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Received: *18 September 2002*
Accepted: *22 November 2002*

doi: 10.1046/j.1469-8137.2003.00710.x

## Summary

• Changes in the defence compounds pyrrolizidine alkaloids (PAs) in roots and shoots of *Senecio jacobaea* are reported in response to nutrient addition in order to investigate whether changes in concentration are adaptive.

• PA concentrations were examined in leaves and roots of 40 vegetative ragwort plants, subjected to four nutrient treatments in a climate chamber study. Roots from 10 plants were subdivided into main root cortex, main root vascular cylinder, lateral roots and root tips and analysed for PA concentrations.

• Increasing nutrients lead to a significant reduction in total PA concentration of both roots and shoots. All individual PAs except jacobine decreased in concentration. The total amount of PA produced in the whole plant was not influenced by nutrient supply. Root tips contained a three times lower concentration than the main and lateral roots. The concentrations in the main root cortex were five times higher than concentrations in the vascular cylinder.

• Changes in biomass rather than changes in production rates can explain alterations in PA concentration of *S. jacobaea* in response to nutrients.

**Key words:** *Senecio jacobaea* (common ragwort), pyrrolizidine alkaloids, nutrients, distribution, allocation.



## Introduction

Defence is an important function of plant secondary metabolism. An array of compounds is produced, which deter herbivore grazing (van Loon & Schoonhoven, 1999; Dyer *et al.*, 2001, Gonzalez *et al.*, 1995; Schmeller *et al.*, 1997). Secondary plant metabolites are highly diverse in structure and abundant throughout all plant genera. Regulation of secondary metabolite production, transformation and allocation in the plant influence defence mechanisms in plants. Secondary metabolite concentration and diversity may vary among locations (Johnson *et al.*, 1985, von Borstel *et al.*, 1989; Ralphs *et al.*, 2000), and within and among seasons (Johnson *et al.*, 1985; O'Dowd & Edgar, 1989; Laus *et al.*, 1997; Hook *et al.*, 1999), and this variation in concentration can have a large impact on resistance to herbivory.

The maintenance of active secondary metabolite concentrations at a certain level may require the production of defence compounds to be very flexible. This should occur independently of the variation in environmental factors such as climate, light, humidity and nutrients. There are also inducible defence compounds, the levels of which are raised when necessary (Karban & Baldwin, 1997). However, our study focuses on the flexibility of a constitutive defence system in response to nutrient supplies. It is generally accepted that increasing nutrient supplies leads to an increased shoot : root ratio. If secondary metabolites production takes place throughout the whole plant, no consequences would be expected from increasing nutrient supply. However, when the production of secondary metabolites is coupled with root growth only, higher nutrient supplies would lead to lower concentrations in shoot and root. We sought to determine whether a plant species is capable of adapting defence levels to increased nutrient supply through different allocation patterns, increased production or both. A plant species was selected which produces secondary metabolites in the roots, allocates them over the whole plant and occurs under a wide range of nutrient conditions. *Senecio* spp. grow in poor sandy dune soils and at

road sites, but are also found as noxious weeds in pastures, where they cause acute and chronic liver damage when ingested by cattle (Mattocks, 1986). The use of *Senecio* spp. in traditional human medicine could give rise to carcinogenic effects (Steenkamp *et al.*, 2001). These effects are due to pyrrolizidine alkaloids (PAs), which are regarded as part of the defence mechanism of the plant against herbivores (Hartmann & Witte, 1995; Van Dam *et al.*, 1995a). Recently, we showed that PAs influence soil borne fungi (Hol & Van Veen, 2002). PA production in *Senecio* spp. takes place in the root (Hartmann & Dierich, 1998) and is related to root growth (Hartmann & Toppel, 1987; Sander & Hartmann, 1989). Therefore it is important to understand the response of PA production and transformation in relation to growth biomass and variation in nutrient supplies.

*S. jacobaea* is a monocarpic perennial and flowering is often delayed for several years because a threshold size has to be reached for vernalization (Van der Meijden & Van der Waals-Kooi, 1979; Prins *et al.*, 1990; Wesselingh & Klinkhamer, 1996). We therefore focused on the vegetative stage.

Differences in the distribution of secondary metabolites over a plant can be considerable. Five-fold differences between different plant parts were observed in PA content of *S. vulgaris* (Hartmann & Zimmer, 1986) and *Cynoglossum officinale* (Van Dam *et al.*, 1995b). Most data reported on this subject concern detailed studies of above-ground plant parts and it is unknown whether the same variation in secondary metabolite concentration will be found below-ground. This may have considerable effect on the defence of the root tissue against insects, fungi, nematodes and bacteria, which is essential for survival to the flowering stage. The older, basal root parts may also be used for storage of PAs. Optimal defence theory predicts that the level of defence in plant parts is related to the importance of that plant part for fitness (Rhoades, 1979). We therefor expect high concentration in the root tips, which are important for root growth.

## Materials and Methods

### Plant species

Pyrrolizidine alkaloids (PAs) in *Senecio* spp. are present throughout development and under all circumstances. Highest concentrations were found in the flowerheads (Hartmann & Zimmer, 1986) of the two annual *Senecio* species *S. vernalis* and *S. vulgaris*. Vrieling *et al.* (1993) demonstrated that 50–100% of the total variation in total PA concentration was due to genetic differentiation under controlled growth conditions. Artificially damaged *S. jacobaea* plants did not increase PA concentrations as one might expect, but instead a significant decrease was found within 6–12 h after damage (Van Dam *et al.*, 1993). PAs are transported via the phloem to the shoot (Hartmann *et al.*, 1989), where they are transformed into other PAs (Hartmann & Dierich, 1998). Turnover of PAs is negligible (Sander & Hartmann, 1989; Hartmann & Dierich, 1998). It is unknown to what extent the allocation of PAs is dependent on the relative biomass above and below-ground.

### Experiment I

A high PA producing genotype was selected from a cross between high and low PA plants of *Senecio jacobaea* (L.). Plants were propagated using tissue culture. At the start of the experiment, 40 plants were weighed and potted in 1.3 l sterilised dune sand. The experiment was carried out over a 16-h photoperiod and day/night temperatures of 20°C/15°C. Relative humidity was 70%. Every plant received the same amount of water three times a week. One week after planting nutrients were given as Steiner nutrient solution (Steiner, 1968) (macronutrients: N 167 mg $l^{-1}$, P 31 mg $l^{-1}$, K 282 mg $l^{-1}$, S 111 mg $l^{-1}$, Ca 180 mg $l^{-1}$, Mg 49 mg $l^{-1}$). The control treatment of 10 plants did not receive nutrients (N0) but the same volume of water, the other treatment groups (N1, N2, N3), also consisting of 10 plant each, received 20 ml Steiner once, twice or three times a week. N1 received twice and N2 once 20 ml water.

Plants were harvested after 61 d and divided into shoot, main roots and minor roots. The main roots were defined as the upper 5 cm under the hypocotyl, without lateral roots. The remainder of the root system with the younger parts was named minor roots. All material was dried at 50°C for 72 h and weighed. The PA and nitrogen contents of all plants were determined as described below.

### Experiment II

This experiment analysed PA distribution in the roots of 10 vegetative plants. Three plants (FI1, FI2 and FI3) were collected from a field site, Meijendel, The Netherlands and seven plants from three genotypes (CE1-1, CE1-2, CE2-1, CE2-2, CE3-1, CE3-2, CE3-3, where CE1, CE2 and CE3 indicated different genotypes, originated from Meijendel) were grown in climate chambers in dune sand with 60 ml Steiner solution per week for 3 months (16-h photoperiod, 20°C/15°C (day/night), rh = 70%). Roots were divided into main and lateral roots and root tips. The main roots were defined as described for experiment I. Fresh material of the main roots was easily separated into cortex and vascular cylinder by hand. Root tips were defined as the last cm of the roots. All plant material was dried for 72 h at 50°C and PAs were measured, as described below.

### PA extraction and identification

Total PA concentration in the shoots and different root parts was determined in 10 mg dried and ground plant material, according to a modified version (de Boer, 1999) of the acid-base extraction method of Hartmann & Zimmer (1986).

PA content was determined on a GC (HP 6890 30 m × 0.25 μm, HP-1) with heliotrine (Latoxan, France) as an internal standard. Conditions of GC analysis were: injector 250°C, temperature programme 0-22-5-250, split mode 1–30, carrier gas N2 0.9 ml $min^{-1}$, pressure 56 kPa; detector FID. PAs were identified by comparing retention times with reference samples.

### Nitrogen analysis

The ground plant material was analysed for total nitrogen. Digestion was done according to Novozamsky *et al.* (1983). Nitrogen was measured by Atomic Emission Spectroscopy as in Troelstra *et al.* (1995).

### Statistics

Differences in biomass, PA concentrations and nitrogen concentrations between treatments were tested with a oneway ANOVA if possible. Post-hoc multiple comparisons between all treatments were made with Bonferroni tests. Because not all data met the assumptions of ANOVA, individual PA concentrations were tested with Kruskal–Wallis and treatments were compared with a multiple comparisons test according to Siegel (1956). The allocation of PAs to the shoot was calculated as the fraction of PAs present in the shoot of the total amount of PAs in the whole plant. Correlation between nutrient supply and PA allocation was tested with Spearman's rank correlation. Correlation between PA content and root biomass was compared with theoretical correlations based on Monte Carlo simulations. We compared the empirical relationship with the theoretical correlation based on random chosen concentrations (Partel *et al.*, 1996). Five thousand times a random concentration between 0 and 1 mg $g^{-1}$ was chosen for each shoot and root and each time the correlation coefficient between total PA production and root biomass was calculated. The correlation is significant when greater than 95% of the simulated correlations. All tests were performed with SPSS 8.0 (SPSS Inc, 1998).

## Results

### Experiment I

As expected, total plant biomass increased significantly with increasing nutrient-supply ($n = 39$ ρ: 0.597 $P$: 0.000). Shoot weight increased 4-fold, but the root biomass was not significantly different between the nutrient treatments. Consequently, the shoot : root ratio increased with increasing nutrient-supply rate from 0.5 (N0) to 1.3 (N4). Nitrogen concentration in roots was much less than in shoots. In the shoots, the N concentration in the N0 was significantly less than in N1–N3.

The PA concentrations in different plant parts decreased with increasing nutrient supply (Table 1). Highest concentrations were found in the main roots of plants N0 and N1. The concentration in the shoots was less than in the main roots. There were no significant differences between the minor roots and shoots except for N0. Total PA content of the whole plant and the different organs was not different between the treatments, yet, there was a significant, positive correlation between nutrient supply and PA allocation to the shoot ($n = 39$, ρ: 0.615, $P$: 0.000). There is a strong positive correlation between total PA and root biomass (ρ: 0.857 $P < 0.05$) (Fig. 1) if all treatments are combined. Since root biomass was not different between treatments, this seems to indicate a

**Table 1** The effect of nutrients on mean (± SE) biomass, pyrrolizidine alkaloid (PA) concentration, total PA and relative allocation of PAs to the shoot in *Senecio jacobaea*

| | | N0 | N1 | N2 | N3 | *F* | d.f. | *P* |
|---|---|---|---|---|---|---|---|---|
| Biomass | Start (g f. wt) | 0.95 ± 0.24a | 0.79 ± 0.10a | 0.90 ± 0.17a | 1.01 ± 0.11a | 0.34 | 3,35 | 0.796 |
| (g d. wt) | Shoot | 0.24 ± 0.03a | 0.45 ± 0.07ab | 0.63 ± 0.05b | 0.94 ± 0.10c | 17.49 | 3,35 | 0.000 |
| | Main roots | 0.18 ± 0.02a | 0.20 ± 0.04a | 0.20 ± 0.02a | 0.26 ± 0.02a | | 3,35 | 0.203* |
| | Minor roots | 0.32 ± 0.05a | 0.36 ± 0.07a | 0.37 ± 0.05a | 0.45 ± 0.03a | 0.90 | 3,35 | 0.450 |
| | Whole plant | 0.74 ± 0.10a | 1.00 ± 0.17a | 1.20 ± 0.12ab | 1.68 ± 0.17b | 6.767 | 3,35 | 0.001 |
| | Shoot/root | 0.51 ± 0.03a | 0.89 ± 0.08b | 1.16 ± 0.10bc | 1.32 ± 0.06c | 21.49 | 3,35 | 0.000 |
| Nitrogen | Shoot | 3.01 ± 0.22a | 3.98 ± 0.19b | 3.84 ± 0.16b | 4.02 ± 0.17b | 6.36 | 3,35 | 0.001 |
| %N d. wt | Main root | 0.71 ± 0.10a | 1.16 ± 0.18ab | 1.11 ± 0.14a | 1.80 ± 0.21b | 7.30 | 3,35 | 0.001 |
| | Minor roots | 2.01 ± 0.43a | 1.79 ± 0.23a | 1.73 ± 0.17a | 2.12 ± 0.13a | | 3,35 | 0.523* |
| PA conc | Shoot | 6.18 ± 0.07b | 5.54 ± 0.41b | 4.77 ± 0.35ab | 3.30 ± 0.39a | 8.38 | 3,35 | 0.000 |
| (mg $g^{-1}$ d. wt) | Main roots | 6.68 ± 0.38cd | 7.64 ± 0.44d | 5.98 ± 0.32ac | 4.61 ± 0.42a | 10.98 | 3,35 | 0.000 |
| | Minor roots | 4.77 ± 0.34b | 5.20 ± 0.32b | 4.03 ± 0.37b | 2.60 ± 0.39a | 10.46 | 3,35 | 0.000 |
| PA (mg) | Whole plant | 4.19 ± 0.61a | 5.78 ± 1.01a | 5.84 ± 0.76a | 5.45 ± 0.72a | 0.88 | 3,35 | 0.461 |
| PA production | mg $g^{-1}$ root | 8.69 ± 0.45ab | 10.98 ± 0.61b | 10.10 ± 0.71b | 7.68 ± 0.60a | 5.90 | 3,35 | 0.002 |
| PA allocation | Shoot/total | 0.36 ± 0.03a | 0.44 ± 0.03ab | 0.53 ± 0.01b | 0.55 ± 0.06b | | 3,35 | 0.001* |

N0: control: 0 mg Steiner $wk^{-1}$; N1: 20 ml Steiner $wk^{-1}$; N2: 40 ml Steiner $wk^{-1}$; N3: 60 ml Steiner $wk^{-1}$. *Kruskal–Wallis test.

**Table 2** The effect of nutrient supply on average (± SE) PA concentrations in the shoot and roots of *Senecio jacobaea*

| | | N0 | N1 | N2 | N3 | *P* KW |
|---|---|---|---|---|---|---|
| Shoot | Senecionine | 1.30 ± 0.16a | 1.04 ± 0.11ab | 0.66 ± 0.05bc | 0.34 ± 0.06c | 0.000 |
| (mg $g^{-1}$ d. wt) | Seneciphylline | 1.67 ± 0.17a | 1.40 ± 0.07ab | 1.07 ± 0.08bc | 0.68 ± 0.08c | 0.000 |
| | Integerrimine | 0.31 ± 0.07ab | 0.41 ± 0.05a | 0.26 ± 0.05ab | 0.16 ± 0.03b | 0.021 |
| | Jacobine | 1.72 ± 0.20a | 1.67 ± 0.21a | 1.81 ± 0.17a | 1.54 ± 0.17a | 0.829 |
| | Erucifoline | 1.10 ± 0.10a | 0.80 ± 0.09ab | 0.73 ± 0.07b | 0.48 ± 0.05b | 0.000 |
| Main roots | Senecionine | 5.72 ± 0.38a | 5.70 ± 0.39a | 3.91 ± 0.28b | 2.87 ± 0.26b | 0.000 |
| (mg $g^{-1}$ d. wt) | Seneciphylline | 0.56 ± 0.07ab | 0.59 ± 0.05a | 0.45 ± 0.04ab | 0.37 ± 0.07b | 0.023 |
| | Integerrimine | 0.48 ± 0.06b | 0.69 ± 0.04a | 0.55 ± 0.4ab | 0.44 ± 0.06b | 0.014 |
| | Jacobine | 0.10 ± 0.00b | 0.33 ± 0.10ab | 0.68 ± 0.14a | 0.77 ± 0.16a | 0.000 |
| | Erucifoline | tr | tr | tr | tr | |
| Minor roots | Senecionine | 3.92 ± 0.26a | 4.14 ± 0.30a | 3.03 ± 0.28ab | 1.91 ± 0.20b | 0.000 |
| (mg $g^{-1}$ d. wt) | Seneciphylline | 0.36 ± 0.06a | 0.30 ± 0.05a | 0.23 ± 0.04a | 0.21 ± 0.04a | 0.146 |
| | Integerrimine | 0.29 ± 0.03b | 0.44 ± 0.03a | 0.38 ± 0.04ab | 0.22 ± 0.05b | 0.006 |
| | Jacobine | 0.10 ± 0.00a | 0.17 ± 0.05a | 0.26 ± 0.05a | 0.25 ± 0.06a | 0.039 |
| | Erucifoline | tr | tr | tr | tr | |

N0: control: 0 mg Steiner $wk^{-1}$; N1: 20 ml Steiner $wk^{-1}$; N2: 40 ml Steiner $wk^{-1}$; N3: 60 ml Steiner $wk^{-1}$; tr: trace amounts different letters indicate significant differences between treatments.

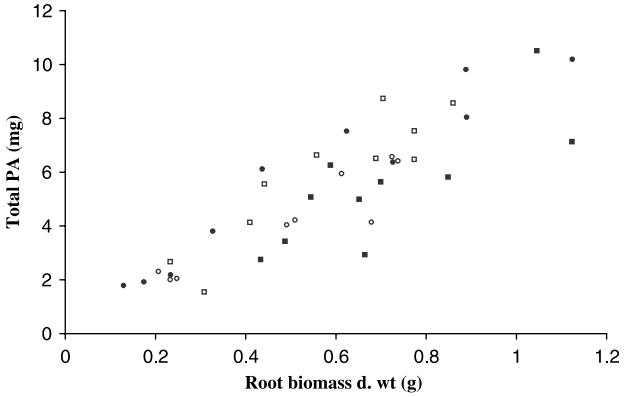


**Fig. 1** Relation between root biomass and total pyrrolizidine alkaloid production in *Senecio jacobaea* plants. Treatments: open circles, N0, control; closed circles, N1, nutrients added once a week; open squares, N2, nutrients added twice a week; closed squares, N3, nutrients added three times a week.

constant production of PAs independent of the nutrient concentration. However, PA production calculated as total PA in the whole plant divided by root biomass was lower in N3 than in N1 and N2, although it was not different from N0. The decrease in total PA content with increasing nutrients holds for most PAs in the shoot (Table 2). In the shoot senecionine, seneciphylline and erucifoline concentration decreased, while the jacobine concentration was not affected by nutrient treatments. In roots the same PAs are found as in the shoot, but less evenly distributed. Erucifoline is found only in trace amounts, whereas senecionine is the single dominant alkaloid (60–80%). Although present at low concentrations, jacobine is the only alkaloid that actually increases in concentration with increasing nutrient supply.

### Experiment II

Differences were observed in PA concentration in roots (Fig. 2). Lateral roots and the exterior of main roots contained high concentrations. PA concentrations in root tips and in the vascular cylinder of main roots were low, at the minimum detection level. Root tips contained about three times less PA than main and lateral roots. Concentrations in the main root cortex were on average five times higher than concentrations in the vascular cylinder.

## Discussion

Decreasing PA concentrations in roots and shoots with increasing nutrient-supply appear to result from a diluting effect. Increasing nutrient supplies increased plant biomass, while PA production remained constant. Other studies on the response of plants to nutrient addition found significant changes in concentration of secondary metabolites (Iason & Hester, 1993; Höft *et al.*, 1996; Baricevic *et al.*, 1999; Salmore & Hunter, 2001). However, statistical analyses suggest that changes or differences in concentration of allelochemicals can sometimes be explained by changes in tissue biomass only (Koricheva, 1999). Thus, changes in concentration may not result from changes in production, but from dilution or concentration due to enhanced or reduced growth in the whole plant or plant parts.

It is unclear whether the same effect may be expected in flowering plants. Brown & Molyneux (1996) did not find an effect of nutrient deficiency on PA concentrations. Both flowerhead tissue biomass and total amount of PAs increased with nutrient supply, while concentrations remained constant. However, they only measured PAs in flowers and therefore it is not certain whether this reflects a change in total PA

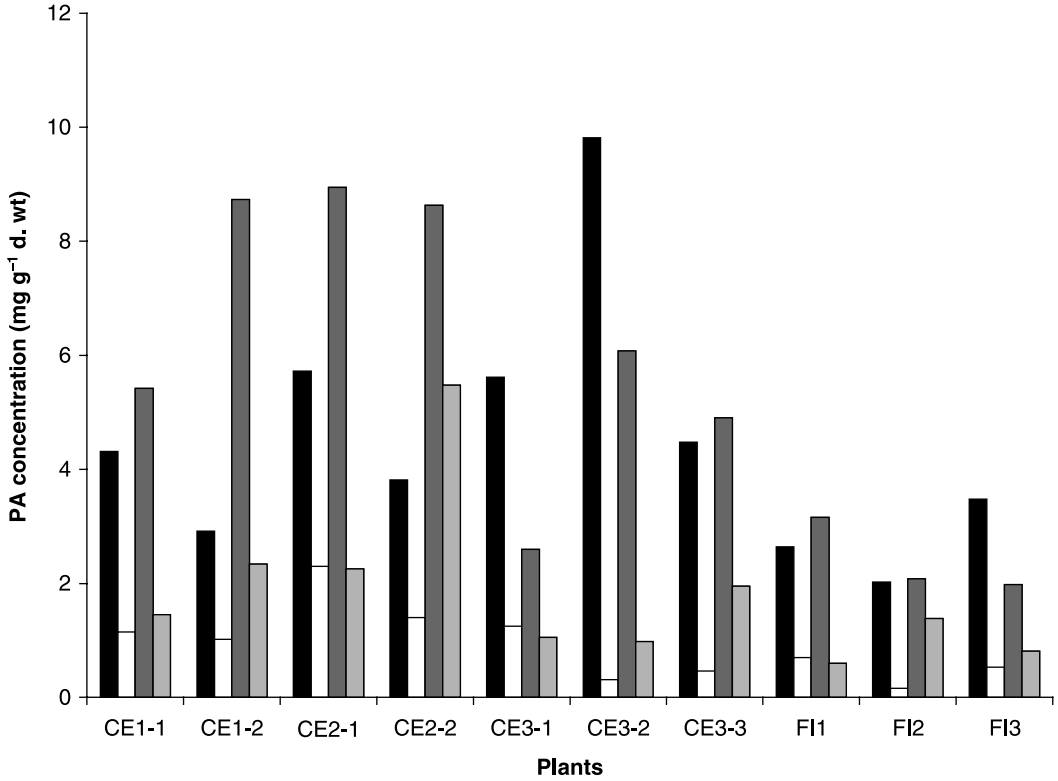


**Fig. 2** Pyrrolizidine alkaloid concentration in different root parts from *Senecio jacobaea*. Genotype CE1, CE2 CE3 were growing in climate chambers, FI1, FI2 and FI3 were collected from the field site, Meijendel, The Netherlands. Solid bars, main root cortex; open bars, main root vascular cylinder; medium grey bars, lateral roots; light grey bars, root tips.

production in the plant, or only a change in allocation to the flowerheads in order to compensate for the dilution effect. In an experiment where resource availability to individual plants was controlled, Vrieling & Van Wijk (1994a) found no effect of N or P limitation on PA concentration.

Although root biomass was not significantly influenced by nutrient supply, concentrations of PAs in the roots decreased as nutrients increased (Table 1). This might be the consequence of a larger shoot resulting in a larger PA sink in the above-ground parts. The correlation between root biomass and PA production was positive and stronger than the simulated correlation. This is what is expected, since PAs in *Senecio* spp. are produced in the roots (Hartmann & Toppel, 1987). However, this reduces flexibility in PA production. If PA production per root is not flexible, and independent of resources like N, then changes in biomass may have a large impact on PA concentrations and thus on the defence of the plant.

Often the negative correlation between plant biomass and secondary metabolite concentration is regarded as evidence for a trade-off. The effect of nutrients on concentrations of secondary metabolites can be explained by two mechanisms. Nutrients, in particular N, are integral components of secondary compounds. Nutrient addition may therefore increase the concentration of PAs. Alternatively, when nutrients are not limiting for the production of PAs, they control to a large degree plant biomass production and secondary metabolites may be diluted when nutrients are supplied at larger rates, which is reflected in a lower concentration.

The resource availability hypothesis (Coley *et al.*, 1985) predicts that under conditions of slow growth, plants should invest more in defence against herbivores. Although large and more nutritious plants may attract more herbivores, there is no need for more defence as long as the growth is faster than the removal of mass by herbivory. This would imply that under conditions of increased plant growth, due to increased availability of nutrients, dilution of secondary plant metabolites is acceptable for a plant explaining the present observations.

Concentrations of individual PAs consistently decreased with one exception. Jacobine remained constant in the shoot and increased in the roots. This might suggest that jacobine is of more importance for the defence of the plant than the other PAs are under the prevalent conditions. Jacobine is unusually toxic to guinea pigs (*Cavia porcellus*), which are generally resistant to PAs (Chung & Buhler, 1995). However, jacobine concentrations in the roots were very low in comparison to the other PAs and therefor would have to be very toxic. We could not find any study in which effects of jacobine on herbivorous insects or pathogens were compared with the effects of other PAs. By contrast, Vrieling & de Boer (1999) found that the jacobine and erucifoline chemotype of *S. jacobaea* are

equally attractive for preference and performance of the specialist herbivore *Tyria jacobaeae.*

PA concentrations in root tips were on average lower than in the other root parts. High concentrations were expected in root tips, which are extremely important for the plant with regard to water and nutrient uptake and at the same time vulnerable to attack by herbivores and pathogens. Optimal defence theory (Rhoades, 1979) argues that the plant should protect the most valuable parts best, implying high concentrations of defence compounds for instance in the flowers, seeds, young leaves and below-ground in fine roots.

Sander & Hartmann (1989) found the root apex to be the site of enhanced biosynthesis of PAs, although production was found in all root parts. In the present experiment PAs could hardly be detected at these production sites, which may indicate a fast transport to storage sites, such as the cortex of the main roots. This is opposite to observations by Hartmann & Toppel (1987), who found significant differences in total PA concentration of root parts in root cultures of the annual *S. vulgaris*. The fine roots contained twice as much PA as the coarse roots. In root cultures there is no transport to aboveground plant parts and also re-allocation in the root cultures may be different from natural circumstances. In this study we used whole plants from the vegetative stage of the monocarpic perennial *S. jacobaea*. Mature roots often contained higher concentrations of alkaloids than the root tips, like hyoscyamine accumulation in *Atropa belladonna* (Falk & Doran, 1996). Also in lupines epidermal cells are preferred sites of storage for quinolizidine alkaloids (Wink *et al.*, 1984). The more mature parts of roots are important for regrowth. Defoliation will not kill *Senecio* spp. (Obeso & Grubb, 1994; Vrieling *et al.*, 1996), but *Longitarsus jacobaeae* larvae, which feed on the roots can be deleterious to *S. jacobaea* (Windig, 1993). Although *L. jacobaeae* is a specialist herbivore, Vrieling & Wijk (1994b) found a negative correlation between PA concentration and herbivory by both adults and larvae. Thus an important part of the root system seems to have a defence in the form of relative high concentrations of PAs, but at present it is unknown against which herbivores or pathogens.

## Acknowledgements

We thank Wiecher Smant for nutrient analysis and T. de Jong, P. G. L. Klinkhamer and E. van der Meijden for valuable comments.

## References

**Baricevic D, Umek A, Kreft S, Maticic B, Zupanic A. 1999.** Effect of water stress and nitrogen fertilization of the content of hyoscyamine and scopolamine in the roots of deadly nightshade (*Atropa belladonna*). *Environmental and Experimental Botany* **42**: 17–24.

**Boer de NJ. 1999.** Pyrrolizidine alkaloid distribution in *Senecio jacobaea* minimises losses to generalist feeding. *Entomologia Experimentalis et Applicata* **91**: 169–173.

**Borstel von K, Witte L, Hartmann T. 1989.** Pyrrolizidine alkaloid patterns in populations of *Senecio vulgaris*, *S. vernalis* and their hybrids. *Phytochemistry* **28**: 1635–1638.

**Brown MS, Molyneux RJ. 1996.** Effects of water and mineral nutrient deficiencies on pyrrolizidine alkaloid content of *Senecio vulgaris* flowers. *Journal of the Science of Food and Agriculture* **70**: 209–211.

**Chung WG, Buhler DR. 1995.** Major factors for the susceptibility of guinea pig to the pyrrolizidine alkaloid jacobine. *Drug Metabolism and Disposition* **23**: 1263–1267.

**Coley PD, Bryant JP, Chapin FS. 1985.** Resource availability and plant herbivore defence. *Science* **230**: 895–899.

**Dyer LA, Dodson CD, Beihoffer J, Letourneau DK. 2001.** Trade-offs in antiherbivore defenses in *Piper cenocladum*: ant mutualists versus plant secondary metabolites. *Journal of Chemical Ecology* **27**: 581–592.

**Falk LR, Doran PM. 1996.** Influence of inoculum morphology on growth of *Atropa belladonna* hairy roots and production of tropane alkaloids. *Biotechnology Letters* **18**: 1099–1104.

**Gonzalez JA, EstevezBraun A, EstevezReyes R, Bazzocchi IL, Moujir L, Jimenez IA, Ravelo AG, Gonzalez AG. 1995.** Biological activity of secondary metabolites from *Bupleurum salicifolium* (Umbelliferae). *Experientia* **51**: 35–39.

**Hartmann T, Dierich B. 1998.** Chemical diversity and variation of pyrrolizidine alkaloids of the senecionine type: biological need or coincidence? *Planta* **206**: 443–451.

**Hartmann T, Ehmke A, Eilert U, von Borstel K, Theuring C. 1989.** Sites of synthesis, translocation and accumulation of pyrrolizidine alkaloid N-oxides in *Senecio vulgaris* L. *Planta* **177**: 98–107.

**Hartmann T, Toppel G. 1987.** Senecionine N-oxide, the primary product of pyrrolizidine alkaloid biosynthesis in root cultures of *Senecio vulgaris*. *Phytochemistry* **26**: 1639–1643.

**Hartmann T, Witte L. 1995.** Pyrrolizidine alkaloids: chemical, biological and chemoecological aspects. In: Pelletier SW, ed. *Alkaloids: chemical and biological perspectives*, *Vol. 9*. Oxford, UK: Pergamon Press, 155–233.

**Hartmann T, Zimmer M. 1986.** Organ specific distribution and accumulation of pyrrolizidine alkaloids during the life history of two annual *Senecio* species. *Plant Physiology* **112**: 67–80.

**Höft M, Verpoorte R, Beck E. 1996.** Growth and alkaloid contents in leaves of *Tabernaemontana pachysiphon* Stapf (Apocynaceae) as influenced by light intensity, water and nutrient supply. *Oecologia* **107**: 160–169.

**Hol WHG, van Veen JA. 2002.** Pyrrolizidine alkaloids from *Senecio jacobaea* affect fungal growth. *Journal of Chemical Ecology* **28**: 1763–1772.

**Hook I, Poupat C, Ahond A, Guenard D, Gueritte F, Adelin M, Wang X, Dempsey D, Breuillet S, Potier P. 1999.** Seasonal variation of neutral and basic taxoid contents in shoots of European Yew (*Taxus baccata*). *Phytochemistry* **52**: 1041–1045.

**Iason GR, Hester AJ. 1993.** The response of heather (*Calluna vulgaris*) to shade and nutrients – prediction of the carbon-nutrient balance hypothesis. *Journal of Ecology* **81**: 75–80.

**Johnson EA, Molyneux RJ, Merril GB. 1985.** Chemistry of toxic range plants. Variation in pyrrolizidine alkaloid content of *Senecio*, *Amsinckia*, and *Crotalaria* species. *Journal of Agricultural and Food Chemistry* **33**: 50–55.

**Karban R, Baldwin IT. 1997.** *Induced responses to herbivory.* Chicago, IL, USA: University of Chicago Press.

**Koricheva J. 1999.** Interpreting phenotypic variation in plant allelochemistry: problems with the use of concentrations. *Oecologia* **119**: 467–473.

**Laus G, Brossner D, Keplinger K. 1997.** Alkaloids of peruvian *Uncaria tomentosa*. *Phytochemistry* **45**: 855–860.

**van Loon JA, Schoonhoven LM. 1999.** Specialist deterrent chemoreceptors enable *Pieris* caterpillars to discriminate between chemically different deterrents. *Entomologica Experimentalis et Applicata* **91**: 29–35.

**Mattocks AR. 1986.** *Chemistry and toxicology of pyrrolizidine alkaloids.* Orlando, FL, USA: Academic Press.

**Novozamsky I, Houba VJG, van Eck R, van Vark W. 1983.** A novel digestion technique for multi-element plant analysis. *Communications in Soil Science and Plant Analysis* **14**: 239–249.

**O'Dowd DJ, Edgar JA. 1989.** Seasonal dynamics in the pyrrolizidine alkaloids of *Heliotropium europaeum*. *Australian Journal of Ecology* **14**: 95–105.

**Obeso JS, Grubb PJ. 1994.** Interactive effects of extent and timing of defoliation, and nutrient supply on reproduction in a chemically protected annual *Senecio vulgaris*. *Oikos* **71**: 506–514.

**Partel M, Zobel M, Zobel K, van der Maarel E. 1996.** The species pool and its relation to species richness: evidence from Estonian plant communitities. *Oikos* **75**: 111–117.

**Prins AH, Vrieling K, Klinkhamer PGL, De Jong T. 1990.** Flowering behaviour of *Senecio jacobaea*: effects of nutrient availability and size dependent vernalization. *Oikos* **59**: 248–252.

**Ralphs MH, Gardner DR, Pfister JA. 2000.** A functional explanation for patterns of norditerpenoids alkaloids levels in tall larkspur (*Delphinium barbeyi*). *Journal of Chemical Ecology* **26**: 1595–1607.

**Rhoades DF. 1979.** Evolution of plant chemical defences against herbivory. In: Rosenthal GA, Hanzen DH, eds. *Herbivores: their interaction with secondary metabolites*. New York, USA: Academic Press, 3–54.

**Salmore AK, Hunter MD. 2001.** Environmental and genotypic influences on isoquinoline alkaloid content in *Sanguinaria canadensis*. *Journal of Chemical Ecology* **27**: 1729–1747.

**Sander H, Hartmann T. 1989.** Site of synthesis, metabolism and translocation of senecionine N-oxide in cultured roots of *Senecio erucifolius*. *Plant Cell, Tissue and Organ Culture* **18**: 19–31.

**Schmeller T, Latz-Brûning B, Wink M. 1997.** Biochemical activities of berberine, palmatine and sanguinarine mediating chemical defence against microorganisms and herbivores. *Phytochemistry* **44**: 257–266.

**Siegel S. 1956.** *Nonparametric statistics for the behavioural sciences*. New York, USA: McGraw-Hill Co.

**SPSS Inc. 1998.** *SPSS Base 8.0 User's Guide*. Chicago, IL, USA: SPSS Inc.

**Steenkamp V, Stewart MJ, van der Merwe S, Zuckerman M, Crowther NJ. 2001.** The effect of *Senecio latifolius* a plant used as a South African traditional medicine, on a human hepatoma cell line. *Journal of Ethnopharmacology* **78**: 51–58.

**Steiner AA. 1968.** Soilless culture. In: International Potash Institute, ed. *The fertilization of protected crops. proceedings of the 6th coll internat potash institute*, Berne, Switzerland: International Potash Institute, 324–341.

**Troelstra SR, Wagenaar R, Smant W. 1995.** Nitrogen utilization by plant species from acid heathland soils. I. Comparison between nitrate and ammonium nutrition at constant low pH. *Journal of Experimental Botany* **46**: 1103–1112.

**Van Dam NM, van der Meijden E, Verpoorte R. 1993.** Induced responses in three alkaloid-containing plant species. *Oecologia* **95**: 425–430.

**Van Dam NM, Vuister LWM, Bergshoeff C, de Vos H, van der Meijden E. 1995a.** The 'raison d'etre of pyrrolizidine alkaloids in *Cynoglossum officinale*: deterrent effects against generalist herbivores. *Journal of Chemical Ecology* **21**: 507–523.

**Van Dam NM, Witte L, Theuring C, Hartmann T. 1995b.** Distribution, biosynthesis and turnover of pyrrolizidine alkaloids in *Cynoglossum officinale*. *Phytochemistry* **39**: 287–292.

**Van der Meijden E, Van der Waals-Kooi RE. 1979.** The population ecology of *Senecio jacobaea* in a sand dune system. *Journal of Ecology* **67**: 131–153.

**Van Loon JA, Schoonhoven LM. 1999.** Specialist deterrent chemoreceptors enable *Pieris* caterpillars to discriminate between chemically different deterrents. *Entomologica Experimentalis et Applicata* **91**: 29–35.

**Vrieling K, de Boer N. 1999.** Host-plant choice and larval growth in the cinnabar moth: do pyrrolizidine alkaloids play a role? *Entomologia Experimentalis et Applicata* **91**: 251–257.

**Vrieling K, de Jong TJ, Klinkhamer PGL, van der Meijden E, van Wijk CAM. 1996.** Testing trade-offs among growth, regrowth and antiherbivore defenses in *Senecio jacobaea*. *Entomologia Experimentalis et Applicata* **80**: 189–192.

**Vrieling K, de Vos H, van Wijk CAM. 1993.** Genetic analysis of the concentrations of pyrrolizidine alkaloids in *Senecio jacobaea*. *Phytochemistry* **32**: 1141–1144.

**Vrieling K, van Wijk CAM. 1994a.** Cost assessment of the production of pyrrolizidine alkaloids in ragwort (*Senecio jacobaea* L.). *Oecologia* **97**: 541–546.

**Vrieling K, van Wijk CAM. 1994b.** Estimating costs and benefits of the pyrrolizidine alkaloids of *Senecio jacobaea* under natural conditions. *Oikos* **70**: 449–454.

**Wesselingh RA, Klinkhamer PGL. 1996.** Threshold size for vernalization in *Senecio jacobaea*: Genetic variation and response to artificial selection. *Functional Ecology* **10**: 281–288.

**Windig J. 1993.** Intensity of *Longitarsus jacobaeae* herbivory and mortality of *Senecio jacobaea*. *Journal of Applied Ecology* **30**: 179–186.

**Wink M, Heinen HJ, Vogt H, Schiebel HM. 1984.** Cellular localization of quinolizidine alkaloids by mass spectrometry (LAMMA 1000). *Plant Cell Reports* **3**: 230–233.