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IMPACT OF POLYPHENOLS ON GROWTH OF THE AQUATIC HERBIVORE *Acentria ephemerella*

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Abstract—Larvae of *Acentria ephemerella* live fully submerged, feeding on submersed aquatic angiosperms such as pondweeds (*Potamogeton* spp.) and *Myriophyllum spicatum.* Only the latter contains high concentrations of hydrolyzable tannins known to interfere with the growth of insect herbivores. We tested whether larvae grow faster on *Potamogeton perfoliatus* or *M. spicatum* and whether this is due to polyphenols in their food source. Larvae originating from the same egg clutch grew faster and larger on *P. perfoliatus* than on *M. spicatum*. The same growth response was observed with larvae that spent winter diapause on either *P. perfoliatus* or *M. spicatum*. These larvae were fed either with their host plant or the other macrophyte. No prior feeding effect was found, but growth of larvae reared on *M. spicatum* was less than when grown on *P. perfoliatus*. Larvae from another egg-clutch reared on *M. spicatum,* either from lake or cultivated in aquaria, exhibited reduced growth on the lake plants. *P. perfoliatus* contained less than 1% and *M. spicatum* (aquarium or field material) between 5 and 9% phenolic compounds. No differences in nitrogen content of leaves were found, but apical shoot sections of *M. spicatum* exhibited a significantly higher nitrogen content than *P. perfoliatus*. Our results indicate that hydrolyzable tannins are responsible for the reduced growth of *Acentria* when fed with *M. spicatum*.

Key Words—Chemical defense, gallotannin, ellagitannin, aquatic angiosperm, freshwater macrophytes, *Myriophyllum*, pyralid moth.

INTRODUCTION

Herbivory on freshwater aquatic angiosperms can be as intense as on terrestrial vegetation (Cyr and Pace, 1993). Biomass loss of macrophytes (Lodge et al.,

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1998) and even changes in macrophyte community composition due to herbivory by both vertebrate (van Donk, 1998) and invertebrate (Johnson et al., 1998; Gross et al., 2001) herbivores have been reported. In the last decade, many studies have shown that chemical defenses of freshwater macrophytes are important, occurring as frequent as in marine or terrestrial macrophytes (Newman et al., 1996; Bolser et al., 1998; Wilson et al., 1999; Kubanek et al., 2000).

Phenolic compounds and especially tannins (polyphenols) are widely recognized as effective herbivore deterrents in terrestrial (Feeny, 1970; Hagermann and Butler, 1991) and marine systems (Steinberg and van Altena, 1992) but not in freshwater plants. Submerged aquatic angiosperms generally contain lower concentrations of phenolic compounds compared to emergent or floating leaved macrophytes (Gross, 1999; Smolders et al., 2000). One exception is *Myriophyllum spicatum* L. (Haloragaceae), which contains high concentrations of phenolics (Smolders et al., 2000), especially gallo- and ellagitannins (Gross et al., 1996; Gross, 1999). Hydrolyzable tannins are the dominant type of phenolics present in *M. spicatum* and other Haloragaceae (Saito et al., 1989; Gross et al., 1996; Nakai et al., 2000). Tellimagrandin II (1,2,3-tri-*O*-galloyl-6 (*S*)-hexahydroxydiphenoyl-ß-D-glucose), the major polyphenol present in *M. spicatum*, has strong algicidal and cyanobactericidal properties (Gross et al., 1996). Its concentration is highest in apical shoot sections where it reaches 1–5% of dry weight and accounts for up to 15% of all hydrolyzable tannins present in milfoil (Gross, 2000; Gross, unpublished results). In addition, condensed proanthocyanidins have been isolated from *M. brasiliense* and *M. spicatum* (Saito et al., 1989; Nakai et al., 2000).

Acentria ephemerella SCHIFFERMÜLLER & DENIS (Lepidoptera; named *Acentria* in the following) are important herbivores on *M. spicatum* (Johnson et al., 1998, Gross et al., 2001). Larvae of this aquatic pyralid moth feed exclusively on aquatic angiosperm tissue and avoid filamentous epiphytic algae (Johnson et al., 1998; Gross et al., 2001). *Acentria* feed on a variety of submerged macrophytes, especially pondweeds (*Potamogeton* spp.), *Ceratophyllum demersum* and *Elodea canadensis*, but avoid others, e.g., *Chara* spp. and *E. nuttallii* (Johnson et al., 1998; Gross and Johnson, unpublished results). Feeding on *M. spicatum* occurs predominantly on the apical shoot sections (Gross et al., 2001, 2002) despite the fact that the highest concentrations of hydrolyzable tannins are present in these plant parts (Gross, 2000). *Acentria* can fully develop on all above-mentioned host plants, but differences exist in the proportion of individuals reaching adulthood (Gross et al., 2001).

Phenolic compounds, especially polyphenols (hydrolyzable and condensed tannins), are well known to interfere with growth and development of many insect herbivores (Feeny, 1976; Hagerman and Butler, 1991). Reduced growth in insects has two major drawbacks. First, a smaller size of female pupae generally results in a lower number of eggs laid by the adult female and a decline in their overall fitness (Haukioja and Neuvonen, 1985; Kaitaniemi et al., 1998; Osier et al.,

2000; Tikkanen et al., 2000). Second, prolonged larval development can result in a higher predation mortality (Feeny, 1976; Benrey and Denno, 1997). However, plant polyphenols may also benefit insect herbivores. Higher tannin content in food plants of *Lymantria dispar* decreased the effectiveness of the bacterial pathogen *Bacillus thuringiensis* (Appel and Schultz, 1994).

We asked whether the polyphenol-rich milfoil would cause changes in growth of larvae similar to that observed in terrestrial lepidopteran larvae. The objectives of our study were to test whether the growth of *Acentria* larvae: (1) is influenced by polyphenol-containing freshwater angiosperms, (2) depends on the amount of polyphenols present in *M. spicatum*, and (3) is determined by prior feeding experiences.

METHODS AND MATERIALS

Plant Samples. Samples of various submerged macrophytes (Table 1) were collected in lakes of the Finger Lakes area of New York, USA, or in northern and southern Germany. The screening for phenolic content in different macrophytes used whole upper shoots (25-cm long). When comparing *P. perfoliatus* and *M. spicatum* from Lake Constance, we dissected the upper 25-cm shoot sections into apical shoot sections, leaves, and stems. Plants were shock-frozen in liquid nitrogen, lyophilized, and stored in the dark at room temperature for further analysis.

Analysis of Plant Chemistry. All analyses were performed with ultrapure water and with analytical-grade (p.a.) or HPLC-grade solvents. Plant material was extracted with 50% aqueous acetone (v/v, 10 mg/ml solvent) over 2 hr and stirred constantly. Solids were centrifuged and the supernatant used for the analysis of the total phenolic content. Phenolic content was measured as described previously (Gross et al., 1996). Extracts were used in concentrations yielding absorbances (A_{750nm}) between 0.1 and 0.5 AU. Calibration curves with tannic acid (Sigma T8406) were used to calculate total phenolic content as tannic acid equivalents (TAE). The PVPP (polyvinylpolypyrrolidone, Sigma P6755) assay was performed to distinguish phenolic compounds from other redox active compounds of non-phenolic origin (Loomis and Battaile, 1966; Gross et al., 1996).

The carbon and nitrogen content of plant samples was measured with an Elementar Analysator NCS 2500 (CE Instruments/Thermoquest) using atropine sulfate as a standard (58.8% C, 4.0% N, C:N molar ratio 17:1). The protein content of leaves was determined by multiplication of the percent nitrogen content by 6.25 (Feeny, 1970). Soluble protein content was not determined because both Lowry and Bradford assays are sensitive to soluble phenolic compounds, and do not provide an exact measurement of protein content in plants with high concentrations of phenolic compounds (Gross, unpublished results).

Growth Assays. We performed three different growth assays using either newly hatched larvae of *Acentria ephemerella* (experiments I and II) from egg

clutches found in summer 1999, or diapausing larvae of *Acentria* (experiment III) found in late September 2000 on senescent shoots of *M. spicatum* and *P. perfoliatus.* All larvae originated from Lower Lake Constance. Macrophytes used in the experiments were either collected weekly in the lake or cultured prior to the experiments for at least three months in aquaria under constant conditions (18°C ± 1°C, 16L:8D photoperiod, 60 μmol PAR/m^2/sec). Head capsule width of larvae was measured with digital imaging at a mean precision of 1.5%.

Experiment I. To test whether polyphenol-rich *M. spicatum* affects the growth of *Acentria* larvae, we used two different treatments. Either newly hatched larvae were fed lake- or aquarium grown *M. spicatum* or *P. perfoliatus*. A clutch of 340 eggs found on *P. perfoliatus* in Lower Lake Constance on July 12, 1999, was used. Larvae that hatched during July 20–23 were randomly distributed to 12-well microtiter plates (50 larvae per treatment, maintained individually), and the remaining larvae were distributed equally to 100-ml glass jars with the respective food. Water and food were renewed twice a week. The head capsule width of the larvae was measured at intervals of 6 to 12 d. Until day 41, larvae that died were replaced with larvae of equal size from jars fed with the same host plant. The experiment ended at day 89.

Experiment II. In this experiment, we compared whether *Acentria* larvae differ in growth on *M. spicatum* derived from the lake or from aquaria. In Lower Lake Constance, on August 6, 1999, a second egg clutch was found on *P. perfoliatus.* The 370 eggs that hatched between August 10 and 13 were divided into two groups according to the procedure described in experiment I. The experiment ended on day 72 (October 21, 1999) because *M. spicatum* became senescent at this time.

Experiment III. This assay was designed to investigate whether previous food experience would influence the outcome of the growth assays with polyphenol-rich and polyphenol-free freshwater macrophytes. *Acentria* hibernates in the larval stage and bores holes for shelter in lower stem sections of tall growing macrophytes, such as *P. perfoliatus* or *M. spicatum* (Gross et al., 2002). Larvae were collected on September 27, 2000, in Lower Lake Constance, near the island of Reichenau, and stored at 4°C. In March 2001, working on ice, larvae were removed from the stems of macrophytes and freed from their hibernicula. They were placed individually in 30-mm-diam Petri dishes and fed with leaves of the macrophytes used in the different treatments submerged in 5 ml of tap water. Larvae that originated from *M. spicatum* were then fed with either *M. spicatum* or *P. perfoliatus* (MSMS or MSPF treatment). Larvae that came from *P. perfoliatus* plants were fed with either one of the two macrophytes (PFMS and PFPF treatment). In each treatment, 24 larvae were used. Larvae were reared for 53 d, and head capsule width was measured at the start of the experiment and then every 6–10 d.

Statistical Analysis. Data were analysed using the Student *t* test, one- or two-way ANOVA, and Tukey HSD *post-hoc* tests (Statistica 99 edition or SigmaStat, version 2.03).

RESULTS

Plant Chemistry. The phenolic content of aquatic angiosperms belonging to several families varied from < 1% to 15%, measured as tannic acid equivalents (TAE) and based on dry mass dm, (Table 1). Several host plants of *Acentria ephemerella*, such as *Ceratophyllum demersum, Elodea canadensis* and *Potamogeton perfoliatus*, had < 1% TAE in their tissue. All members of the Haloragaceae (*Myriophyllum* spp., *Proserpinaca* sp.) contained > 6.5% dm phenolic compounds, approximately one order of magnitude higher than all other species investigated.

We tested the nutritional quality of the plants used in the growth experiments during late summer 1999. A comparison of leaves from the upper 25-cm shoot sections of *P. perfoliatus* and *M. spicatum*, sampled during late summer of 1998 and 1999, demonstrated that in both years the latter had a significantly higher phenolic content than *P. perfoliatus* (Table 2, 9% vs. 1% TAE in both years, $P < 0.006$). More than 95% of all tannic acid equivalents in *M. spicatum* were phenolic compounds and precipitated with PVPP. Approximately 50% of the tannic acid equivalents in *P. perfoliatus* were of nonphenolic origin. *M. spicatum* plants kept for several months in aquaria exhibited a lower leaf phenolic content than those taken directly from the field (aquarium: $5.5 \pm 0.4\%$ TAE, mean $\pm$ 2 SEM, $N = 9$; lake, samples taken in summers 1998–2000 at peak biomass of

TABLE 1. COMPARISON OF TOTAL PHENOLIC CONTENT OF VARIOUS SUBMERGED MACROPHYTES[a]

Aquatic angiosperms	Tannic acid equivalents (mg/g dm)		Different locations
	Mean $\pm$ 1 SD	Range	
Haloragaceae			
Myriophyllum alterniflorum	79.8 ± 11.0	67.6–88.9	3
M. heterophyllum	66.7 ± 6.6	62.4–74.3	3
M. spicatum	83.2 ± 8.8	68.7–101.7	15
M. verticillatum	67.5	65.4–69.6	2
Proserpinaca palustris	148.6	148.6	1
Other families			
Ceratophyllum demersum	8.3 ± 1.8	6.5–10.1	3
Elodea canadensis	6.0 ± 1.3	4.9–7.0	3
Najas flexilis	3.6	3.6	1
Potamogeton perfoliatus	9.4 ± 4.5	4.5–12.1	3
Ranunculus trichophyllus	18.9 ± 1.7	16.7–21.0	3

[a] Only data from plants taken during maximum plant biomass at different sites/in different lakes were taken into account. Upper 25 cm of shoot sections were taken for the analysis. Data are presented as tannic acid equivalents based on plant dry mass. Data represent means, 1 SD, and range of samples taken in late summer from different locations.

TABLE 2. COMPARISON OF TOTAL PHENOLIC CONTENT OF SUBMERGED MACROPHYTES USED AS HOST PLANTS IN OUR GROWTH EXPERIMENTS[a]

	Tannic acid equivalents (mg/g dm)			
	M. spicatum	*P. perfoliatus*	*t*	*P*
1998	88.0 ± 16.0	11.6 ± 7.8	8.5	0.001
1999	94.6 ± 30.0	12.1 ± 7.7	5.3	0.006

[a] Only leaves from the upper 25 cm of shoot sections were taken for the analysis. *M. spicatum* and *P. perfoliatus* were collected in Lake Constance during the maximum growing period (July–September) of 1998 and 1999 ($N = 3$ each; mean ± 2 SEM).

M. spicatum: 9.0 ± 1.0% TAE, $N = 9$, Student's t test, $t = 6.0$, $P < 0.001$). Whereas the phenolic content of aquarium plants stayed more or less the same, we observed a steady decline in field milfoil from 12% at the beginning of August to 7% at the end of September and < 5% TAE at the end of October 1999 (linear regression, TAE = 333.5 − 0.99 × (time); $R = -0.97$; $P < 0.001$). When comparing the phenolic content of *M. spicatum* samples taken in late September to late October 1998–2000 with those of aquarium plants, no difference was found (*M. spicatum* fall 5.8 ± 1.3% TAE, $N = 6$; aquarium samples as above, $t = 0.5$, $P = 0.7$).

Nitrogen content is commonly used as a proxy for protein content and thus nutritional quality of host plants for herbivores. Leaves of *P. perfoliatus* and both field- or aquarium grown *M. spicatum* had a nitrogen content of 3.6–3.8% dm, and were not significantly different (Table 3; one-way ANOVA, $P = 0.8$). The apical shoot sections of *P. perfoliatus* exhibited a significantly lower nitrogen content (3.3%) than those of either aquarium or field *M. spicatum* (4.8–5.4%; Table 3; one-way ANOVA, $P < 0.001$). Using the conversion factor of 6.25 (Feeny, 1970), to estimate the content of soluble protein, we calculated a content of 22–24% in

TABLE 3. NITROGEN CONTENT (% OF DM) OF MACROPHYTES USED IN GROWTH EXPERIMENTS WITH *Acentria* LARVAE[a]

	Nitrogen					
	M. spicatum		*P. perfoliatus*			
	Field	Aquarium	Field	*MS*	*F*	*P*
Leaves	3.6 ± 0.4 a	3.6 ± 0.4 a	3.8 ± 0.3 a	0.1	0.3	0.8
Apical shoots	4.8 ± 0.4 a	5.4 ± 0.5 a	3.3 ± 0.3 b	8.6	20.1	<0.001

[a] Data represent means ± 2 SEM of 6 to 12 independent samples. Results of one-way ANOVAs for each plant part are presented. Different letters indicate treatments that vary significantly at $\alpha = 0.05$.

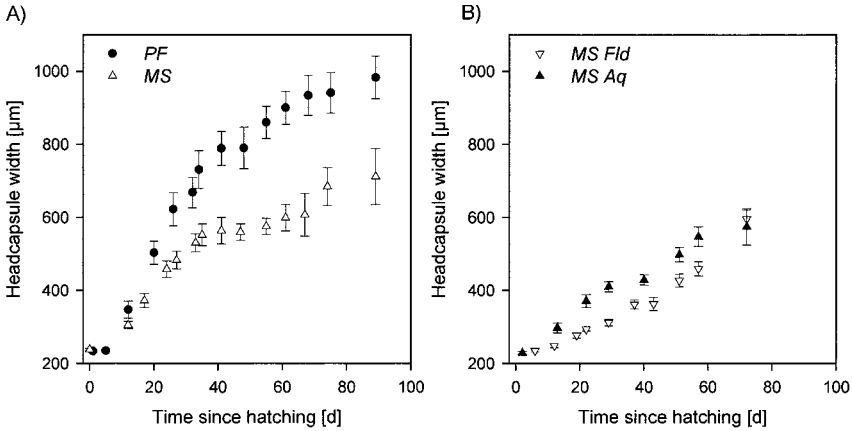

FIG. 1. Newly hatched *Acentria* larvae reared on different freshwater macrophytes. Growth was measured as headcapsule width. A) Experiment I: *Acentria* reared on *Potamogeton perfoliatus (PF)* and *Myriophyllum spicatum (MS)*. B) Experiment II: *Acentria* reared on fresh *M. spicatum* harvested weekly from the lake *(MS Fld)* or cultured in aquaria *(MS Aq)*. $N = 50$ each per treatment in both experiments, $N = 14$–15 per treatment at the end of the experiments. Data represent means $\pm$ 2 SEM.

leaves of *P. perfoliatus* and *M. spicatum*. The carbon content of all plants ranged between 35 and 42% dm and the C:N molar ratio varied from 8:1 to 14:1.

Growth Experiments. In experiment I, larvae developed differently on *P. perfoliatus* and *M. spicatum* (Fig. 1A). Larvae did not differ in size at the beginning of the experiment (*M. spicatum* head capsule widths $= 238 \pm 2\mu$m; *P. perfoliatus* $= 234 \pm 2\mu$m, mean $\pm$ 2 SEM, $N = 50$ each, $P = 1.0$). The average head capsule width of larvae reared on *P. perfoliatus* was larger than when fed with *M. spicatum* (Figure 1A). The two-way ANOVA showed a significant treatment effect ($F_{1,554} = 225.5$, $P < 0.001$), a significant time effect ($F_{7,554} = 180.2$, $P < 0.001$), and the interaction of treatment and time was also highly significant ($F_{7,554} = 9.6$, $P < 0.001$). From the third week until the end of the experiment, larvae were larger on *P. perfoliatus* than on *M. spicatum* ($P < 0.05$). The largest larvae on *P. perfoliatus* reached a head capsule width of 1190 μm; the average size being $983 \pm 58\mu$m ($N = 14$). Some larvae fed with *M. spicatum* also reached a head capsule width of more than 900 μm, but not until the end of the experiment; others stayed well below 600 μm, resulting in the lower mean size ($712 \pm 76\mu$m, $N = 15$). In both treatments, larval size reached a plateau after 40 days, indicating the end of growth.

Experiment II was performed with another egg clutch found on *P. perfoliatus*. This was divided into two treatment groups; one group was fed fresh *M. spicatum* collected weekly in the field, and one was fed *M. spicatum* from aquaria. During most of the experiment, larvae fed with aquarium plants grew better than those

reared on field *M. spicatum* (Figure 1B). Again, in the two-way ANOVA we observed a significant treatment ($F_{1,242} = 199.8$, $P < 0.001$), a significant time ($F_{4,241} = 51.0$, $P < 0.001$), and a significant interaction effect ($F_{4,241} = 5.0$, $P < 0.001$). *Post-hoc* tests revealed significant differences at days 13 to 57 ($P < 0.005$), but not at the end of the experiment (day 72, $P = 0.98$). At this time (end of October) we had to stop the experiment, because milfoil started to decay in the lake. During September and October 1999, field milfoil no longer had a higher phenolic content compared to plants from the aquaria (field 5.8%, aquarium 5.5% TAE, $t = 0.5$, $P = 0.7$).

In experiment III, we investigated whether previous feeding experience would influence the growth of *Acentria* on polyphenol-rich or polyphenol-free macrophytes. This experiment was conducted with larvae that had started winter diapause in either *P. perfoliatus* or *M. spicatum*, and we assumed that these were the plants the larvae fed on previously. Larvae dissected from *P. perfoliatus* or *M. spicatum* stems did not differ in size (mean head capsule width all larvae 556 $\pm$ 16 μm; mean $\pm$ 2 SEM; range 400–707 μm). Larvae then developed differently depending on the macrophyte being offered for food. The two-way ANOVA showed a significant treatment effect ($F_{3,362} = 18.8$, $P < 0.001$), a significant time effect ($F_{4,362} = 293.1$, $P < 0.001$) and a significant time $\times$ treatment interaction effect ($F_{12,362} = 6.9$, $P < 0.001$). Starting with day 15, larvae fed with *P. perfoliatus* (treatments PFPF and MSPF) were significantly larger than those shifted from *P. perfoliatus* to *M. spicatum* (PFMS) ($P = 0.015$). At the end of the experiment, larvae were either large (PFPF/MSPF), or small (PFMS/MSMS) with mean head capsule widths of 1014/989 or 891/834 μm, respectively (Figure 2). *Post-hoc* tests revealed that these treatments differed significantly at day 40 ($P < 0.02$) and day 53 ($P < 0.005$).

DISCUSSION

Our study shows that the performance of a true aquatic shredder–herbivore, *Acentria ephemerella*, is correlated with the presence and amount of phenolic compounds in their host plants. In our screening, all members of the Haloragaceae contained high concentrations of phenolic compounds, approximately one order of magnitude higher than other macrophytes investigated. Smolders et al. (2000) also found that *M. spicatum* had a high phenolic content, reporting a mean concentration in leaves of 8.4%. Phenolic compounds in this species are predominantly gallo- and ellagitannins (Gross et al., 1996). In contrast, according to our assays, *P. perfoliatus* always contained less than 1% phenolic compounds. In another study, *P. perfoliatus* contained less than 2% phenolic compounds (Smolders et al., 2000), and no tannins were found in this species (McClure, 1970).

The mean nitrogen content of 3.6–3.8% dm in leaves of the freshwater macrophytes used in our experiments fits well within the range found in other studies

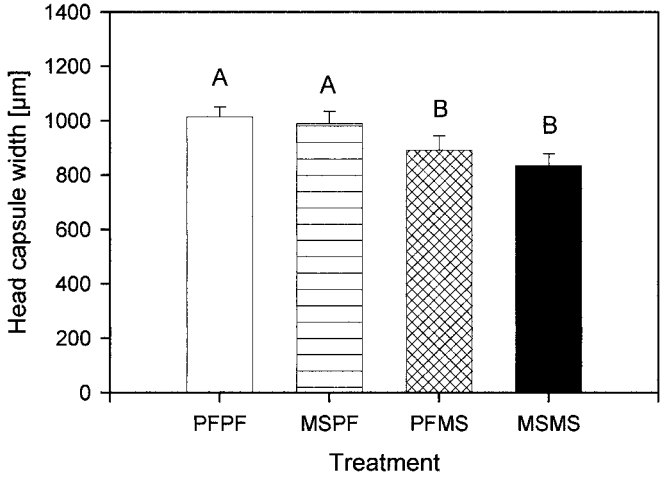

FIG. 2. Experiment III: final head capsule width of *Acentria* larvae that had a winter diapause in their life cycle in shoot sections of either *Potamogeton perfoliatus (PF)* or *Myriophyllum spicatum (MS)*. Larvae were fed in a reciprocal design with either the macrophyte they originated from or the other macrophyte (PFPF, PFMS; MSPF, MSMS). Data represent means $\pm$ 2 SEM of their size at day 53. Different letters indicate treatments that vary significantly at $\alpha = 0.05$.

(Nichols and Keeney, 1976; Spencer and Ksander, 1999). Nitrogen content was the same in all host plants, and was apparently not limiting the growth of *Acentria*. Therefore, the decreased size of *Acentria* in our experiments seems to be a result of the high concentration of polyphenols in *M. spicatum*. Two different experimental designs demonstrated that *Acentria* grew faster on *P. perfoliatus* than on *M. spicatum*. In experiments I and II, larvae differed significantly in size 3 wk after hatching, depending on the source of food offered. In these experiments, larvae originated from the same egg clutch. In experiment III, we used diapausing larvae retrieved from either milfoil or *P. perfoliatus*, suggesting they had used the respective plant for feeding prior to hibernation. Based on our laboratory findings, one would expect field larvae to be smaller on milfoil than on *P. perfoliatus* in fall. However, diapausing larvae from both macrophytes did not differ in size. This can be explained by examining the differences in life-cycle between the plants. Larvae first colonize *P. perfoliatus* in spring, and later move to *M. spicatum* at the end of summer (Gross et al., 2002). This is probably related to the earlier emergence of *P. perfoliatus* and later senescence of *M. spicatum* in Lake Constance. Thus, larvae on milfoil have a longer time for development and may reach the same size for hibernation as those already diapausing in lower stems of *P. perfoliatus* (Gross et al., 2002). Our results in experiment III indicate that larvae stay longer

at different instar stages with *M. spicatum*, thus reaching a similar size later than larvae fed with *P. perfoliatus*.

Apparently, not only the presence but also the amount of polyphenols present in *M. spicatum* determine the final size of *Acentria* larvae. *M. spicatum* from the field or from aquaria exhibited no difference in their nitrogen content. However, field plants contained, on average, twice as much polyphenols as aquarium plants. In the fall at the end of experiment II, this difference disappeared. At that time, larvae in both treatments were the same size. Unfortunately, we could not extend the experiment due to the senescence of *M. spicatum* in the lake. The final proof that polyphenols, especially hydrolyzable tannins, are solely responsible for the reduced growth of *Acentria* can only be obtained with a defined artificial diet to which selected polyphenols are added in various concentrations. Unfortunately, *Acentria* can not be reared on artifical diets. We have tested several commonly used lepidopteran diets (wheat germ-, pinto bean-based diets) or alginate- and agar-based diets used in marine studies. Larvae did not accept any of these and did not survive (Gross, unpublished results).

Feeding preferences of the semi-aquatic moth *Munroessa gyralis* (Pyralidae), were not related to phenolic compounds, protein concentration or the leaf toughness of macrophytes (Dorn et al., 2001). Field observations and laboratory tests show that *Acentria* larvae prefer the apical meristems of *M. spicatum* compared to lower shoot sections (Gross et al., 2001). Apical meristems contain significantly higher concentrations of polyphenols and tellimagrandin II than lower shoots (Gross, 2000). Despite the reduced growth observed in these experiments, larvae can fully metamorphose on *M. spicatum* (Johnson et al., 1998, 2000; Gross et al., 2001, 2002), and are frequently found associated with this macrophyte (Painter and McCabe, 1988; Newman and Maher, 1995; Johnson et al., 2000). Larvae may benefit from feeding at apical tissue of milfoil because of the higher nutrient content and a better protection from visually oriented predators (Gross et al., 2002). Additionally, the antimicrobial activity of polyphenols (Walenciak et al., 2002) may protect *Acentria* from bacterial and fungal pathogens and thus compensate for reduced growth. Whether milfoil polyphenols affect the overall fitness of *Acentria* remains to be elucidated.

In this study, we have shown that *Acentria* exhibits a reduced growth on the polyphenol containing *M. spicatum* compared to *P. perfoliatus.* This may ultimately lead to a lower female fecundity, as observed in many other lepidopteran studies (Haukioja and Neuvonen, 1985; Kaitaniemi et al., 1998; Tikkanen et al., 2000). The antinutritional effects of dietary tannins result most often in a depressed growth rate and feed utilization efficiency (Hagerman and Butler, 1991). Apparently, gut symbiontic microorganisms are also involved in the allelochemical interaction of polyphenols and herbivores (Walenciak et al., 2002). Nonetheless, the exact mechanism of tannin interference with digestion in Lepidoptera is still unknown.

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