Leaf surface chemistry: the interface between herbivores and plants

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Introduction

Adult herbivorous insects that do not consume plants, like moths and butterflies, have to rely mainly on the outside of the leaf for assessing the quality of the inside of the leaf.

This offers the opportunity for the plant to disguise itself.

Is the leaf surface chemistry a reliable cue for the inside chemistry of the leaf?

We measured the pyrrolizidine alkaloid (PA, fig. 1) profile on the leaf surface and inside the leaf in 44 F2 hybrids of *Jacobaea vulgaris* and *Jacobaea aquatica.*

Previous studies showed that PAs act as oviposition stimulants for one of the main specialist herbivores of *Jacobaea* plants, the cinnabar moth (Fig. 1).

Fig. 1: Structure of the PA senecionine in *Jacobaea* plants in its N-oxide and its free base form

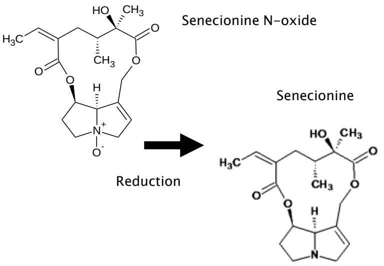


Fig. 2: Cinnabar moth *Tyria jacobaeae*

Results

	Inside leaf	leaf surface
Number of PAs	41	30
Average PA conc.	3168 ug/g	319 pg/cm^2

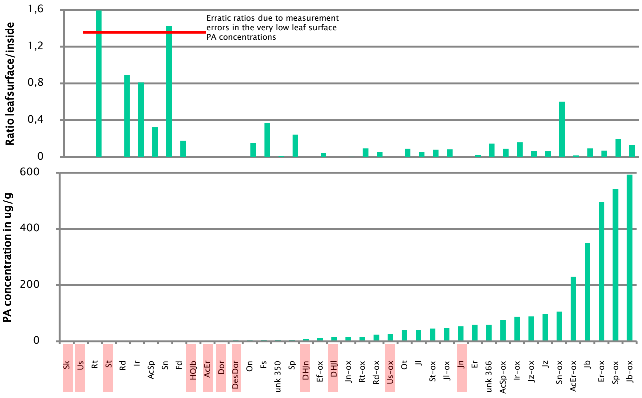
The ratio of (PAs on the leaf surface/PAs inside the leaf) varied for the different PAs especially for Senecionine N-oxide (Fig 3)

13 out of 30 individual PAs were significantly correlated between inside and leaf surface

The PAs of *Jacobaea* plants can be divided into four structural groups based on their biosynthesis. Previous research (Cheng et al, 2011) showed that PAs within this groups are covarying. The correlation of PA groups on inside and leaf surface showed that these are significant for Jacobine-like and Otonecine-like PAs but not for Senecionine- and Erucifoline-like Pas (fig. 4).

Fig 3 Below: Average PA concentration of individual PAs inside the leaf of 44 *Jacobaea* plants. PAs in red are not present on the leaf surface

Above: ratio of PAs on the leaf surface (pg/cm^2) / PAs inside the leaf (ug/g dry)



Material and Methods

Plant growth

44 F_2 hybrid genotypes derived from a cross between *J. vulgaris* and *J. aquatica* were grown in a climate chamber for 15 weeks.

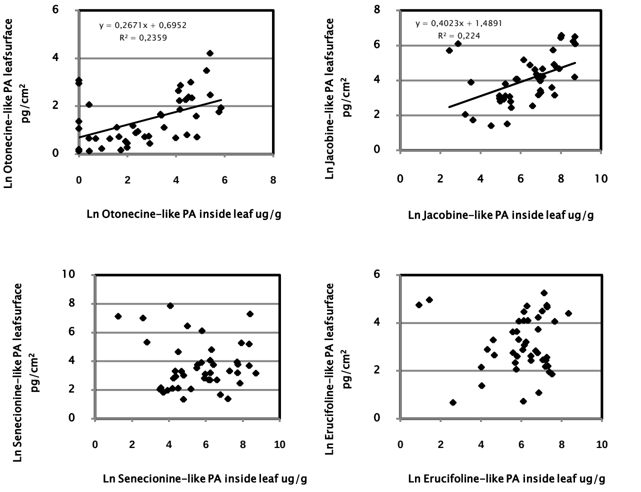
Experimental design

A fully developed leaf of each plant was dipped for 50 s in 40 ml of 0,004% formic acid. The leaf was flash frozen in liquid nitrogen, freeze dried and analyzed for PA content. The liquid used for the dipping was reduced to 0,5 ml with SPE and analyzed for PA content.

PA measurement

Pas were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using heliotrine and heliotrine N-oxide as internal standards

Fig. 4 Scattergrams of the four structural groups of PAs inside the leaf against PAs on the leaf surface



Conclusion

- The majority of individual Pas inside the leaf are also found on the surface of the leaf but with variable ratios especially for Senecionine N-oxide .
- Jacobine- and Otonecine-like PAs are significantly correlated between inside of the leaf and the leaf surface. Senecionine- and Erucifoline-like PAs are not significantly correlated between inside and leaf surface in *Jacobaea* plants. Interestingly Jacobine- or Otonecine-like PAs were the only PAs correlated with preference or performance of three tested herbivores (Cheng et al. press/prep).

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