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PHENOTYPIC PLASTICITY OF *Senecio vulgaris* FROM CONTRASTING HABITAT TYPES: GROWTH AND PYRROLIZIDINE ALKALOID FORMATION

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Abstract—The growth–differentiation balance hypothesis (GDB), which postulates a physiological trade-off between growth and differentiation (morphological and chemical), has been tested almost exclusively for carbon-based secondary metabolites. Little attention has been paid to N-based compounds. In this study we aimed to test the predictions of the GDB hypothesis under field conditions for growth and pyrrolizidine alkaloid (PA) formation in *Senecio vulgaris*. We conducted a reciprocal transplant experiment at two sites differing widely in their nutrient supply. These included a conventionally managed vineyard (V) and a strip of local wild flowers between crop fields, which was established to promote species diversity in agroecosystems (C). No fertilizer or pesticides are allowed in such ecological compensation areas. In C, we expected lower growth but higher PA formation than in V. Due to differentiated selection regimes in the two habitat types with regard to nutrient (nitrogen) availability in the soil, we also expected different N-allocation patterns for the genotypes of the two collection sites. Plants of V produced more biomass and were taller than the plants of C. The relatively poor nitrogen conditions in C favored an earlier differentiation towards generative organs. In plants of C, higher concentrations of PAs were found than in plants of V. There exists a close negative correlation between growth and PA formation, indicating a trade-off. The origin of the plant

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material had only a little effect on PA formation. The observed phenotypic reaction of PA formation in *S. vulgaris* in the two habitats fits quite well the predictions of GDB theory. It is shown that this general response is overlaid by physiological factors leading to a pattern of PA accumulation, which is not readily predictable by nonmechanistic theories.

Key Words—Environment, growth, growth–differentiation balance hypothesis, phenotypic plasticity, pyrrolizidine alkaloids, secondary metabolism, *Senecio vulgaris*.

INTRODUCTION

It is widely accepted that phytochemicals play an important role in ecological interactions by, for example, protecting plants against herbivores and pathogens, providing specialized herbivores with ecological niches, or by modulating litter decomposition (for reviews see Rosenthal and Berenbaum, 1992; Harborne, 1993). Inversely, plant secondary metabolism is known to be influenced by environmental parameters. Since these are increasingly changed by human activities, it is important to understand the related phenotypic reactions or adjustments of secondary metabolism. Specifically, the increase of both CO_2 and nitrogen forms available to plants is a new and relevant issue to be investigated in this context (Heyworth et al., 1998).

Two basic concepts dealing with the phenotypic response of secondary metabolism to the above-mentioned parameters have been proposed. The carbon–nutrient balance hypothesis (CNB) (Bryant et al., 1983) postulates limited growth and reduced N-based phytochemicals, but favored accumulation of carbon-based secondary metabolites such as phenolics, terpenoids, and tannins when carbons dominate over nutrients (primarily nitrogen). An excess of nitrogen (over carbon) results in preferential allocation of nitrogen-based compounds such as alkaloids or cyanogens. Due to such imbalances, growth may remain suboptimal.

The growth–differentiation balance hypothesis (GDB) (Loomis, 1953; Herms and Mattson, 1992) centers on a physiological trade-off between growth and differentiation. Plant tissue grows either by division or expansion of cells, or differentiates chemically and/or morphologically. As both growth and differentiation are dependent on photosynthetic products, they are, in terms of metabolism, negatively correlated with each other, whereby differentiation is thought to include secondary metabolism. The hypothesis states that: when resource availability is low, both growth and differentiation are reduced; at intermediate resource levels differentiation is favored; and at high resource levels growth prevails.

Unfortunately the CNB and GDB hypotheses have been tested almost exclu-

sively for carbon-based secondary metabolites and little attention has been paid to N-based compounds. Nevertheless, the formation pattern of phenolics appears to be in accordance with both hypotheses. Elevated CO_2 led to an increase in the concentration of phenolic compounds, and their concentration was found to decrease after fertilization (e.g., Muzika, 1993; Gebauer et al., 1998; Peñuelas and Estiarte, 1998).

The majority of studies do not support either theory with regard to nitrogen-based secondary metabolites. For instance, *Nicotiana attenuata* is homeostatic in its patterns of nitrogen allocation to nicotine and to growth. Under laboratory conditions, an eightfold nitrogen supply had no effect on either the constitutive or methyljasmonate-induced nicotine accumulation when allometrically related to growth (Lynds and Baldwin, 1998). In *N. sylvestris*, nicotine accumulation continues during nitrogen stress (Baldwin et al., 1993). It was concluded that nitrogen allocation to nicotine is not dependent on nitrogen in excess of growth requirements (Baldwin et al., 1993; Ohnmeiss and Baldwin, 1994). For pyrrolizidine alkaloids (PAs) in *Senecio jacobaeae*, no negative relationship between growth and differentiation could be found (Vrieling and van Wijk, 1994). Deficiencies of mineral nutrients including N did not significantly alter PA concentrations in flower heads of *Senecio vulgaris* under growth chamber conditions (Brown and Molyneux, 1996). GDB theory is supported by the work of Höft et al. (1996) who found that nitrogen supply resulted in enhanced alkaloid accumulation in *Tabernaemontana pachysiphon* under greenhouse conditions. Moderate fertilization was more effective than abundant nitrogen supply.

The above-mentioned studies testing CNB and GDB hypotheses in view of nitrogen-based secondary metabolites were carried out under greenhouse or laboratory conditions and were not conclusive. It is essential to investigate these models under field conditions so that the plants are exposed to the entire array of physical factors. Clarifying the validity of these hypotheses also is important from a nature conservation view. The fulfilment of the GDB predictions in heavily fertilized soil, for instance, suggests a decrease of the allelochemical concentration in the plant body. This will influence not only plant survival but also the fitness of associated specialist insect herbivores (for reviews see Rosenthal and Berenbaum, 1992; Harborne, 1993).

In an agroecosystem of northern Switzerland, we examined GDB in *Senecio vulgaris* by choosing two habitats differing widely in their nutrient supply. These were a conventionally managed vineyard and a strip of local wild flowers between crop fields, which was established to promote species diversity in agroecosystems. No fertilizer or pesticides are allowed in such ecological compensation areas. The Swiss government financially subsidizes farmers for setting aside arable land for ecological purposes.

PA formation in the predominantly inbreeding *S. vulgaris* provides an excellent model to study phenotypic plasticity of PA formation and possible

effects on specialized herbivores. PAs are feeding deterrents for most herbivores (Hartmann, 1999) and are hepatotoxic to vertebrate (Mattocks, 1986) and mutagenic to insects (Frei et al., 1992). The use of PA by specialist insects is well documented (Boppré, 1995; Hartmann, 1999). Specialized insect herbivores such as *Arctia caja* and *Thyria jacobaea* (Lepidoptera: Arctiidae) (Hartmann and Witte, 1995), which were, among other species, associated with *S. vulgaris*, were favored. Both species are recorded in the study area and are on the red list of endangered species (Bundesamt für Naturschutz, 1998).

Sites of PA biosynthesis are the roots, where the key compound senecionine *N*-oxide is produced (Sander and Hartmann, 1989) and its formation is quantitatively controlled. Senecionine *N*-oxide is translocated to the above-ground plant parts (Hartmann et al., 1989) and subsequently subjected to chemical diversification specific to species and populations (von Borstel et al., 1989; Witte et al., 1992). The total amount of alkaloids is not changed by this diversification. Senecionine *N*-oxide and the transformation products are spatially mobile but do not underlie degradation (Hartmann and Dierich, 1998).

A reciprocal transplant experiment was carried out with seed material originating from a vineyard and an ecological compensation area, which were closely located and ecologically similar to the experimental sites. The first aim was to test the predictions of the GDB hypothesis under field conditions. In the compensation area we expected lower growth but higher PA formation than in the vineyard. Secondly, due to differential selection regimes in the two habitat types with regard to nitrogen availability in the soil, we also expected different N-allocation patterns among the genotypes of the two collection sites. Thirdly, we aimed to clarify whether plants growing in compensation areas with low N input may allocate higher levels of PAs and benefit specialized insect herbivores.

METHODS AND MATERIALS

Plant Material and Design of Field Trials. Achenes of *S. vulgaris* were randomly sampled in 1997 in the Klettgau region of northern Switzerland (Canton of Schaffhausen) in both a vineyard and an ecological compensation area ca. 5 km apart. Achenes from 10 individual plants per site were grown for one generation in the greenhouse at the University of Fribourg to reduce possible maternal effects. In April 1998, achenes from five individuals (maternal seed families) from each site were sown in seed trays containing nutrient-poor soil in the greenhouse. After 19 days, seedlings were planted into two field plots (1×3.5 m) located in an ecological compensation area (C) and in a vineyard (V), respectively. All vegetation was removed in these plots before planting the seedlings. Field plots were in close (200 m) to each other and located near where the original material had been collected. In each habitat, 10 individuals from each

seed family and from both origins (C and V) were randomly assigned to plant positions in a regular grid of 15 × 15 cm, resulting in 100 plants per site. In the vineyard trial it was necessary to replant, since all seedlings suffered from herbivory (most probably slugs) during the first night. Replanting resulted in a delay of 14 days for the whole experiment.

Harvesting and Determination of Growth Parameters. Above-ground biomass from each of three randomly selected plants from each family was harvested around midday at 18 and 37 days after planting, i.e., 37 days and 56 days after sowing. After determination of plant height, leaf numbers, and fresh weight, the material was dried at 70°C to measure the dry weight. At the second harvest, plants were dissected into vegetative (stem and leaves) and generative parts (flower heads).

Soil Nitrogen Analysis. At the second harvest, five soil samples were randomly collected from the upper 30 cm of soil and pooled to determine total mineralized nitrogen (N_{min}, NO_3, and NH_4). The analysis was carried out photometrically by Schweizer AG, (Laboratory of Soil Analytics and Environmental Technologies, Thun, Switzerland), according to the N_{min} method of Scharf (1977).

Extraction of PA. The method of Hartmann and Toppel (1987) was slightly modified: the dried and finely powdered plant material was extracted with 0.05 M H_2SO_4 (ca. 25–50 mg/ml) at room temperature for 1 hr by sonication. An aliquot of the extract was transferred into a 2-ml Eppendorf tube and centrifuged. Then, 500 μl of the supernatant was mixed with 300 mg freshly reground (in a mortar) zinc dust and vigorously shaken for 5 hr in order to reduce the PA N-oxides. After centrifugation, 400 μl of the extract was removed and the pH adjusted with concentrated ammonia to ca. 8.0, as estimated by dotting on an indicator stripe. The extract was applied onto a Kieselgur column (Extrelut, Merck, Darmstadt, Germany) in a Pasteur pipet, and after 5 min, elution was carried out with 6 ml CH_2Cl_2. After passive evaporation of the solvent at room temperature, the residue was dissolved in 400 μl methanol. In a few cases, where the plant sample weight was below the standard extraction measure, the volume parameters were adjusted. The purification procedure resulted in a loss of 16, 31, and 27% of retrorsine, seneciphylline, and senecionine, respectively, as determined by reference compounds. The presented values were adjusted accordingly.

HPLC. Separation of PA was performed on a Nucleosil 100-5 ODS (Stagroma) column (5 μm; 4.6 × 250 mm; precolumn 4 × 20 mm) with 50 mM KPi buffer (with 1% THF), pH 2.5 (buffer A) and MeOH (with 1% THF) (buffer B) at a total flow rate of 1 ml/min and by the following gradient (% B over A): 0 min (5), 0–14 min (10), 14–25 min (50), 25–26 min (5); and 26–35 min (5). Parameters were controlled by a Hewlett Packard liquid chromatography (model HP 1090) equipped with a diode array detector set at 216 nm. Peak identification was achieved by comparing UV spectrum and R_t of authentic standards (library

established under separating conditions). Quantification was made by standard curves (external standards). The R_ts (minutes) were as follows: retrorsine (20.5); seneciphylline (23.6); senecionine (26.6). The latter includes the *E* isomer integerrimine.

Statistical Analysis. A nested ANOVA design was adopted as a model with origin (site where mother plants had been collected), genotype (original seed family, individual plant) and environment (experimental site) as main factors. The effect of origin was tested against the genotype within origin, the origin × environment interaction against the genotype × environment interaction, and all other factors and interactions against the residual term.

RESULTS

Genetic Effects. Nested analysis of variance showed no significant effect of origin and origin × environment on both the plant growth and PA formation (Tables 1 and 2). Significant genotype effects on growth were detected for plant fresh and dry weight, plant height, and fruit fresh weight (Table 1). With regard to PA formation, the effects were low and, with the exception of PA concentration related to dry weight, not significant (Table 2). Genotype × environment effects were only found for plant height and for dry weight of flowers at the second harvest (Table 1) but not for PA formation (Table 2).

Environment Effects on Growth Parameters. After 37 days of development, no environmental effect could be observed on plant height, but dry weight differed significantly (Table 1). Plants of C had 51% higher dry weight than plants of V. At this developmental stage no reproductive organs were visible.

After 56 days of development, plants in V were significantly taller than plants in C and the fresh weight of plants of V was 70% higher than those of C (Figure 1). Generally, differences in dry weight were more prominent at the first harvest and differences in fresh weight at the second harvest (Table 1).

Fresh weight and dry weight of flower heads were significantly different (Table 1). Flowers of C had 60% more fresh weight (Figure 1) and 135% more dry weight than the flowers of V due to an advanced stage of differentiation at the same age in C. More than twice as many flower heads had developed in plants of C compared to plants of V (results not shown). The fresh and dry weights of an individual flower head, however, was higher in plants of V.

Plants of V were more succulent at both sampling dates. The dry–fresh weight ratios were higher in plants of C than of V.

Environment Effects on Alkaloid Formation. A highly significant environmenal effect on PA concentration was found when referred to the fresh weight (Table 2). At both harvest times, PA concentrations were about twice as high in plants of C than in plants of V (Figure 2). On a dry weight basis the PA

TABLE 1. RESULTS FOR NESTED ANALYSIS OF VARIANCE: GROWTH AND DEVELOPMENTAL PARAMETERS[a]

Source of variation	First harvest time (37 days)				Second harvest time (56 days)			
	df	*SS*	*F*	*P*	*df*	*SS*	*F*	*P*
Fresh weight								
Origin	1	0.11	0.28	NS	1	0.32	0.01	NS
Genotype within origin	8	3.09	2.37	<0.05	8	347.60	2.60	<0.05
Environment	1	0.40	2.48	NS	1	344.87	20.60	<0.001
Origin × environment	1	0.26	1.00	NS	1	1.28	0.05	NS
Genotype × environment	6	1.58	1.62	NS	8	190.31	1.42	NS
Residual	36	5.87			40	669.72		
Dry weight								
Origin	1	0.00	0.00	NS	1	0.07	0.10	NS
Genotype within origin	8	0.07	3.66	<0.01	8	5.57	2.93	<0.05
Environment	1	0.03	15.03	<0.001	1	0.11	0.46	NS
Origin × environment	1	0.00	0.00	NS	1	0.20	0.07	NS
Genotype × environment	6	0.03	2.27	NS	8	2.54	1.34	NS
Residual	36	0.08			40	9.50		
Plant height								
Origin	1	1.68	0.21	NS	1	101.40	1.36	NS
Genotype within origin	8	63.27	3.05	<0.05	8	597.41	6.48	<0.001
Environment	1	1.30	0.50	NS	1	1026.72	89.06	<0.001
Origin × environment	1	0.95	0.21	NS	1	1.35	0.02	NS
Genotype × environment	6	27.34	1.75	NS	8	602.80	6.54	<0.001
Residual	36	93.49			40	461.15		

[a] NS = not significant.

TABLE 1. CONTINUED

Source of variation	First harvest time (37 days)				Second harvest time (56 days)			
	df	*SS*	*F*	*P*	*df*	*SS*	*F*	*P*
Dry wt/fr. wt ratio								
Origin	1	0.00	1.06	NS	1	0.00	1.18	NS
Genotype within origin	8	0.01	2.01	NS	8	0.00	2.05	NS
Environment	1	0.05	169.18	<0.001	1	0.07	238.67	<0.001
Origin × environment	1	0.00	0.10	NS	1	0.00	0.02	NS
Genotype × environment	6	0.00	0.32	NS	8	0.00	0.77	NS
Residual	36	0.01			40	0.01		
Fr. wt flowers								
Origin					1	0.43	0.18	NS
Genotype within origin					8	19.41	2.41	<0.05
Environment					1	5.40	5.36	<0.05
Origin × environment					1	0.12	0.08	NS
Genotype × environment					8	12.88	1.60	NS
Residual					40	40.28		
Dry wt flowers								
Origin					1	0.00	0.01	NS
Genotype within origin					8	0.39	2.01	NS
Environment					1	0.83	33.79	<0.001
Origin × environment					1	0.00	0.00	NS
Genotype × environment					8	0.58	2.97	<0.05
Residual					40	0.98		

TABLE 2. RESULTS FOR NESTED ANALYSIS OF VARIANCE: PA FORMATION[a]

Source of variation	First harvest time (37 days)				Second harvest time (56 days)			
	df	*SS*	*F*	*P*	*df*	*SS*	*F*	*P*
PA concentration (% dry wt)								
Origin	1	8.52	3.90	NS	1	2.91	3.87	NS
Genotype within origin	8	17.5	1.53	NS	8	6.03	2.28	<0.05
Environment	1	4.62	3.24	NS	1	3.96	11.97	<0.01
Origin × environment	1	1.75	0.90	NS	1	0.64	1.63	NS
Genotype × environment	6	11.72	1.37	NS	8	3.14	1.19	NS
Residual	36	51.42			40	13.23		
PA concentration (% fr. wt)								
Origin	1	0.02	0.44	NS	1	0.05	2.28	NS
Genotype within origin	8	0.39	2.01	NS	8	0.18	1.93	NS
Environment	1	2.53	104.28	<0.001	1	0.82	68.72	<0.001
Origin × environment	1	0.03	0.61	NS	1	0.03	2.26	NS
Genotype × environment	6	0.31	2.10	NS	8	0.09	0.95	NS
Residual	36	0.87			40	0.48		
PA total								
Origin	1	1.22	2.12	NS	1	0.13	0.07	NS
Genotype within origin	8	4.61	1.26	NS	8	15.01	1.71	NS
Environment	1	2.18	4.79	<0.05	1	1.46	1.33	NS
Origin × environment	1	0.6	0.44	NS	1	0.10	0.13	NS
Genotype × environment	6	0.84	0.31	NS	8	6.22	0.71	NS
Residual	36	16.4			40	43.78		

[a] NS = not significant.

TABLE 2. CONTINUED

Source of variation	First harvest time (37 days)				Second harvest time (56 days)			
	df	*SS*	*F*	*P*	*df*	*SS*	*F*	*P*
PA concentration (% fr. wt)								
flowers								
Origin					1	0.26	1.60	NS
Genotype within origin					8	1.29	1.52	NS
Environment					1	0.02	0.22	NS
Origin × environment					1	0.04	0.48	NS
Genotype × environment					8	0.68	0.80	NS
Residual					39	4.14		
PA concentration (% dry wt)								
flowers								
Origin					1	41.79	1.65	NS
Genotype within origin					8	202.67	2.03	NS
Environment					1	1.59	0.13	NS
Origin × environment					1	11.39	0.59	NS
Genotype × environment					8	155.05	1.55	NS
Residual					39	486.62		

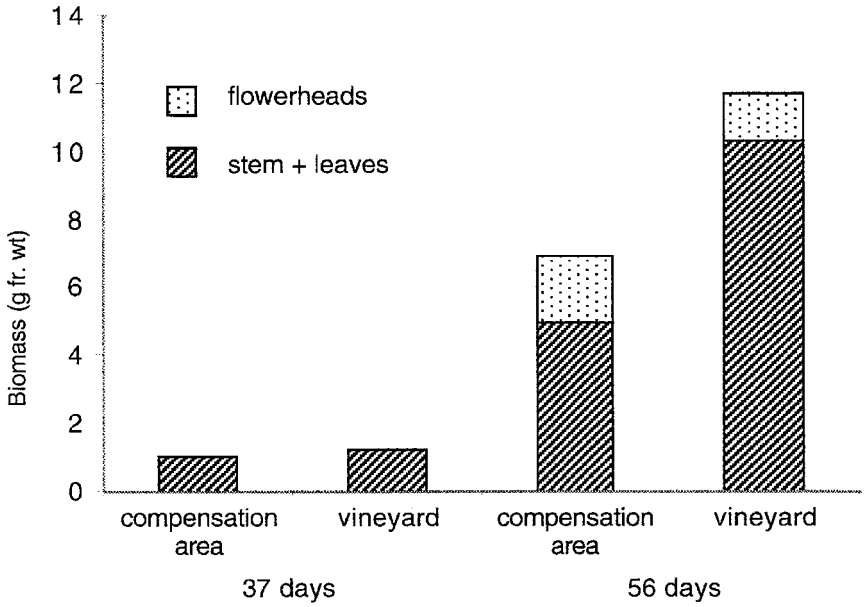

FIG. 1. Biomass (g fr. wt) of *Senecio vulgaris* at 37 days and 56 days of development.

concentrations were 15% and 24% (first and second harvest times, respectively) higher in plants of C than in plants of V. The effect was significant only after 56 days. Total PA content was higher in plants of C than in plants of V (about 70% after 37 days and about 12% after 56 days). The effect was significant only at the first harvest time.

At both sites, PA concentration decreased from the first to the second harvest by a factor of almost 2 (from 0.90 to 0.48 mg/g fresh wt in C and from 0.44 to 0.24 mg/g fresh wt. in V; Figure 2). No environmental effect on PA formation could be found in fruits (Table 2).

The main alkaloid compounds were seneciphylline and senecionine (including the *E* isomer integerrimine) in varying proportions. The portion of retrorsine was below 10%. Correlating with the habitat, no significant alkaloid pattern was found.

Soil Nitrogen. Mineral nitrogen of V was twice as high as of C (33 kg N_{min}/ha and 16 kg/N_{min}/ha, respectively). Compared to sites in the neighborhood, the soil nitrogen content of V was relatively low, because no fertilization was carried out during the experiment. The nitrogen content of C is within the range of sites nearby (Serena Rigamonti, personal communication).

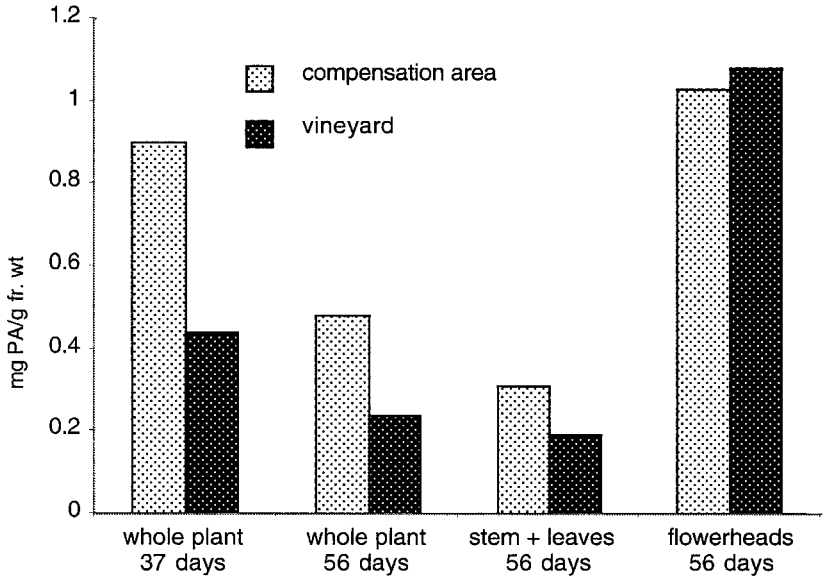

FIG. 2. Alkaloid concentration (mg PA/g fr. wt) in *Senecio vulgaris* at 37 days and 56 days of development.

DISCUSSION

In general, the observed results fit the GDB hypothesis (Loomis, 1953; Herms and Mattson, 1992) quite well. Plants of V with a larger soil nitrogen supply were oriented to growth whereas plants of C were oriented to differentiation. After 56 days, plants of V had a higher fresh weight, were taller, and had more leaves (result not shown) than plants of C. The number of flower heads, however, was twice as high in plants of C than in those of V. The relatively poor nitrogen conditions favored an earlier differentiation towards generative organs. After 37 days, the measured growth parameters (fresh and dry weight, height) did not yet demonstrate higher growth of V and dry weight was even lower in V.

PA formation also followed the GDB hypothesis: higher concentrations were found in plants of C than of V (Figure 2). In accordance with growth development, the difference is more pronounced after 56 days than after 37 days. There exists a significant negative correlation between log biomass and milligrams of PA per gram of biomass ($R^2 = 0.52$, $P < 0.001$ for fresh weight; $R^2 = 0.31$, $P < 0.001$ for dry weight) indicating a trade-off between growth and differentiation (Figure 3). Such a significant negative correlation was also found within each site (for fresh weight: V: $R^2 = 0.43$, $P < 0.001$; C: $R^2 = 0.23$, $P = 0.007$).

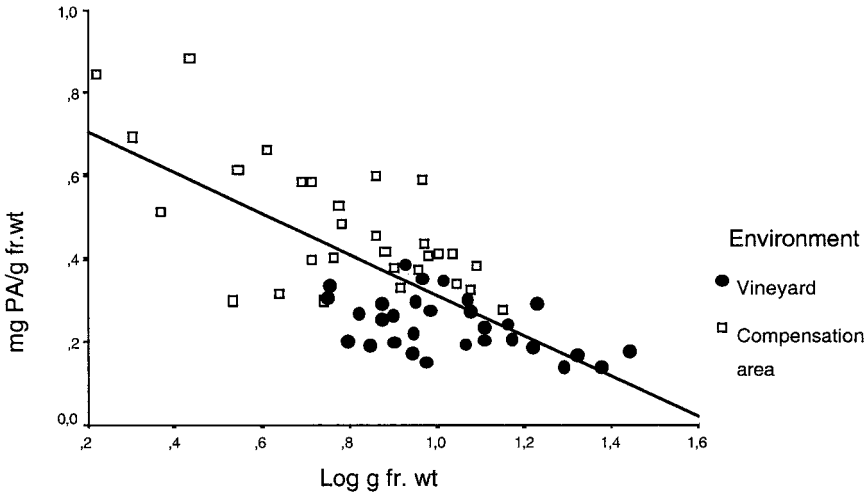

FIG. 3. Correlation between of log fresh weight and alkaloid formation at 56 days of development ($R^2 = 0.52$, $P < 0.001$).

The CNB hypothesis (Bryant et al., 1983) was not supported. Higher availability of nitrogen did not lead to an increased accumulation of PAs.

The fact that seed origin had no significant effect on the various growth and PA parameters assessed is not surprising. A recent plant molecular study carried out on these *S. vulgaris* populations suggests that ecological compensation areas were only recently colonized from adjacent corn fields, with a nitrogen status similar to the vineyard sites (Müller-Schärer and Fischer 2000). The significant genotype effects observed for some of the growth and PA variates indicate, however, genetic variation for these traits and, therefore, the potential for local adaptation.

PAs are synthesized in the roots. In organ cultures their formation is strictly linked to root growth and ceases when root growth stops (Hartmann et al., 1988; Sander and Hartmann, 1989). The shoot–root ratio in *S. vulgaris* correlates positively with the availability of soil nitrogen (Van der Kooij et al., 1998); therefore, plants of C might have a relatively better developed root system than plants of V. If the postulated link between root growth and PA formation exists under field conditions, increased PA formation in C could be an indirect consequence of a morphophysiological adaptation to low mineral nutrient conditions and not a reaction in the sense of the GDB hypothesis. A significant negative correlation between shoot–root ratio and PA concentration could be detected in experiments with seedlings of *S. vulgaris*. A similar trend, although not significant, was found in seedlings of *S. jacobaea*. In both species, a significant positive cor-

relation was found between root biomass and total PAs per seedling (Schaffner et al., unpublished manuscript). The relationship between root growth and PA formation should be further studied under field conditions.

Physiological and biochemical (Hartmann, 1999; Hartmann and Dierich, 1998; Hartmann and Zimmer, 1986) as well as genetic studies (Vrieling et al., 1993) indicate that two different sections of PA formation exist. The root, where biosynthesis of the key compound, senecionine N-oxide, occurs under highly constrained conditions, and the above-ground parts, where a large number of subsequent biosynthetic transformations and tissue- and development-specific accumulations take place. This study supports the model of Hartmann and Dierich (1998) also for an ecological factor. Nitrogen supply is a high constraint for the production of the total amount of PAs (relative to biomass) in the roots. The flexible translocation and allocation of PAs in the above-ground plant parts is independent of the production in the roots. Although alkaloid production relative to biomass was lower in V than in C, alkaloid content in the generative plant parts is equal in V and in C. In an ecological sense, it is reasonable that alkaloid accumulation in vulnerable and valuable plant organs as flowers and fruits are, within a certain range, independent of the supply. Therefore, although the phenotypic reaction of PA formation in *S. vulgaris* in the two sites fits quite well the predictions of GDB theory, the response in detail is overlaid by physiological factors leading to a pattern of PA accumulation, which in its details is hardly predictable by nonmechanistic theories.

The hypothesis that ecological compensation areas with low soil nitrogen may be beneficial to endangered insect specialists that sequester PAs has to be tested further. The results clearly show that PA concentration is higher in plants of C than V, which is in agreement with this hypothesis. The internal allocation pattern may compensate for this reduced production as the level of PA concentration in flowers was found to be independent of the soil nitrogen conditions. Therefore, only specialized insects consuming vegetative plant parts would be able to benefit from increased PA concentrations of ecological compensation sites.

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