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Attract and deter: a dual role for pyrrolizidine alkaloids in plant–insect interactions

Mirka Macel

Abstract Pyrrolizidine alkaloids (PAs) are the major defense compounds of plants in the *Senecio* genus. Here I will review the effects of PAs in *Senecio* on the preference and performance of specialist and generalist insect herbivores. Specialist herbivores have evolved adaptation to PAs in their host plant. They can use the alkaloids as cue to find their host plant and often they sequester PAs for their own defense against predators. Generalists, on the other hand, can be deterred by PAs. PAs can also affect survival of generalist herbivores. Usually generalist insects avoid feeding on young *Senecio* leaves, which contain a high concentration of alkaloids. Structurally related PAs can differ in their effects on insect herbivores, some are more toxic than others. The differences in effects of PAs on specialist and generalists could lead to opposing selection on PAs, which may maintain the genetic diversity in PA concentration and composition in *Senecio* species.

Keywords Evolution · Performance · Preference · *Tyria jacobaeae* · *Jacobaea vulgaris*

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Introduction

Pyrrolizidine alkaloids (PAs) are constitutive plant defenses against herbivores. They are present in several different plant species, amongst which the genera *Eupatorium* (Asteraceae), *Crotolaria* (Fabaceae), and *Senecio* (Asteraceae) (Hartmann 1999). These secondary plant compounds are infamous for their hepatotoxic and pneumotoxic effects on some mammals, like cows and horses. Cattle can contract alkaloid poisoning primarily through the consumption of hay poisoned with *Senecio* material (Mattocks 1986; Cheeke 1988). The livers of mammals can be severely damaged by toxic pyrrols, the breakdown products of PAs that are formed in the gut (Mattocks 1986). In the field, mammals generally avoid PA-containing plants of the *Senecio* genus. The role of the alkaloids in *Senecio* as defense against mammalian herbivores seems therefore unequivocal. Their role in the defense against insects, however, is less straightforward. Unlike most mammals, insect herbivores are more selective feeders. The majority of insects are specialized feeders of some sort, specializing on particular plant species, genera or families or on particular plant organs. The varying degree of host plant specialization in insects has led to different ways of insects to deal with and respond to plant secondary compounds (Deprés et al. 2007). Some of the specialist insect herbivores with a narrow host range have evolved physiological adaptations to the specific secondary metabolites in their host plants

(Aplin and Rotschild 1972; Naumann et al. 2002; Wittstock et al. 2004). They often even use these compounds to find their host plant and use these chemicals for their own defense. On the other hand, generalist herbivores that feed from many different plant species may be less adapted and could be negatively affected by plant secondary compounds.

Here I will review the different ways that insect herbivores cope with PAs of the plant genus *Senecio*, as well as their behavioral response to these compounds. There is a list of around 40 insect herbivores found on the PA-containing *Senecio jacobaea* (Harper and Wood 1957). Some of those insect herbivores, like the cinnabar moth *Tyria jacobaeae* and the flea beetle *Longitarsus jacobaeae*, feed exclusively on *Senecio jacobaea* and close relatives. Others, like *Arctia caja*, feed on PA-containing plants from different genera. There are also some insect herbivores that feed from all kinds of plants, regardless of their plant secondary compounds. Thus, the diet breath of the insects on *Senecio* is rather diverse which reflects in the ways they deal with PAs.

The structure of PAs is important for their activity to insects. PAs in *Senecio* are of the senecionine type (Hartmann and Witte 1995) (Fig. 1). The backbone structure of most PAs in *Senecio* species is senecionine *N*-oxide. Other PAs that occur in *Senecio*, such as seneciphylline, jacobine, and senkirkine are all derived from senecionine (Fig. 1) (Hartmann and Dierich 1998). There is a great diversity of PAs within *Senecio* species. Each species usually contains multiple PAs and has a species-specific PA pattern (Hartmann and Witte 1995). In addition, also within species different PA chemotypes can be found (Witte et al. 1992). PAs are not induced in shoots after herbivory (Van Dam et al. 1993; Hol et al. 2004) but they are induced in the roots when roots are damaged (Hol et al. 2004). PAs can occur in two forms, the tertiary free base and the *N*-oxide. Both forms are present in plants (Joosten et al. 2009). In the roots, the PAs were almost exclusively present as *N*-oxides and not as free bases while in the shoots around 35% of the PAs were tertiary free bases. The *N*-oxide can be broken down to the tertiary PA in the gut of insects and mammals, where it is absorbed and further reduced to highly unstable toxic pyrrols. The PA *N*-oxide itself, which is non-toxic per se, cannot be directly converted into toxic pyrrols and is hydrophylic and unable to passively pass through membranes (Hartmann 1999).
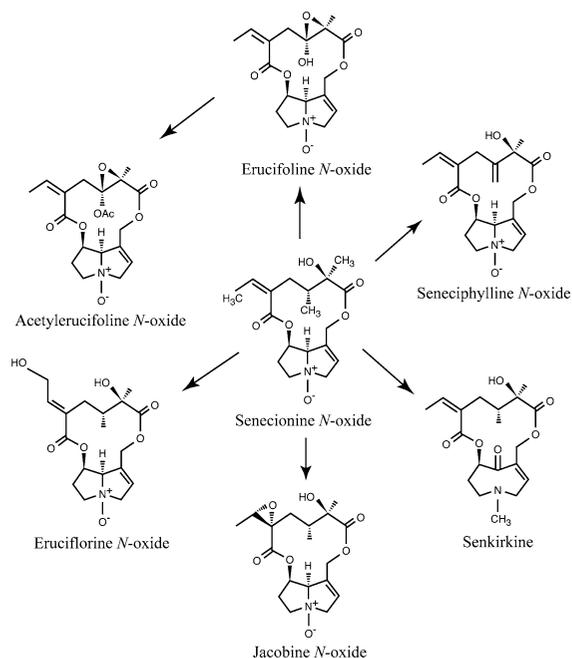

Fig. 1 Examples of PAs found in *Senecio* species. Senecionine *N*-oxide is the basic structure from which other PAs are formed. These senecionine type PAs have an otonecine (senkirkine) or retronecine ester base (e.g., senecionine) and a 12-membered macro-cyclic ring. Drawings: Martin Brittijn

Effects of PAs on specialized insects

Metabolism of PAs

Some specialized insects are able to detoxify PAs in their host plant through *N*-oxidation (Lindigkeit et al. 1997; Naumann et al. 2002). By *N*-oxidation potentially toxic free bases are converted back into PA *N*-oxides that cannot be transformed into toxic pyrrolic derivatives (Hartmann 1999). For the specialist *Tyria jacobaeae* (Fig. 2), feeding on *S. jacobaea* and related *Senecio* species, the detoxification mechanism is well studied. This moth species efficiently *N*-oxidizes the PAs in the haemolymph with the enzyme senecionine *N*-oxygenase (SNO), which is a flavin-dependent monooxygenase (Lindigkeit et al. 1997; Naumann et al. 2002). Similar to detoxification mechanisms of insects in other systems, the enzymes involved are of the P450 family (Naumann et al. 2002; Li et al. 2003; Wittstock et al. 2004). These P450 enzymes occur in all kingdoms of life and are amongst others involved in the xenobiotic metabolism of an organism. In specialized insects,

Fig. 2 a Larvae of the specialist moth *Tyria jacobaeae* feeding on their host plant *Senecio jacobaea*, **b** adult *T. jacobaeae*. Both larvae and adults contain pyrrolizidine alkaloids and show aposematic warning coloring. Photo's: Eric Thomassen

P450 enzymes have evolved to detoxify specific metabolites (Deprés et al. 2007). The SNO in *T. jacobaeae* is highly specific to pyrrolizidine alkaloids. Its activity is highest on senecionine type PAs which occur in the host plant *S. jacobaea*, other types of PAs are less well converted (Lindigkeit et al. 1997; Naumann et al. 2002). Specialist *Longitarsus* flea beetles (Chrysomelidae) can also *N*-oxidize tertiary PAs (Naberhaus et al. 2003). The enzymes involved are unidentified but are likely also P450s. SNO was also found in other insects such as the arctiid moths *Creatonotos transiens* and *Arctia caja*. Both moth species have a wider host range than *Tyria* and feed from multiple plant genera (PA adapted generalists). The SNO from *C. transiens* and *A. caja* has a broader substrate affinity than the SNO from *Tyria* (Lindigkeit et al. 1997).

Specialist insect herbivores but also PA adapted generalists may even sequester PAs for their own defense (Rothschild et al. 1979; Von Nickisch-Rosenegk and Wink 1993; Dobler 2001; Pasteels

et al. 2003; Naberhaus et al. 2003). Larvae of *T. jacobaeae* store PAs from their host plants primarily in their haemolymph and the integument. In fact, all life stages of *T. jacobaeae* contain PAs; eggs, larval, pupae and adults (Van Zoelen and Van der Meijden 1991). Several arctiid moths, like *T. jacobaeae* and *Arctia caja*, also transform plant acquired PAs into insect specific PAs like callimorphine (Aplin and Rothschild 1972; Ehmke et al. 1990; Hartmann 1999). *Longitarsus* flea beetles also sequester PAs (Dobler 2001; Naberhaus et al. 2003). PAs are sequestered as *N*-oxides (Hartmann 1999) and the concentrations of PAs in insects can exceed the concentrations in plants (Aplin and Rotschild 1972). The sequestered PAs protect the arctiid moth *Utetheisa ornatrix*, which feeds from PA containing *Crotolaria* species, against predators (Eisner and Eisner 1991). PAs can thus affect the preference and performance of the third (and higher) trophic levels (see a review by Trigo in this issue). PA sequestration is not solely limited to specialists within the arctiids and chrysomelid families. Some danaine and ithomiine butterflies, ctenuchiid moths and the African grasshopper *Zonocerus variegatus* also sequester PAs (Boppré 1986; Nishida 2002). Not all PA adapted insects acquire PAs from larval host plants. Adults may also obtain PAs pharmacophagously from withered and decomposing plants or PA containing nectar (Boppré 1986). Some PA adapted insects use the alkaloids as pheromone precursors and PAs can be transferred from males to females during mating (for reviews see Hartmann 1999; Nishida 2002). Most, if not all PA sequestering insects show aposematic warning coloration (Hartmann 1999).

Effects of PAs on specialist preference and performance

The physiological adaptations to PAs and their ability to sequester these compounds, suggests that specialist insects may even depend on PAs to find their host plants. Bioassays using isolated PAs showed that *T. jacobaeae* indeed uses PAs as oviposition cue. Not all PAs stimulated oviposition; while senecionine, seneciphylline and monocrotaline were attractive, retrorsine, senkirkine, and heliotrine were not stimulatory (Macel and Vrieling 2003). Surprisingly a mixture of alkaloids for a non-host plant (*Senecio inaequidens*) was equally attractive as a PA mixture

from *S. jacobaea* (Macel and Vrieling 2003). The danaid butterfly *Idea leuconoe*, a specialist on *Parsonia laevigata* (Apocynaceae), can also use the PAs in its host plant as oviposition stimuli (Honda et al. 1997). Besides *Tyria*, it is unknown if other specialists on *Senecio* use PAs to find their hosts. Larvae of *T. jacobaeae* have PA specific taste receptors (Bernays et al. 2004). However, caterpillars will only feed from *Senecio* leaves and will not accept any neutral substrates such as lettuce disks and artificial diets with added alkaloids (Macel unpublished data), indicating that other factors are needed to stimulate feeding in *Tyria*. Similar PA specific taste receptors have been found in other arctiid moths that feed on PA containing plants, such as the specialists *U. ornatrix*, and PA adapted generalists *Estigmene acrea* and *Grammia geneura* (Bernays et al. 2002a, b, 2003). *Utetheisa ornatrix* larvae preferred artificial diets with PAs to diets without PAs (Kelley et al. 2002) which indicates that PAs stimulate feeding in this species.

Despite the stimulatory effects of PAs on feeding and oviposition of adapted insects, correlations between specialist insect preference and PA concentration and profiles of *Senecio* plants are not always apparent. The diversity of PAs in *Senecio* species did not determine host plant preference of *Tyria jacobaea* (Macel et al. 2002). PA chemotype of *S. jacobaea* also did not affect preference of this specialist (Vrieling and de Boer 1999; Macel et al. 2002). In the field, total alkaloid concentration in *S. jacobaea* was not an important factor in selection of food plants for the cinnabar moth (Van der Meijden et al. 1989). Thus, although this specialist can clearly recognize PAs, the role of the alkaloids for host plant selection is less clear. Possibly, it uses PAs as a general cue to recognize its host plant amongst other plants species, but within the host plant species, selection depends on other factors such as nutrients (Van der Meijden et al. 1989). The relation between damage by the flea beetle *Longitarsus jacobaeae* and PA concentrations in plants ranged from positive to negative (Vrieling and van Wijk 1994; Stastny et al. 2005). These findings are all based on field studies where various factors can determine insect behavior, which could have masked any potential correlation between alkaloids and host preference. The presence of the specialist thrips *Haplothrips senecionis* was not correlated with PA concentration (Vrieling et al.

1991a). *Senecio jacobaea* plants infested with the specialist aphid *Aphis jacobaeae* had a lower alkaloid concentration than plants that were not infested by these aphids, which suggests that this specialist may be negatively affected by high PA concentrations. Here, however, the third trophic level could also have played a role in host plant selection. The ants tending these aphids would probably benefit from lower PA concentrations in the honeydew (Vrieling et al. 1991b).

Surprisingly little studies have investigated whether PAs affect specialist insect performance, i.e., survival, growth, development time and pupal weight. The PA seneciphylline added in artificial diets did not affect development of two specialist leaf beetles (Hägele and Rowell-Rahier 2000). Similar to its adult preference, the performance of the larvae of the cinnabar moth *T. jacobaeae*, measured as pupal weight and development time, was not affected by PA profile or concentration of the host plant (Vrieling and de Boer 1999; Macel et al. 2002). For other specialists on *Senecio* it is still largely unknown whether PAs affect performance.

Effects of PAs on generalists

Metabolism of PAs

Although the toxicity of PAs to mammals is extensively studied, the toxicity and the mode of action of PAs towards non-adapted generalist insects are less known. PAs are genotoxic and mutagenic to *Drosophila melanogaster* (Frei et al. 1992). The structure–activity relationship in *Drosophila* is similar to the effects of PAs on mammals, which suggest that the same mechanisms may be involved (Frei et al. 1992). The LD_{50} of PAs in mammals is partly determined by the presence and structure of the macrocyclic ring (Wink et al. 1998). The base of the PA is also important. PAs with an otonecine base (e.g., senkirkine) can have a different effect than PAs with a retronecine base (e.g., senecionine) (Fig. 1) (Frei et al. 1992). PAs are likely to be broken down to toxic pyrrols through P450 enzymes in the insect's gut (Hartmann 1999). Studies using ^{14}C-labeled senecionine *N*-oxide showed that the generalist caterpillar *Spodoptera littoralis* is very efficient in excreting PAs. Senecionine *N*-oxide was easily

reduced to the tertiary alkaloids in the gut of *S. littoralis*, passively absorbed in the haemolymph and then quickly excreted (Lindigkeit et al. 1997). Other generalist insect species may be less efficient in excreting PAs and suffer similar mutagenic effects of PAs as *Drosophila*, but there are no data to support this yet.

Effects of PAs on generalist preference and performance

PAs can deter generalist insects (Bentley et al. 1984; Dreyer et al. 1985; Van Dam et al. 1995; Hägele and Rowell-Rahier 2000; Macel et al. 2005). In choice tests using artificial substrates such as lettuce disks or artificial diets, some but not all generalist herbivores avoided the PA-containing substrates. For example, larvae of the cabbage moth *Mamestra brassicae* were not deterred by PAs in artificial diets (Macel et al. 2005). On the other hand, all tested PAs deterred feeding of the migratory locust *Locusta migratoria*. Interestingly, when given the choice between senecionine and seneciphylline, the locusts preferred senecionine. This indicates that some PAs may be more deterrent than others (Macel et al. 2005). Similarly, the pea aphid *Acyrthosiphon pisum* was also more deterred by seneciphylline than by senecionine (Dreyer et al. 1985). Two stereo-isomers differed in their effects on spruce-budworm (Bentley et al. 1984) again indicating that small structural changes can affect the activity of PAs. Larvae of the noctuid *Spodoptera exigua* were only deterred by a mixture of senecionine type PAs and not by single PAs, indicating synergistic effects (Macel et al. 2005). In contrast, individual PAs of a different structural type (from *Cynoglossum officinale*) did deter feeding of *S. exigua* (Van Dam et al. 1995). The migratory locust was also more deterred by a mixture of PAs than individual alkaloids (Macel et al. 2005). The PA *N*-oxides are generally less deterrent than the tertiary PAs (Dreyer et al. 1985; Van Dam et al. 1995; Macel et al. 2005).

In plants, generalist insects usually prefer feeding on old leaves of *Senecio* rather than younger leaves (Van Dam et al. 1995; De Boer 1999). These younger leaves have higher PA concentrations than the older leaves (De Boer 1999). The amount of damage by the generalist thrips *Frankliniella occidentalis* was negatively correlated with PA concentrations in the leaves of *S. jacobaea* (Macel 2003). Optimal defense theory predicts that these most valuable plant parts should be better protected against herbivory (Zangerl and Bazzaz 1992; Van Dam et al. 1996). In the case of *Senecio* this seems certainly the case, young leaves and also the inflorescences have relatively high concentrations of alkaloids (Hartmann and Zimmer 1986; De Boer 1999).

Direct effects of PAs on the performance of generalist insect herbivores can be tested using artificial diets with and without alkaloids. Senecionine *N*-oxide strongly decreased the survival of larvae of the eri silk moth *Philosamia ricini* (Saturniidae) (Narberhaus et al. 2005). Survival of the generalist aphid *Myzus persicae* was strongly affected by some PAs while other structurally related PAs had no effect (Macel et al. 2005). Thrips (*F. occidentalis*) survival was only affected by very high concentrations of PAs. The survival of the thrips on a diet with the PA retrorsine *N*-oxide was significantly higher than on the diet with the tertiary retrorsine (Macel et al. 2005). Interestingly the PA that had no effect on the aphid, senkirkine, had the strongest negative effect on the thrips (Macel et al. 2005). Such specific differences in effects between herbivores could lead to differential selection pressure on PAs in the field where herbivore presence and abundance shows spatiotemporal variation. Ultimately, this could contribute to the evolution of the diversity of these metabolites.

While bioassays with artificial diets clearly show an effect of PAs on the performance of some generalist insects, the relationship between PAs and generalist insect performance in plants is not always apparent and not well studied. In the field, the growth rate of the generalist aphid *Brachycaudus cardii* was negatively correlated with PAs of *S. jacobaea* (Vrieling et al. 1991a). In the greenhouse, population growth rate of the peach aphid *Myzus persicae* was extremely poor on *S. jacobaea* plants, but there was no correlation between PA concentration and aphid population growth (Macel 2003). The survival of larvae of *M. brassicae* was not correlated with PA concentration in host plants (Joshi and Vrieling 2005). It is likely that other factors besides PAs also determine performance of generalist herbivores on *Senecio* plants. Such factors could be other chemicals like sesquiterpenoids, nitrogen and sugars. Metabolomics, the untargeted screening of many metabolites of different classes, could shed light on all the

(combinations of) compounds involved in host plant resistance. Using such a metabolomics approach (^{1}H-NMR), Leiss et al. (2009) showed that thrips resistance in *Senecio* hybrids was amongst others related to the concentration of the PA jacobine *N*-oxide but also to the flavonoid kaempferol.

Specialist–generalist dilemma

Natural plant populations often show genetic variation in levels of defense chemistry (Denno and McClure 1983; Mauricio and Rausher 1997). Van der Meijden (1996) proposed that the contrasting effects of plant secondary compounds on specialist vs. generalist insect herbivores would maintain this genetic variation in concentration of plant defense compounds. While generalists can be deterred by high levels of defense compounds, specialists may be attracted by the same compounds. Specialists also have evolved efficient adaptations to cope with the toxin in their hosts. The herbivore community in a plant population is likely to show spatial and temporal variation (Van der Meijden and Van Wijk 1997). The abundance of specialists vs. generalists, for example, will shift through time. Therefore, the direction of selection on plant defense will shift accordingly. An example of opposing selection on a defense trait is the contrasting selection imposed on sinigrin (glucosinolate) levels in *Brassica nigra* by a specialist aphid and generalist snails and slugs (Lankau 2007). By manipulating the herbivores in the field (removing either the specialists or the generalists), it was shown that in the presence of specialists there was selection for low levels of sinigrin, with generalists there was selection for high levels of sinigrin and when both generalist and specialist herbivores present selection was neutral.

In natural *Senecio* populations there is often considerable genetic variation in PA concentrations and profiles (Vrieling et al. 1993). Although not explicitly tested, it is likely that fluctuating ratios of generalist vs. specialist in the field will cause shifting selection pressure on PAs and thus could maintain the genetic variation in PA concentration. As I outlined earlier, studies have shown that specialists on *Senecio* are generally well adapted to the PAs in their host plant and can use these compounds as host finding cue. Generalists on the other hand may be deterred by PAs. In a field experiment using different *S. jacobaea* clones, there was little damage to the plants in the site with mainly generalist herbivores, while in the site that harbored also specialist herbivores damage was considerable. At the site with specialist herbivores, PA concentration and damage were positively correlated, but there was no correlation between plant damage and PA concentration at the generalist site (Macel and Klinkhamer 2010). The question is whether these correlations between PAs and damage lead to a fitness effect in *Senecio* plants. Only then, the strength of the selection by herbivores on PAs can be determined.

In addition to the specialist–generalist dilemma, a plant is also faced with the dilemma of the different sensitivity of herbivores towards individual PAs. While alkaloid X can act as defense towards insects species A, alkaloid Y may act as a better defense against insect species B, as was shown for the contrasting effects of the PAs senecionine and senkirkine on thrips and aphids (Macel et al. 2005). Such opposing selection pressures of different herbivores could explain the maintenance of the diversity of PAs found in *Senecio* species (Witte et al. 1992). It is also thought that the ongoing evolutionary arms-race between plants and herbivores has led to the diversity of plant chemical compounds (Ehrlich And Raven 1964). The differences in activity of closely related PAs to insect herbivores indicate that small structural changes can turn PAs more or less effective as defense chemicals. Finally, the diversity of PAs in *Senecio* could also be maintained through synergistic effects of PAs on generalist herbivores. Of course, other factors such as defense against fungi (Hol and Van Veen 2002) or effects of PAs on higher trophic levels (Vrieling et al. 1991b) can also play a role in the evolution and maintenance of the diversity of PAs. Thus far, allocation costs of producing PAs have not been detected (Vrieling and van Wijk 1994). It is also possible that the diversity of PAs is selectively neutral, if the production of various PAs within a single plant does not lead to extra costs (Jones and Firn 1991). However, as outlined in this review, PAs can differ in their ecological costs and benefits towards insect herbivores. Insect herbivores may thus contribute to the evolution and maintenance of the diversity of secondary compounds such as PAs.

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