Pyrrolizidine alkaloid composition in the plant and its interaction with the soil microbial community

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General introduction

Plants are attacked by a variety of (micro)organisms. In order to cope with the great variety of potential attackers many plants synthesize a diversity of repellent, deterrent and/or toxic compounds as a defence against herbivores and pathogens.

The evolution of compound diversity is supposed to be driven by herbivore insects. Herbivore populations change over time and consequently the selection pressure on the defence chemistry of pants will change as well (Macel et al. 2005). Less adapted generalist herbivores are supposed to be more sensitive to differences in chemical compounds. Thus generalists may play a greater role in the evolution and maintenance of diversity of secondary plant metabolites than adapted specialists (Miller and Feeny 1983, Lindroth et al. 1988, van Dam et al. 1995, Agrawal 2002, Macel et al. 2002, Macel et al. 2005).

There are several possible reasons for the variety of secondary metabolites in plants. The first reason is that new compounds are synthesized because of a continuous evolutionary arms race between the production of new defence chemicals by plants and the adaptation to these compounds by attackers. The selective pressure forced by adaptation of the attacker forces the plant to continuously select different compounds. This hypothesis implies that evolutionary older compounds are less effective than more recently developed ones (Berenbaum and Feeny 1981, Miller and Feeny 1983, Berenbaum et al. 1989, Macel et al. 2005).

The second reason for the diversity of compounds might be the synergistically effect of different defence compounds (Adams and Bernays 1978, Lindroth et al. 1988, Berenbaum et al. 1989, Berenbaum et al. 1991). This implies that plants with a more diverse or complex defence compound composition are more successful in deterring attackers than plants with a simpler composition.

The third reason is that this diversity of compounds can be maintained by selection pressure by several attackers (Simms 1990). The plant contains a wide variety of defence compounds, whereby the relative importance of the different compounds differs locally and on the prevailing selective environment. Thereby it is expected that those compounds differ in their toxic and deterrent effects on generalist insects and other enemies such as nematodes (van Dam et al. 1995, Macel et al. 2002; Macel et al. 2005, Dominguez et al. 2008; Thoden et al. 2009).

Pathogens such as fungi and bacteria also are a serious threat to plants and therefore they may also influence the selection of defence mechanisms (Hol and van Veen 2002). Historically, long before herbivores existed on this planet, plants had to cope with microbial pathogens. However, the existing evidence on the role secondary metabolites play in plant defence against microorganisms, like soil pathogens, is scarce especially in comparison to ecological studies with insects on plants. We will try to get a better understanding in the plant chemical defence with the focus on the interaction with soil-borne microorganisms.

Pyrrolizidine alkaloids

Alkaloids represent one of the largest groups of secondary metabolites. From the more than 50.000 secondary metabolites described, ca. 12.000 are alkaloids (Wink and Roberts 1998; Wink 2003). Only around 600 alkaloids are studied in detail on for instance biochemical properties and eco-physiological roles (Wink and Roberts 1998) such as pyrrolizidine alkaloids (PAs) and quinolizidine alkaloids.

Pyrrolizidine alkaloids (PAs) are a well-known class of defence compounds with a wide variety of structures. From several genera of *Asteraceae, Boraginaceae, Orchidaceae* and *Fabaceae*, more than 360 structurally different PAs have been isolated (Rizk 1991; Hartmann and Witte 1995). It is known that PAs in plants are present as mixtures of the tertiary alkaloids and the respective *N*-oxides (Rizk 1991). The number of structurally different PAs actually almost doubles when the PA-state is taken into account since most PAs, but not all, can be present in both forms.

One of the most diverse classes of PAs is the macrocyclic senecionine type with more than 100 structures (Hartmann and Dierich 1998). This type of PAs is abundantly found in species of the tribe *Senecioneae* of the family *Asteraceae* (e.g. Genus *Senecio* and *Jacobaea*).

PAs have toxic, deterrent and/or repellent effects on a wide range of generalist herbivores, which helps the plant to reduce or prevent damage, but also have attractive effects on specialist herbivores (van Dam et al. 1995; Hartmann 1999; Hartmann and Ober 2000; Ober 2003; Macel et al. 2005). These PA effect on organisms depend on the PA concentration and composition in the plant (Macel 2011).

Jacobaea vulgaris Gaertn. (syn *Senecio jacobaea* L.) is known to be one of the suitable systems to study the chemical defence mechanisms of PAs in plants. In previous studies about 14 different PAs were detected in *J. vulgaris* (Witte et al. 1992; Macel et al. 2004; Kowalchuk et al. 2006). The concentration and composition of PAs in *J. vulgaris* depend on the genotype (Vrieling et al. 1993) and its environment (Hol et al. 2003; Macel et al. 2004; Hol et al. 2004; Macel and Klinkhamer 2010).

In *Jacobaea* species, such as *J. vulgaris*, PAs are synthesized in the roots primarily as senecionine *N*-oxide (Hartmann and Toppel 1987; Toppel et al. 1987). Subsequently, senecionine *N*-oxide is transported to the shoot, where further diversification into different individual PAs takes place by specific enzymes (Hartmann and Dierich 1998). All PAs are derived from the senecionine *N*-oxide core-structure and represent end-products, which show no degradation or turnover. Therefore, the total amount of PAs in the plant is controlled by the senecionine *N*-oxide formation in the roots, which is closely linked to root growth (Hartmann et al. 1988; Sander and Hartmann 1989).

Based on their PA composition individual *J. vulgaris* plants can be distinguished into different chemotypes (Witte et al. 1992; Macel et al. 2004). Senecionine-chemotypes contain mainly senecionine-like PAs and largely lack jacobine- and erucifoline-like PAs, Erucifoline-chemotypes contain mainly senecionine- and erucifoline-like PAs and lack jacobine-like PAs, Jacobine-chemotypes contain high levels of jacobine-like PAs and mixed-chemotypes containing both high levels of jacobine-like PAs as well as erucifoline-like PAs (Figure 1).

Two PA forms in *Jacobaea* plants

PAs occur in plants in two forms: *N*-oxides and its reduced tertiary amines. The water soluble *N*-oxide form is considered to be the best form for slow allocation between tissues by phloem transport (Hartmann et al. 1989) and storage in cell vacuoles (von Borstel and Hartmann 1986; Ehmke et al. 1988). Generalist insect herbivores reduce *N*-oxides in the gut to tertiary PAs, where these are passively taken up into the body (Lindigkeit et al. 1997; Hartmann 1999) and converted into pyrroles. Pyrroles are toxic by acting as highly reactive alkylating agents to organisms like mammals (Mattocks 1986) and fruit flies (Frei et al. 1992). Individual PA *N*-oxides show less deterrent or toxic effects for some generalist insect herbivores compared to their tertiary PA form (Dreyer et al. 1985; van Dam et al. 1995; Macel et al. 2005). Specialist insects, adapted to PAs, take up the tertiary amines and actively convert them into *N*-oxides. They even store PA *N*-oxides and use these compounds for their own defence (Boppré1986; Lindigkeit et al. 1997; Dobler 2001; Nishida 2002; Narberhaus et al. 2003).

Plants developed other mechanisms to face serious attacks by specialists. *J. vulgaris* contains high food reserves in the root systems for vegetative reproduction and re-growth to survive complete defoliation by, for instance, specialist herbivores (Verkaar 1987; van der Meijden et al. 1989). Therefore it is to be expected that plant species, using this survival strategy, protect their roots strongly against belowground herbivores and pathogens.

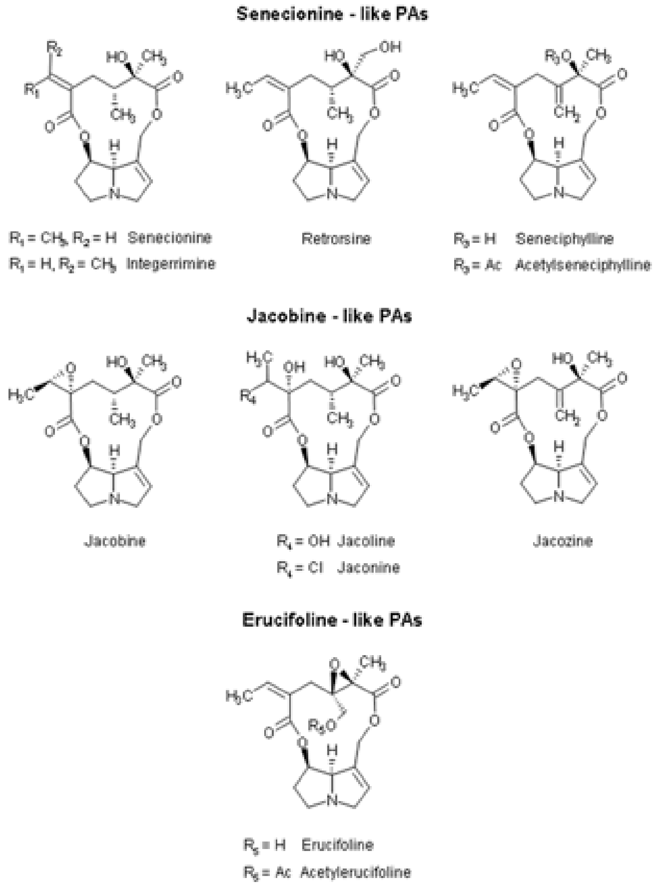

Figure 1. The structure of the major PAs detected in *Jacobaea vulgaris* plants and which occur in both tertiary amine and *N*-oxide form. Only the structures of the tertiary amine form are shown.

Induction of PAs by soil-borne microorganisms

Plants are attacked most times simultaneously above- and belowground. When the root system is exposed to belowground organisms (e.g. herbivore insects, nematodes, root pathogens and mycorrhizal fungi) plants may show several defence responses in the shoots that may also affect aboveground herbivores and, thus plant fitness (van Loon et al. 1998; Paul et al. 2000; van der Putten et al. 2001; Gange et al. 2002; Dicke and Hilker 2003; van Dam et al. 2003; Bezemer et al. 2005; Bezemer and van Dam 2005). Less is known about PA induction by pathogens. Artificial root damage has shown to result in an increased PA concentration in the shoots of *J. vulgaris* (Hol et al. 2004). Macel and Klinkhamer (2010) noticed that the composition of PAs in genotypes of *J. vulgaris* changed in the field compared to the initial composition in laboratory clones. The PA composition also differed between the aboveground parts of clones grown on two different experimental field sites. Bezemer et al. (2006) found that aboveground herbivory was related to the fungal community belowground. They suggested that the fungal community directly or indirectly changed the concentration of different PAs in the shoots and in this way affected the above-ground insect community.

The possible impact of soil-borne microorganisms on the defence system of the plant may have ecological consequences. If the aboveground defence compounds are affected this may influence plant resistance by attracting or deterring herbivores (McEvoy et al. 1993; Macel and Vrieling 2003; Macel et al. 2005, Macel and Klinkhamer 2010). It could also have considerable consequences for other relevant processes, for instance, the success of invasive plants and the biological control of plants.

Aims

The aim of this thesis is to gain better understanding on the PA defence system, with the primary focus on the interaction with soil-borne microorganisms. A novel PA analysis has been used that allows for obtaining better knowledge of the composition of PAs in above- and belowground plant parts and the chemical forms in which they occur. Emphasis is on the impact of soil-borne microorganisms on the above- and belowground PA defence system and its ecological consequences. Molecular techniques have been applied to get a better insight in the influence of PAs on the soil-borne microbial community in the rhizosphere and roots of the plant.

The main Research Questions addressed here are:

- Is the novel extraction and detection method reliable, applicable and sensitive enough for studying PAs, by comparing the traditional method with the novel method, in particular with respect to the detection of reduced PAs?
- Are PAs present in tertiary amine form in *J. vulgaris*? Is the presence of PAs in tertiary amine form in *J. vulgaris* genotype and/or PA-type dependent?
- Is the PA production inducible by soil-borne microorganisms? Do soil-borne microorganisms affect the aboveground metabolic profiles and so the defence against thrips in *J. vulgaris*?
- Do PAs affect the soil-borne microbial community living in the rhizosphere and roots of *J. vulgaris*?

Thesis outline

In **Chapter 2**, a review is presented on the available literature on defensive properties of PAs against microorganisms.

In **Chapter 3**, a new technique to extract and detect PAs is described. In this study two different approaches for the analysis of PAs in plant material are compared. The questions that are addressed in this chapter are: (A) does, detection of (reduced) PAs with GC-PND and LC-MS/MS give similar results with respect to the concentration and composition in plant material, irrespective of the sample preparation procedure?, (B) is formic acid extraction an effective alternative to sulphuric acid extraction ?, (C) could PA *N*-oxide reduction with sodium metabisulfite ($Na_2S_2O_5$) be an alternative to the commonly used zinc dust for analysis by GC based methods? and (D) does the traditional and the novel analytical method give similar results regarding PA concentration and composition in plant material?

In **Chapter 4** I tested whether high concentrations of tertiary PAs are present in the plant material of *J. vulgaris* or just an artefact of this novel approach for PA measurements. By analysing the PAs in both forms in different *J. vulgaris* genotypes, we observed the PA state to be genotype and PA-type dependent. In order to assess the genetic basis of this variation, we analysed PAs in F_2 hybrids of *J. vulgaris* and *Jacobaea aquatica* ((Hill) P. Gaertn).

In **Chapter 5** information is presented on the impact of soil-type and soil-borne microorganisms on PA concentrations and composition in roots and shoots of *J. vulgaris*. In **Chapter 6** I report that the results in the previous study are reproducible and that the defence system of different *J. vulgaris* chemotypes is changed in a similar way as a response to soil-borne microorganisms. To test if the changed PA composition has any influence on a higher trophic level, I introduced a generalist thrips (*Frankliniella occidentalis* (Pergande)) on the plants, and measured aboveground herbivore resistance. The following research questions are addressed: (A) does the soil-borne microbial community affect plant growth?, (B) does the soil-borne microbial community affect PA concentration and PA composition in shoots? and as a consequence (C) does the soil-borne microbial community affect resistance against thrips? and (D) are the effects of the soil-borne microbial community on growth, PAs and thrips resistance, genotype depended?
The effects of intra-specific differences in the PA defence system of different *J. vulgaris* genotypes on the microbial community structure belowground are addressed in **Chapter 7**. I assessed the general fungal and bacterial soil communities in the rhizosphere and in the root tissue as well as Arbuscular Mycorrhizal Fungi, AMF, in the roots of five different *J. vulgaris* genotypes grown on two different soil-types, löss and sand. General bacterial and fungal community structures were analysed by DGGE and AMF by T-RFLP.
The results of the different studies are summarized, discussed and evaluated in **Chapter 8**.

References

Adams C, Bernays EA (1978) The effect of combinations of deterrents on the feeding behavior of *Locusta migratoria* (L.). Entomol. Exp. Appl. 23:101-109

Agrawal AA, Conner JK, Johnson MTJ, Wallsgrove R (2002) Ecological genetics of an induced plant defence against herbivores: additive genetic variance and costs of phenotypic plasticity. Evolution 56:2206-2213

Berenbaum M, Feeny P (1981) Toxicity of angular furanocoumarins to swallowtailbutterflies: escalation in a coevolutionary arms race? Science 212:927-929

Berenbaum M, Nitao OJK, Zangerl AR (1991) Adaptive significance of furanocoumarin diversity in *Pastinaca sativa* (Apiaceae). J. Chem. Ecol. 17:207-215

Berenbaum MR, Zangerl AR (1998) Chemical phenotype matching between a plant and its insect herbivore. Proc Natl Acad Sci USA 95:13743-13748

Bezemer TM, de Deyn GB, Bossinga TM, van Dam NM, Harvey JA, van der Putten WH (2005) Soil community composition drives aboveground plant-herbivore-parasitoid interactions. Ecol. Lett. 8:652-661

Bezemer TM, van Dam NM (2005) Linking aboveground and belowground interactions via induced plant defenses. Trends Ecol Evol 20:617-624

Bezemer TM, Harvey JA, Kowalchuk GA, Korpershoek H, van der Putten WH (2006) Interplay between *Senecio jacobaea* and plant, soil, and aboveground insect community composition. Ecology 87:2002-2013

Boppré M (1986) Insects pharmacophagously utilizing defensive plant-chemicals (pyrrolizidine alkaloids). Naturwissenschaften 73:17-26

Dicke M, Hilker M (2003) Induced plant defenses: from molecular biology to evolutionary ecology. Basic Appl. Ecol. 4:3-14

Dobler S (2001) Evolutionary aspects of defense by recycled plant compounds. Basic Appl. Ecol. 2:15-26

Dominguez DM, Reina M, Santos-Guerra A, Santana O, Agullo T, Lopez-Balboa C, Gonzalez-Coloma A (2008) Pyrrolizidine alkaloids from Canarian endemic plants and their biological effects. Biochem. Syst. Ecol. 36:153-166

Ehmke A, von Borstel K, Hartmann T (1988) Alkaloid *N*-oxides as transport and vacuolar storage compounds of pyrrolizidine alkaloids in *Senecio vulgaris* L. Planta 176:83-90

Frei H, Lüthy J, Brauchli J, Zweifel U, Würgler FE, Schlatter C (1992) Structure/activity relationships of the genotoxic potencies of sixteen pyrrolizidine alkaloids assayed for the induction of somatic mutation and recombination in wing cells of *Drosophila melanogaster*. Chem. Biol. Interact. 83:1-22.

Gange AC, Stagg PG, Ward LK (2002) Arbuscular mycorrhiza fungi affect phytophagous insect specialism. Ecol. Lett. 5:11-15

Hartmann T, Zimmer M (1986) Organ-specific distribution and accumulation of pyrrolizidine alkaloids during the life history of two annual *Senecio* species. J. Plant Physiol. 122:67-80

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, Sander H, Adolph R, Toppel G (1988) Metabolic links between the biosynthesis of pyrrolizidine alkaloids and polyamines in root cultures of *Senecio vulgaris*. Planta I 75:82-90

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

Hartman T, Witte L (1995) Chemistry, Biology and Chemoecology of the Pyrrolizidine Alkaloids. In: Pelletier SW (Ed), Alkaloids: Chemical and Biological Perspectives 9, Pergamon Press, Oxford, pp 155-233

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 (1999) Chemical ecology of pyrrolizidine alkaloids. Planta 207:483-495

Hartmann T Ober D (2000) Biosynthesis and Metabolism of Pyrrolizidine Alkaloids in Plants and Specialized Insect Herbivores. In: Leeper FJ, Vederas JC (Eds) Topics in current Chemistry: Biosynthesis: Aromatic Polyketides, Isoprenoids, Alkaloids, Vol. 209. D-Berlin: Springer, pp 207-243

Hol WHG, van Veen JA (2002) Pyrrolizidine alkaloids from *Senecio jacobaea* affect fungal growth. J. Chem. Ecol. 28:1763-1772

Hol WHG, Vrieling K, van Veen JA (2003) Nutrients decrease pyrrolizidine alkaloid concentrations in *Senecio jacobaea*. New Phytol. 158:175-181

Hol WHG, Macel M, van Veen JA, van der Meijden E (2004) Root damage and aboveground herbivory change concentration and composition of pyrrolizidine alkaloids of *Senecio jacobaea* Basic Appl. Ecol. 5:253-260

Kowalchuk GA, Hol WHG, van Veen JA (2006) Rhizosphere fungal communities are influenced by *Senecio jacobaea* pyrrolizidine alkaloid content and composition. Soil Biol. Biochem. 38:2852-2859

Lindigkeit R, Biller A, Buch M, Schiebel HM, Boppré M, Hartmann T (1997) The two faces of pyrrolizidine alkaloids: The role of the tertiary amine and its *N*-oxide in chemical defense of insects with acquired plant alkaloids. Eur. J. Biochem. 245:626-636

Lindroth RL, Scriber JM, Hsia SMT (1988) Chemical ecology of the tiger swallowtail: mediation of host use by phenolic glycosides. Ecology 69:814-822

Macel M, Klinkhamer PGL, Vrieling K, Van der Meijden E (2002) Diversity of pyrrolizdine alkaloids in *Senecio* species does not affect the specialist herbivore *Tyria jacobaeae*. Oecologia 133:514-550

Macel M, Vrieling K (2003) Pyrrolizidine alkaloids as oviposition stimulants for the cinnabar moth, *Tyria jacobaeae*. J. Chem. Ecol. 29:1435-1446

Macel M, Vrieling K, Klinkhamer PGL (2004) Variation in pyrrolizidine alkaloid patterns of *Senecio jacobaea*. Phytochemistry 65:865-873

Macel M, Bruinsma M, Dijkstra SM, Ooijendijk T, Niemeyer HM, Klinkhamer PGL (2005) Differences in effects of pyrrolizidine alkaloids on five generalist insect herbivore species. J. Chem. Ecol. 31:1493-1508

Macel M, Klinkhamer PGL (2010) Chemotype of *Senecio jacobaea* affects damage by pathogens and insect herbivores in the field. Evol. Ecol. 24:237-250

Macel M (2011) Attract and deter: a dual role for pyrrolizidine alkaloids in plant-insect interactions. Phytochem. Rev. 10:75-82

Mattocks AR (1986) Chemistry and Toxicology of Pyrrolizidine Alkaloids. Academic Press, London UK

McEvoy PB, Rudd NT, Cox CS, Huso M (1993) Disturbance, competition, and herbivory effects on ragwort *Senecio jacobaea* populations. Ecol. Mono. 63:55-75

Miller JS, PP Feeny (1983) Effects of benzylisoquin-oline alkaloids on the larvae of polyphagous Lepidoptera. Oecologia (Berlin) 58:332-339

Narberhaus I, Theuring C, Hartmann T, Dobler S (2003) Uptake and metabolism of pyrrolizidine alkaloids in *Longitarsus* flea beetles (Coleoptera: Chrysomelidae) adapted and non-adapted to alkaloid containing host plants. J. Comp. Physiol. B 173:483-491

Nishida R (2002) Sequestration of defensive substances from plants by Lepidoptera. Annu. Rev. Entomol. 47:57-92

Ober D (2003) Chemical ecology of alkaloids exemplified with the pyrrolizidines. In: Romeo JT (Ed) Integrative Phytochemistry: From Ethnobotany to Molecular Ecology, Vol 37. Pergamon, Amsterdam, pp 203-230

Paul ND, Hatcher PE, Taylor JE (2000) Coping with multiple enemies: an integration of molecular and ecological perspectives. Trends Plant Sci. 5:220-225

Rizk AM (1991) Naturally occurring Pyrrolizidine Alkaloids. CRC Press, Boca Raton, Florida, USA

Sander H, Hartmann T (1989) Site of synthesis, metabolism and translocation of senecionine *N*-oxide in cultured roots of *Senecio erucifolius*. Plant Cell Tissue Organ Cult. 18:19-32

Simms EL (1990) Examining selection on the multivariate phenotype: Plant resistance to herbivores. Evolution 44:1177-1188

Thoden TC, Boppré M, Hallmann J (2009) Effects of pyrrolizidine alkaloids on the performance of plant-parasitic and free-living nematodes. Pest Manag. Sci. 65:823-830

Toppel G, Witte L, Riebesehl B, von Borstel K, Hartmann T (1987) Alkaloid patterns and biosynthetic capacity of root cultures from some pyrrolizidine alkaloid producing *Senecio* species. Plant Cell. Rep. 6:466-469

van Dam NM, Vuister LWM, Bergshoeff C, de Vos H, van der Meijden ED (1995) The 'raison d'etre' of pyrrolizidine alkaloids in *Cynoglossum officinale.* Deterrent effects against generalist herbivores. J. Chem. Ecol. 21:507-523

van Dam NM, Harvey JA, Wäckers FL, Bezemer TM, van der Putten WH (2003) Interactions between aboveground and belowground induced responses against phytophages. Basic Appl. Ecol. 4:63-77

van der Meijden E, Wijn H, Verkaar J (1988) Defence and regrowth, alternative plant strategies in the struggle against herbivores. Oikos 51:355-363

van der Putten WH, Van Dijk C, Peters BAM (1993) Plant specific soil-borne diseases contribute to succession in foredune vegetation. Nature 362:53-56

van der Putten WH, Vet LEM, Harvey JA, Wäckers FL (2001) Linking above- and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. Trends Ecol. Evol. 16:547-554

van Loon JJA, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 36:453-483

Verkaar HJ (1987) Population dynamics-the influence of herbivory. New Phytol. 106:49-60

von Borstel K, Hartmann T (1986) Selective uptake of pyrrolizidine *N*-oxides by cell suspension cultures from pyrrolizidine alkaloid producing plants. Plant Cell Rep. 5:39-42

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

Wink M, Roberts MF (1998) Compartmentation of Alkaloid Synthesis, Transport, and Storage. In: Alkaloids: Biochemistry, Ecology, and Medicinal Applications. Plenum, NewYork, pp. 239–262

Wink M (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64:3-11

Witte L, Ernst L, Adam H, Hartmann T (1992) Chemotypes of 2 pyrrolizidine alkaloid-containing *Senecio* species. Phytochemistry 31:559-565