/cultivars.

Results are presented in Figs 1–8 and Table 1. The levels and distribution of phytoecdysteroids vary considerably between ecdysteroid-positive species in the Family Chenopodiaceae, ranging from low (e.g. *Kochia scoparia*; Dinan, 1994) to very high levels (e.g.

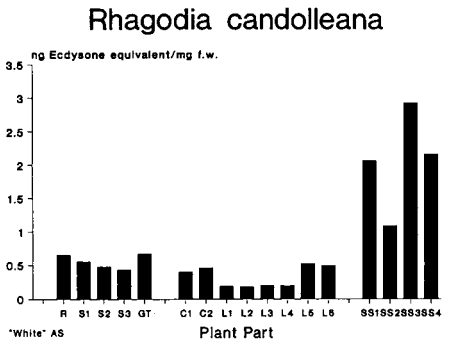

Fig. 7. Distribution of phytoecdysteroids in *Rhagodia candolleana*. Plant sections designated as in previous figures, with the addition of SS1–4 – side-shoots (numbered sequentially up the plant). Ecdysteroids quantified with the "White" antiserum.

Rhagodia candolleana). Levels in seed of the tested species vary between species and, in some cases, between samples from different sources (Table 1). The latter indicates that genetic or environmental factors may influence ecdysteroid levels. Importantly, this implies that it should be possible to identify individual plants of the same species with different maximal levels of phytoecdysteroid for the assessment of their differential susceptiblity to insect predation. The general distribution pattern observed previously in plants of *Chenopodium album* (Dinan, 1992a,b) is seen in other members of that genus and also *Spinacia oleracea* (see also Grebenok & Adler, 1991). These are rather rapid growing species with relatively soft tissues. The highest concentrations of phytoecdysteroid are associated with the softest tissues (i.e. youngest leaves, growing tips and root tips). In *Rhagodia candolleana* (as in all species of *Rhagodia*) high levels of ecdysteroid are found. This is a slow-growing perennial woody species. In the specimen examined, highest levels were associated with the soft, newly-developing side-shoots. In *Axyris amaranthoides*, very high ecdysteroid concentrations are observed in the roots, with much lower concentrations associated with the aerial portions. Perhaps the distribution in this species reflects a requirement (past or present) to protect itself against soil invertebrates. Conversely, in *Atriplex oblongifolia* the highest concentrations are found in the aerial portions, with an almost uniform concentration along the stem. The data for *Beta patellaris*, while partial, indicate a distinctly different distribution, with the highest levels in the leaves being assosciated with

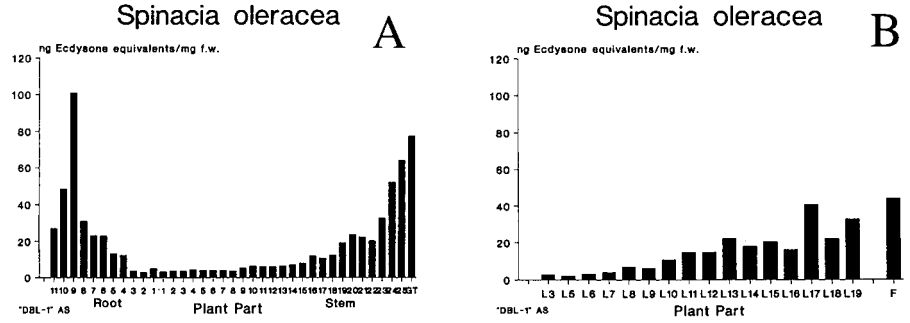

Fig. 8. Distribution of phytoecdysteroids in *Spinacia oleracea*. A – 1 cm sections of the roots and stem; B – leaves (L3–L19) and flowers (F). Ecdysteroids quantified with the "DBL-1" antiserum.

the lower portion of the plant. Perhaps in a perennial species such as this it is more important that the large energy-producing leaves are protected to ensure the survival of the plant into the next growing season, than to protect the growing tips and the associated reproductive organs, as appears to be the strategy in *C. album*. In the genus *Beta*, the majority of species do not contain ecdysteroids. The only two exceptions we have found so far are *B. patellaris* and *B. webbiana*, which together with *B. procumbens* (ecdysteroid-negative) form the section Procumbentes. On the basis of morphological and cytological characteristics, it has previously been suggested that these three species should be in a separate genus from the rest of the *Beta* species (Williams et al., 1976). These studies on phytoecdysteroids would support this idea.

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