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THE INFLUENCE OF PYRROLIZIDINE ALKALOIDS OF
SENECIO JACOBAEA ON *TYRIA JACOBAEA*E,
BRACHYCAUDUS CARDII AND *HAPLOTHRIPS SENECIONIS**

by

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SUMMARY

The effect of natural differences in pyrrolizidine alkaloid (Pa) concentration in *Senecio jacobaea* on attack of performance of three herbivores was studied. Differences in larval performance and development of *Tyria jacobaeae* were not correlated with total Pa-concentration or the concentration of the different Pa's. The population growth rate of the aphid *Brachycaudus cardii* was negatively correlated with total Pa-concentration of the *S. jacobaea* plants on which they were reared. In our study area *S. jacobaea* plants with and without *Haplothrips senecionis* did not differ in total Pa-concentration, indicating that food plant choice of the thrips was not affected by Pa-concentration.

Thus Pa's in *S. jacobaea* act as a chemical defence against the polyphagous aphid *B. cardii*, whereas the monophagous *T. jacobaeae* and *H. senecionis* are not influenced by Pa's in their diet. These results are in agreement with the predictions of the plant apparency theory which states that specialist herbivores (monophagous) are adapted to a qualitative chemical defence (Pa's) and generalists (polyphagous) herbivores are not.

KEY WORDS: *Senecio jacobaea*, *Tyria jacobaeae*, *Brachycaudus cardii*, *Haplothrips senecionis*, pyrrolizidine alkaloids, chemical defence.

INTRODUCTION

Although *Senecio jacobaea* L. plants are known to possess pyrrolizidine alkaloids (Pa's), they are frequently defoliated by the larvae of *Tyria jacobaeae* L. (DEMPSTER & LAKHANI, 1979; VAN DER MEIJDEN, 1979; MYERS, 1980; CRAWLEY & GILLMAN, 1989) and they are attacked by at least fifty other (insect) herbivores (HARPER & WOOD, 1957). Pa's are reported to be not only toxic to vertebrates (BULL *et al.*, 1968; MATTOCKS, 1986) but also to insects (BERNAYS & CHAPMAN, 1977; BENTLEY *et al.*, 1984; DREYER *et al.*, 1985; BOPPRÉ, 1990; VRIELING *et al.*, 1991). We were interested to know whether the Pa's of *S. jacobaea* function as a defence against its numerous herbivores.

S. jacobaea contains at least seven different Pa's (APLIN *et al.*, 1968; SEGALL, 1978; PIETERS *et al.*, 1989), which can be present as free bases

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and as N-oxides (JOHNSON *et al.*, 1985). The Pa N-oxides are reported to be less toxic (DREYER *et al.*, 1985) than the free bases. The concentration of total Pa's varies widely between plants (0.1%-1.5% dry weight), as does the concentration of the individual Pa's (APLIN *et al.*, 1968). For both concentration and composition genetic variation is present (VRIELING, 1991).

We chose three herbivores, *T. jacobaeae*, *Brachycaudus cardii* L. and *Haplothrips senecionis* Bagnall, to investigate the role of different concentrations of Pa's on their feeding behaviour or development. *T. jacobaeae* and *H. senecionis* commonly feed on *S. jacobaea* in Meijendel (dunes near The Hague, The Netherlands); they are reported to be respectively monophagous and oligophagous in Great Britain (HARPER & WOOD, 1957). In Meijendel *H. senecionis* can be considered monophagous, as the other reported food plant, *Senecio aquaticus* L., does not occur in this area. According to the plant-apparency theory, specialists like *T. jacobaeae* and *H. senecionis* should have broken through the qualitative chemical defence (Pa's) in *S. jacobaea* (FEENY, 1976; RHOADES & CATES, 1976). We therefore expect these herbivores to be adapted to Pa's in their diet. *B. cardii* is known to feed also on taxa of the Boraginaceae (H. C. BURGER, pers. comm., VRIELING, pers. obs.). Following the plant-apparency theory the polyphagous *B. cardii* is expected to be repelled by Pa's.

Although some butterflies are reported to be repelled by Pa's (BENTLEY *et al.*, 1984; BOPPRÉ, 1990) it is well known that *Danaus* species depend on Pa's for the biosynthesis of sexpheromones (EDGAR & CULVENOR, 1974). Several butterflies feeding on Pa-containing plants are known to sequester Pa's for their own defence against predators (ROTHSCHILD *et al.*, 1979; BOPPRÉ, 1990). Pa's are also sequestered by *T. jacobaeae* (APLIN *et al.*, 1968, EHMKE *et al.*, 1990), the most common and most important consumer of *S. jacobaea* in the dunes of Meijendel (PRINS, 1990). In choice experiments, oviposition preference by *T. jacobaeae* was not influenced by artificially raised total Pa-concentration (VAN DER MEIJDEN *et al.*, 1989). Neither did plants in the field with egg batches of *T. jacobaeae* differ in total Pa-concentration from their nearest neighbour without egg batches (A.M. VAN ZOELLEN, pers. comm.), although VAN DER MEIJDEN *et al.* (1984) did not rule out effects of high Pa-concentrations in similar experiments. VAN DER MEIJDEN *et al.* (1984) found that plants with intermediate nitrogen concentration were preferred for oviposition and that nitrogen was positively correlated with Pa-concentration. They suggested that this pattern was due to moths selecting for plants with high nitrogen concentration and at the same time rejecting plants with very high Pa-concentration (and therefore very high nitrogen concen-

tration). Feeding and choice experiments, in which Pa's were artificially added, showed that jacobine had a phagostimulatory effect on young larvae and repelled fifth instar larvae. The combined addition of senecionine, integerimine and seneciphylline had no effect on feeding and choice in third and fifth instar larvae (A.M. VAN ZOELEN, pers. comm.). In this paper the influence of naturally varying Pa-concentrations and Pa-composition of *S. jacobaea* plants on growth and development of *T. jacobaeae* is studied.

From several studies it is known that certain aphids are deterred by alkaloids in their diet (ZÚÑIGA *et al.*, 1985; ZÚÑIGA & CORCUERA, 1986; WINK, 1987). Pa's are also reported to affect aphids (DREYER *et al.*, 1985; VRIELING *et al.*, 1991). We studied the aphid *B. cardii* and expected *B. cardii* to be deterred by high Pa-concentrations in its diet.

At present no knowledge of Pa-concentrations on feeding of thrips species is available. We studied *H. senecionis* because it is commonly found on *S. jacobaea* and can have a considerable impact on the plant's reproductive success (VRIELING & VIERBERGEN, unpubl.).

MATERIALS AND METHODS

Tyria jacobaeae

Growth and developmental time of larvae of *T. jacobaeae* were recorded and correlated with total Pa-concentration in their food plants. In May 1989, fifty large flowering plants of *S. jacobaea* were collected from natural populations in the dunes of our study area Meijndel and brought into a growth room (temp. 18-23 °C, RH c. 70%, 16L/8D, c. 25 W/m² PAR at plant surface). Plants were potted in 10 l pots filled with dune sand and given 75 ml STEINER (1968) nutrient solution weekly and ample water. All plants were covered with a cage of transparent plastic with gauze on top and were allowed two weeks to acclimatize before the experiment. Batches of eggs of *T. jacobaeae* were collected in Meijndel. Larvae of seven egg batches were used and randomly distributed over 49 plants. From 3 June on, 9 or 10 newly hatched larvae from the same egg batch were weighed together and placed on a randomly selected plant. Just before larvae were placed on a plant, two fully developed and healthy-looking leaves from the lower part of the stem from each plant were harvested for Pa analysis. Separate Pa's were determined by gas-liquid chromatography (GLC).

After 7 days all larvae were weighed and losses recorded. From each plant three randomly chosen larvae were returned to their original host. The number of larvae was reduced in order to avoid food shortage in a later stage. Two to three days before pupation larvae stop feeding and leave the food plant. These larvae, together with some leaves of their food plant, were placed in glass jars covered with a cloth. Jars were inspected daily for pupating larvae. The pupae were placed in a climate room (8L/16D, 5 °C). Seven days after pupation pupae were weighed. After 11 months in a cold room (*cf.* VAN ZOELEN & KUSTERS, 1985) percentage emergence was recorded.

Three of the total of 49 plants were excluded from the statistical analyses: two developed a severe aphid infestation and the sample from one plant was lost during the Pa analysis. From another plant data on pupal weight and developmental time are missing because larvae accidentally drowned. On five plants ants (*Lasius niger* L.

and *Lasius alienus* L.) or sow-bugs (*Oniscus asellus* L.) were observed. Mortality in the first seven days was significantly higher on these plants, probably due to predation on the first instar larvae (VAN DER MEIJDEN, 1979; DEMPSTER, 1971). When mortality was calculated these plants were omitted from the analysis. Other characters were probably not affected by the presence of ants or sow-bugs.

Brachycaudus cardii

In order to determine the effect of total Pa-concentration on the growth of the aphid *B. cardii*, aphids were reared in small cages on leaves of *S. jacobaea* rosettes in the laboratory.

Plants were grown in the laboratory in 0.8 l pots filled with a 50% mixture of dune sand and leaf mould. Plants received ample nutrients and water. On 31 July 1987 nineteen small cages with two aphids each, from a clone reared in the laboratory, were attached to a leaf. The population density of aphids was recorded on four occasions. After three weeks the experiment was stopped and the leaf on which the aphids had been reared was harvested for total Pa determination.

Haplothrips senecionis

In Meijden a field experiment was carried out to examine the influence of Pa-concentration on food plant choice of *H. senecionis*. From 12 nearest-neighbour pairs of bolting *S. jacobaea* plants with and without *H. senecionis* two fully grown leaves were harvested for analysis of total Pa-concentration. Both the distance between the two plants and the height of each plant were measured.

Pa determination

Pa's were extracted as described earlier in VRIELING *et al.* (1991). Total Pa-concentration was determined by the spectrophotometric method of MATTOCKS (1967). Monocrotaline was used as a reference compound.

GLC analysis

All GLC analyses were performed on a Packard 433 GC equipped with a 25 m fused silica column 0.32 mm ID (CP-sil 8 Chrompack) and a split-splitless injection system used in the split mode (1:30): temperature program 240 °C isotherm, injector block and detector 250 °C. Hydrogen was used as a carrier gas with a flow rate of 2 ml/min. Samples were dissolved in 200 µl ethanol with 5 mg/ml atropine as an internal standard. The amount of extract injected was 0.3 µl. Pa peaks were identified with GC-MS and ¹³C NMR spectroscopy (PIETERS *et al.*, 1989).

Calculations

We calculated relative growth rates (RGR's) of the weight of *T. jacobaeae* larvae using average weight of all larvae on the plant. Two RGR's were calculated: a) from day 1 to day 7 (RGR1), based on the average weight of 7-10 individuals per plant, and b) from day 7 to pupation (RGR2), based on the average of 1-3 individuals per plant. RGR was calculated as

$$\text{RGR} = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

(W_1 , W_2 = average weight in mg at time 1 and 2, respectively, t_1 , t_2 = time 1 and 2 in days).

Mortality was calculated from days 1 to 7 as the percentage losses of the total number of larvae initially present.

Population growth rate of *B. cardii* was calculated as the slope of the linear regression of the logarithm of the number of aphids against time.

RESULTS

T. jacobaeae

1) Differences between egg batches.

Oneway and nested analyses of variance yielded significant differences between egg batches in all measured characters except pupal weight (tables I and II). On average the food plants of larvae from different egg batches did not differ with regard to Pa-concentrations (table III), organic nitrogen concentration and sugar concentration (SOLDAAT & VRIELING 1991).

TABLE I

Differences in growth and development of larvae of *T. jacobaeae* between different egg batches, tested with a oneway analysis of variance.

	Df	F	
Weight at day 1	6, 39	55.1	***
RGR1	6, 39	7.7	***
RGR2	6, 38	28.6	***
Mortality	6, 34	2.4	* ¹⁾

¹⁾ Arcsine-root transformed. * $p < 0.05$,
*** $p < 0.001$.

2) Differences between plants.

Weight after 7 days, pupal weight and developmental time differed significantly between plants (table II) when tested with a nested analysis of variance. We conclude that plant characteristics of *S. jacobaea* influence growth and developmental time of larvae of the cinabar moth. Total Pa-concentration also showed large variation between plants, ranging from 0.19-1.17% dry weight.

3) Correlations.

Correlations of separate and total Pa-concentration with several

TABLE II

Differences in growth and developmental time of larvae of *T. jacobaeae* among egg batches and plants of *S. jacobaea* tested with a nested analysis of variance (plants in egg batches).

NS Not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Weight day 7			Weight of pupa			Developmental time					
	Df	F	%var	Df	F	%var	Df	F	%var			
Among egg batches	6	5.5	**	22	6	0.7	NS	0	6	31.7	***	71
Among plants, within egg batches	42	5.2	***	27	40	3.0	***	44	40	1.8	*	7
Within plants	333			51	72			56	76			22

TABLE III

Analysis of variance of total Pa-concentration of plants of *S. jacobaea* between egg batches. NS = not significant, $n = 46$.

	SS	Df	MS	F	
Between egg batches	7.19	6	1.198	1.404	NS
Within egg batches	33.27	39	0.853		

growth characteristics of the larvae of *T. jacobaeae* are all non-significant when corrected for the number of tests performed with the sequential Bonferroni test (HOLM, 1979; RICE, 1989) (table IV). Pa's in the host plant therefore do not influence development of *T. jacobaeae*.

Multiple correlation with growth characteristics (corrected for egg batch) and individual concentrations of Pa's did not yield significant results when corrected for the number of tests carried out with the sequential Bonferroni test (table IV).

B. cardii

The consumption of Pa's by aphids became apparent from the analysis of three samples of approximately one hundred aphids each and two samples of honeydew: both aphids (0.003% dry weight) and honeydew (0.02% dry weight) contained Pa's. Pa-concentration in the honeydew is on average one hundred times lower than in leaves and probably reflects the concentration in the phloem.

In three cages *B. cardii* did not produce offspring. In four other cages offspring was produced but thereafter the populations went ex-

TABLE IV

Correlations between Pa-concentrations in the food plant *S. jacobaea* and growth, developmental time and mortality of *T. jacobaeae*.

	<i>Total Pa</i>	<i>sn</i>	<i>sp</i>	<i>ir</i>	<i>jb</i>	<i>jz</i>	<i>jl</i>	<i>jn</i>	<i>n</i>
Wt. at day 1	0.19	0.05	-0.09	0.02	0.20	0.15	0.25	0.08	46
Wt. at day 7	-0.13	0.16	0.38	0.17	-0.18	0.12	-0.04	0.34	46
Wt. of pupa	¹⁾ 0.07	-0.38	-0.12	-0.33	0.06	0.02	0.07	0.10	46
Develop. time	¹⁾ -0.05	-0.13	-0.16	-0.18	-0.06	-0.00	-0.07	0.07	41
RGR1	-0.15	0.12	0.34	0.13	-0.19	0.09	-0.05	-0.03	45
RGR2	0.06	0.11	0.18	0.18	0.06	0.02	0.07	-0.05	45
Mortality	²⁾ -0.27	0.11	0.28	0.29	-0.30	-0.13	-0.29	-0.14	45

¹⁾ Weight of pupa and development time were log transformed.

²⁾ Percentage mortality was arcsine-root transformed. □ Sn, sp, ir, jb, jz, jl and jn concentrations of the Pa's senecionine, seneciphylline, integerimine, jacobine, jacozine, jacoline and jaconine respectively. n number of items. All Pa-concentrations were log transformed. None of the correlations is significant when corrected for the total number of correlations calculated with the sequential Bonferroni test.

tinct. Pa-concentrations from these 7 leaves were not significantly different from the other plants ($F = 2.56$, $Df = 1, 17$, $p = 0.128$, $n = 19$).

Population growth rate of aphids was negatively correlated with Pa-concentration of the leaf on which they were reared (fig. 1). No population growth rate could be calculated for the seven cages in which the aphids died; in the statistical analysis they were treated as a population with growth rate zero.

H. senecionis

A paired t-test on total Pa-concentration yielded no significant differences between plants with and without *H. senecionis* (fig. 2). Also plant height did not differ between infested and non-infested plants.

DISCUSSION

Although there are significant differences between egg batches in growth characteristics of larvae of *T. jacobaeae*, these differences are not caused by differences in Pa-concentrations. Separate as well as total Pa-concentrations in plants did not differ between egg batches (table III). Also organic nitrogen and sugar concentration did not differ in plants between egg batches (SOLDAAT & VRIELING 1991). Therefore, as plant quality is on average equal between egg batches, differences in growth characteristics of larvae from different egg batches are most likely to be due to genetic differences (including maternal effects) be-

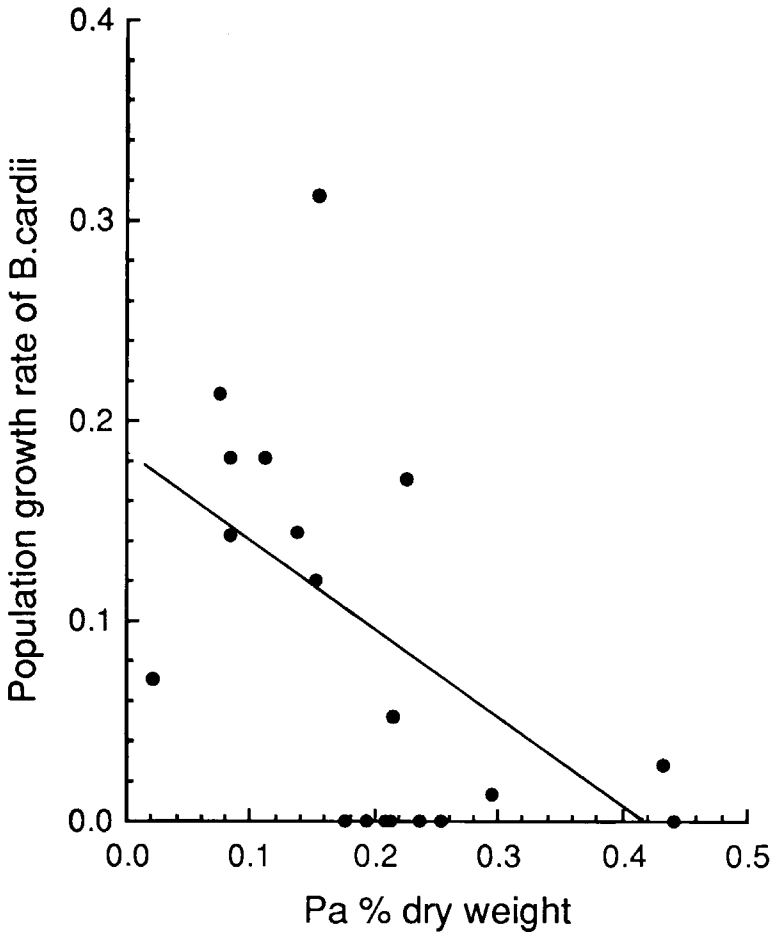


Fig. 1. The correlation between total Pa-concentration of leaves of *S. jacobaea* and population growth rates of *B. cardii* reared on these leaves. $r = -0.54$, $n = 19$, $p < 0.02$ ($Y = -0.46X + 0.177$).

tween egg batches. Differences in performance of larvae from different egg batches of *T. jacobaeae* were also observed by ROSE (1978).

Larval growth and development of *T. jacobaeae* were influenced by the food plant (table II). However, differences in performance of larvae feeding on different plants were not correlated with Pa-concentrations (table IV). Organic nitrogen appeared to be the important factor in this case (SOLDAAT & VRIELING 1991). The earlier men-

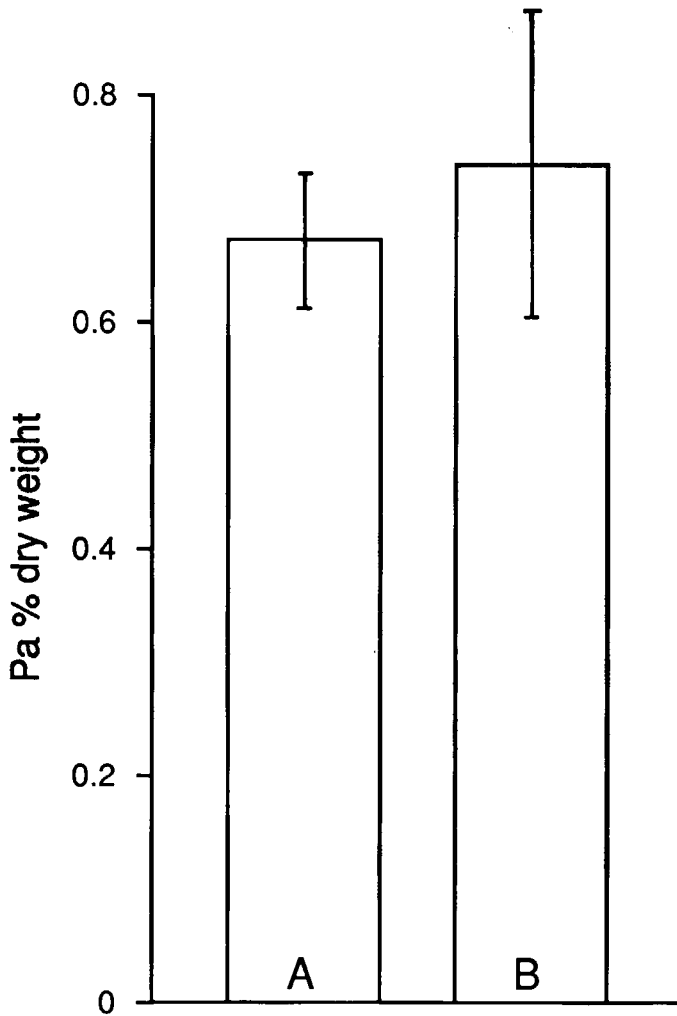


Fig. 2. Total Pa-concentration of leaves of nearest neighbour pairs of *S. jacobaea* in the field. One plant of each pair was infested with the thrips *H. senecionis*. Bars indicate standard errors. A = Pa-concentration of plants infested with *H. senecionis* ($n = 12$), B = Pa-concentration of plants without *H. senecionis* ($n = 12$).

tioned phagostimulatory effect of jacobine could not be detected with respect to larval growth.

In conclusion, Pa's do not act as chemical defence substances against feeding of larvae of *T. jacobaeae*. Larvae of *T. jacobaeae* are likely to be adapted to Pa's in their food; it is known that *T. jacobaeae* is able

to metabolize Pa's (APLIN *et al.* 1968; EHMKE *et al.* 1990). Probably detoxification and excretion are performed without large physiological costs, as larval performance does not differ between plants with low and high Pa-concentrations.

Total Pa-concentration had a negative effect on the population growth rate of *B. cardii*. Pa-concentration was measured in the total leaf and not in the phloem. WINK & WITTE (1984), however, found a correlation between the concentrations of quinolizidine alkaloids in the phloem and the leaves of *Lupinus*. *B. cardii* does not seem to sequester Pa's in large quantities, as the concentration in the body is low. Measurements made on the aphid *Aphis jacobaeae* Schrank showed that the Pa-concentration in the honeydew was the same as in that of *B. cardii*, but the Pa-concentration in the body was approximately twenty times higher (VRIELING *et al.*, 1991). Aphids seem to be very sensitive to Pa's in their diet (DREYER *et al.*, 1985; VRIELING *et al.* 1991; this study).

Food plant choice of *H. senecionis* was not affected by total Pa-concentration in *S. jacobaea* plants, as thrips were found on plants with low and high Pa-concentrations. The average distance between a pair of plants was only 16 cm, hence distance between plants has probably not affected the thrips' choice. *H. senecionis* had infested about 90% of the plants in the sampled area and is probably adapted to Pa's in its diet.

Pa-concentrations act as chemical defence against some herbivores (aphids) but not to others. The oligophagous (*H. senecionis*) and monophagous species (*T. jacobaeae*) are adapted to high Pa-concentrations (APLIN *et al.* 1968; ROTHSCHILD *et al.* 1979; EHMKE *et al.* 1990), whereas the polyphagous species (*B. cardii*) is not. This is in agreement with the plant apparency theory that generalists (polyphagous) are deterred by qualitative defences like Pa's and that specialists (monophagous/oligophagous) have broken through such a defence (FEENY, 1976; RHOADES & CATES, 1976).

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