EXTREME DIFFERENCES IN PYRROLIZIDINE ALKALOID LEVELS BETWEEN LEAVES OF *CYNOGLOSSUM OFFICINALE*

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Abstract—Optimal defence theories predict that the most valuable parts of a plant should be best protected. This theory was tested with pyrrolizidine alkaloids (PAs) in the leaves of rosette plants of *Cynoglossum officinale*. The youngest leaves contained up to 190 times higher levels of PAs than older leaves. This skewed distribution of PAs may be a strategy to divert herbivores from the photosynthetically active young leaves and thus to optimize growth.

INTRODUCTION

Theories on optimal defences in plants postulate that the most valuable parts of a plant should be most protected against herbivores [1]. The value of a plant part is by definition related to its contribution to the plant's fitness. Especially in monocărpic plants during the reproductive phase, flowers and seeds represent the closest approximation to fitness. Indeed it has been found that these organs often contain the highest levels of defensive secondary metabolites within the plant [2–5].

Facultative biennials spend one or more years of their life as vegetative rosettes. During this stage, it is difficult to estimate straightforwardly how much individual leaves contribute to total fitness. Because in most plant species seed and pollen production are closely related to plant size, the contribution of leaves to plant growth can be used as a good approximation. Within the shoots the young, fully expanded leaves have the highest photosynthetic rates and thus contribute most to growth [6, 7]. Commensurate with high photosynthetic rates, these leaves also contain the highest levels of nitrogen and are at the outside of the canopy [8]. The youngest leaves thus are the most valuable for the plant and at the same time the most vulnerable to herbivory. Consequently, we expect that the youngest leaves of a rosette contain more defence chemicals than the oldest leaves.

Cynoglossum officinale L. is a facultative biennial that contains several pyrrolizidine alkaloids (PAs) such as heliosupine, echinatine, and viridiflorine [9]. PAs are toxic to several vertebrate herbivores and deterrent to generalist invertebrate herbivores [10–12]. In this paper we examine the leaf-for-leaf distribution of pyrrolizidine alkaloids in rosette plants of *C. officinale*.

RESULTS AND DISCUSSION

The youngest leaves of rosette plants of *C. officinale* contained significantly higher PA concentrations than the oldest leaves (Figs 1 and 2). Dead leaves did not have detectable amounts of PAs (i.e. <0.4 mg g^{-1} DW). The mean PA concentration of the youngest leaf of the plants in the growth room was 190 times higher than that of the sixth leaf of the same plants. In the plants grown in the field the difference was less extreme, but still the PA concentration of the youngest leaf was 53 times higher than that of the oldest leaf. This difference might be caused by the genetic background of the plants. The plants in the growth room originated from one selfed mother plant and thus were genetically more uniform than the plants in the field. The variation in total PA concentrations in *C. officinale* is known to have a genetic component [13]. Moreover, the growth room plants were grown under uniform conditions, while the field plants came from stands with different environmental conditions. However, all individual plants, in both field and growth room experiments, showed a negative correlation of PA content with leaf number (Spearman rank correlation).

The low PA concentration in older leaves was not due only to dilution by increasing leaf weight during ageing. After having reached a maximum in the fully expanded leaves, also the absolute amount of PAs decreased in the ageing leaves (Figs 1 and 2). It is known that several alkaloid-containing plant species have higher alkaloid levels in young leaves than in mature leaves (listed in ref. [14]). However, the differences in concentration between these leaf age-groups mostly are far less extreme than that we have found in *C. officinale*. For example, young,
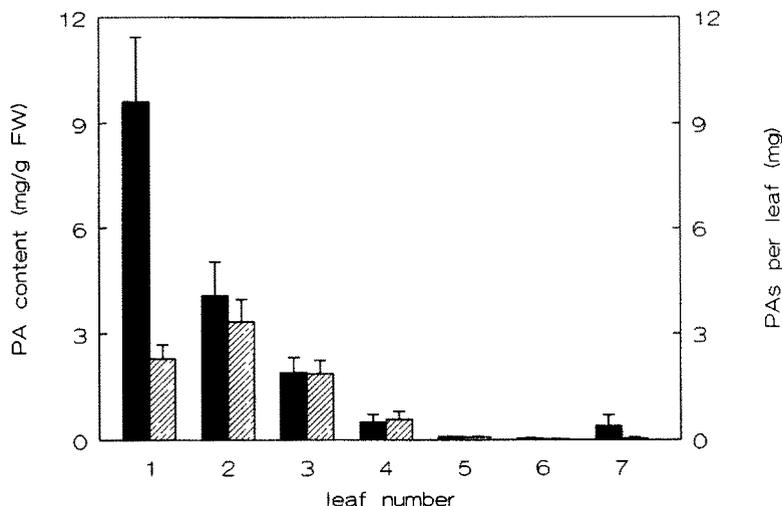
Fig. 1. Pyrrolizidine alkaloid (PA) content (+s.e.m., $n = 3$) per leaf of *Cynoglossum officinale* rosette plants grown in a growth room. Number 1 is the youngest leaf.
Black bars: PA content (mg g^{-1} FW), left Y-axis. Friedman analysis, $\chi^2 = 16.6$, P = 0.01. Dashed bars: total PAs per leaf (mg), right Y-axis. Friedman analysis, $\chi^2 = 16.6$, P = 0.01.
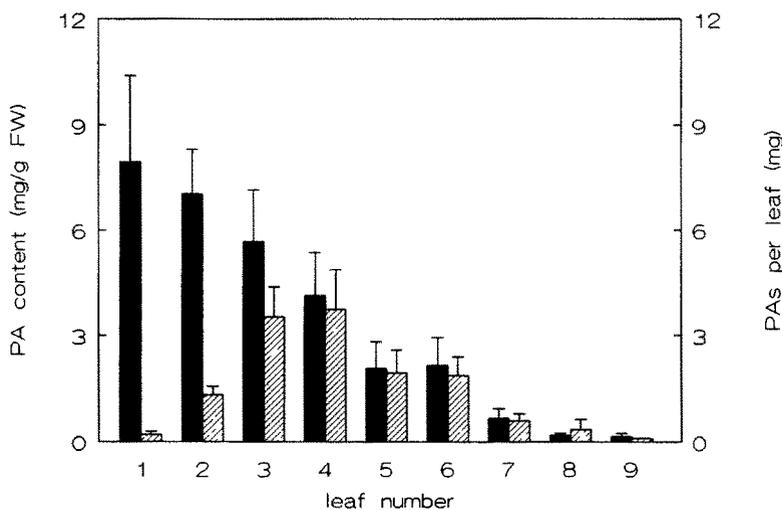

Fig. 2. Pyrrolizidine alkaloid (PA) content (+s.e.m., $n = 7$) per leaf of *Cynoglossum officinale* rosette plants sampled in the coastal dunes near the Hague (NL), 22 July 1993. Number 1 is the youngest leaf.
Black bars: PA content (mg g^{-1} FW), left Y-axis. Friedman analysis (from leaf 1 to 5), $\chi^2 = 18.3$, P = 0.001. Dashed bars: total PAs per leaf (mg), right Y-axis. Friedman analysis (from leaf 1 to 5), $\chi^2 = 17.8$, P = 0.001.

unopened leaves of *Veratrum album* contain five times higher alkaloid percentages than mature leaves [15].

In the literature, we have found only one example of a plant that showed a decrease in alkaloid content with leaf age comparable to that in *C. officinale*. The youngest leaves of *Coffea liberica*, the coffee-plant, had 75 times higher caffeine levels than the oldest leaves [16]. Caffeine biosynthesis is closely related to metabolic activity in young leaves [4]. The decrease of caffeine content with

age is due to catabolism of the alkaloid [17, 18]. In *Cynoglossum officinale* rosette plants, PAs are not catabolized but reallocated from the ageing leaves into the youngest leaves [19].

The distribution of PAs in rosette plants of *Cynoglossum officinale* is in accordance with what we expected based on optimal defence theories: the youngest leaves had higher PA levels than the oldest. We had two reasons to expect this distribution. In the first place, young leaves

have the highest future contribution to total plant assimilation in terms of photosynthetic rate × life expectancy [6]. Removal of young leaves may cause a greater loss of total photosynthetic output, and thus growth, than removal of old leaves [20]. Secondly, young leaves contain more nitrogen and water than older leaves and thus provide food of better quality for herbivores [1, 8]. In general, survival and fecundity of herbivores raised on young leaves is greater than on old leaves [21]. Specialist herbivores, which have overcome the disadvantages of their host's defences, generally prefer young leaves over old leaves [22]. In contrast, most generalist herbivores prefer older leaves [22]. This may indicate that plants can influence the food choice of generalist herbivores within the plant, in order to maximize growth and fitness. This was confirmed by preliminary experiments with generalist herbivores on rosette plants of *C. officinale* (van Dam, unpublished results). Our results should alert chemical ecologists that one might lose a lot of ecologically important information when expressing levels of defence compounds on the basis of total biomass of the plant. Preferably, the levels of the various plant parts should be expressed separately.

EXPERIMENTAL

Plants from the growth room. Seeds of *Cynoglossum officinale* L. (Boraginaceae) originating from one, selfed mother that was grown in a growth room, were scarified and imbibed for 2 weeks (20°/16 hr L, 10°/8 hr D). The seedlings were potted in 1 l pots containing a mixt. of dunesand and compost (1:1) and placed in a growth room (20°/16 hr L, 15°/8 hr D). The plants were provided with sufficient nutrient solution [23] to allow exponential growth. After 6 weeks the individual leaves of 3 plants, which had 7 leaves each, were weighed to measure their fr. wt (FW). The fresh leaves were ground with some sand (Merck) and subsequently extracted for PAs. In addition, we sampled dead leaves from older plants, to assess whether they contained PAs. Brown and dry leaves of 5 plants in the growth room were sampled, ground, and extracted for PAs.

Field plants. On 22 July 1993, 7 undamaged *C. officinale* plants with at least 5 leaves each were randomly sampled in their natural habitat, the coastal dunes near The Hague (The Netherlands). The individual leaves were weighed to obtain their FW and were dried at 50° for 2 days. After determining the dry wt (DW), the leaves were ground with pestle and mortar and extracted for PAs.

PA extraction and determination. PAs were extracted as described in ref. [24]. A spectrophotometric colour reaction was used to determine PA concns [25]. Monocrotaline (99% pure, Sigma) was used as reference.

Statistical analysis. All statistical tests were performed with Statgraphics 5.0 (Statgraphics Corporation 1992). Differences between the leaves in PA contents and total PAs were analysed with the Friedman analysis of variance, since the data were not independent. The Friedman analysis requires equal numbers of observations per

group. The field plants did not have equal numbers of leaves. However, they all had 5 leaves at least, so we applied the Friedman analysis for leaf 1 to 5 only.

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