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## Leaf Beetles (Insecta: Coleoptera: Chrysomelidae) Suffer From Feeding on Fern Leaves<sup>1</sup>

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**Abstract.** Two age groups of larvae of *Agelastica alni* (Linnaeus, 1758) and *Phratora vitellinae* (Linnaeus, 1758) were provided with treated or untreated leaves of their actual host plant (*Alnus glutinosa* or *Salix spp.*, respectively) or of the ferns *Athyrium filix-femina*, *Dryopteris austriaca* and *D. filix-mas*. "Treated" leaves were covered with an aqueous extract of host plant or fern leaves. Mortality of young larvae (one-day old) of *A. alni* was significantly higher when provided with treated or untreated fern leaves and treated food plant leaves compared to untreated food plant leaves. Mortality of older larvae (six-day old) was significantly lower than that of young larvae. Young larvae did not feed on fern leaves at all, while older larvae ingested material from treated fern leaves, however at low rates. *Phratora vitellinae* individuals showed abnormal elytra and irregular melanisation patterns when they had taken up fern substances. In both species, the larvae produced less defensive secretion if they had ingested an extract of *Athyrium filix-femina*.

**Key words.** *Agelastica alni*, *Phratora vitellinae*, Filicatae: Aspidiaceae, phytophagy, larval development

### 1. INTRODUCTION

There are 12,500 species of ferns and 230,500 species of seed plants (SPECK 1992). Some 140,000 species of Phytophaga (= Pseudotetramera = Curculionoidea + Cerambycidae + Bruchidae + Chrysomelidae; Lexikon der Biologie, Urania-Tierreich) mean a proportion of one species of pseudotetramerous beetles per 1.7 species of spermatophyte plant, but only 105 species of Phytophaga are known to feed on ferns (BALICK et al. 1978), producing a proportion of 1:119.0. According to HENDRIX (1980) of 726,000 species of insects known at that time, 338,000 are phytophagous (1:0.7), but only 465 feed on ferns (1:26.9). Of the 413 arthropod species reported as found on ferns, 93 attack bracken (*Pteridium aquilinum*) exclusively, making the proportion 1:33.3. Even if these numbers must be corrected for uneven numbers of individuals or/and different availability of biomass, it is clear that there are much less leaf beetles (Chrysomelidae) feeding on ferns than could be expected according to species numbers. There might be regional exceptions (e.g., Veracruz, Mexico: BALICK et al. 1978; Hawaii: SWEZEY 1922). Especially arthropods with chewing mouthparts are remarkably underrepresented on ferns as compared to the large percentage of insects with sucking mouthparts (Thysanoptera, Heteroptera, Homoptera; HENDRIX 1980; HILL 1998; D'ROZARIO & BERA 2003).

Data from bracken (e.g., MARTINS et al. 1995; GILMAN & COOPER-DRIVER 1998) can be generalised only with care since this fern species is (1) certainly the most abundant fern species on earth, and (2) is regarded a pest since it causes illness to cattle when fed. Thus, bracken is definitively the most intensively studied fern species.

In other taxa, for example, Lithinine moths, there might have been a phylogenetic adaptation towards fern feeding (WEINTRAUB et al. 1995). The sawfly *Strongylogaster osmundae* (Takeuchi, 1941) (Hymenoptera: Tenthredinidae) develops obligatorily on the osmund fern, *Osmunda japonica*. In leaf beetles, however, there is no indication that any species is especially adapted to the exploitation of this food resource. For example, WINTERBOURN (1987) found beetles of seven families on bracken, amongst them no leaf beetles, a result also supported by MESIBOV (2001). MUKHOPADHYAY & THAPA (1994) list 19 beetle species, among them seven species of Chrysomelidae as "associated with ferns", however without providing any information as to the intensity of this "association". Since among the seven leaf beetles listed by MUKHOPADHYAY & THAPA there is also *Aspidimorpha sanctaerucis* (Fabricius, 1792), which is definitely restricted to Convulvulaceae (GHATE et al. 2003), one should draw conclusions very cautiously. Possible reasons for the strikingly lower number of phytophagous beetles on ferns than on angiosperms could be that ferns are either toxic, or have repellents, or lack attractants.

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In order to shed some light on these questions for three species of Central European ferns (*Athyrium filix-femina* (Lady Fern), *Dryopteris austriaca* (Wood Fern) and *D. filix-mas* (Male Fern), we conducted feeding experiments with two species of leaf beetles, *Agelastica alni* (Linnaeus, 1758) and *Phratora vitellinae* (Linnaeus, 1758).

## 2. MATERIALS AND METHODS

Fifteen adult individuals of *Agelastica alni* were collected from Common Alder (*Alnus glutinosa*) in the surroundings of Freiburg im Breisgau, Germany. They were kept in plastic containers on a layer of gypsum and fed with fresh alder leaves. Their eggs were collected and the hatched larvae were fed on fresh leaves of their original food plant until they were used in the tests. We conducted two series of tests: (1) with "young" – i.e., freshly hatched – larvae, and (2) with "old" – i.e., six-day old – larvae. In both age groups feeding traces and

mortality were registered on day 3, 8, and 10 after the beginning of the experiment.

Adults of *Phratora vitellinae* were collected from willows and poplars growing in a park area on the outskirts of Freiburg im Breisgau. They were kept and treated as the individuals of *Agelastica alni*. However, since considerably less larvae of *Phratora vitellinae* could be obtained, meaningful statistical analysis was not possible.

Leaves of *Athyrium filix-femina*, *Dryopteris austriaca* and *D. filix-mas* were collected in a forest close to the city of Freiburg im Breisgau ("Sternwald"), always from the same area. Aqueous extracts were made from fresh leaves and from those dried for four weeks at room temperature. The leaves were put into tap water for 30 minutes and then squeezed with broad forceps in order to gain not only water-soluble but also lipophilous substances. The test leaves were soaked with the respective extracts for 30 minutes in order to mask their contact chemical properties.

Table 1. Experimental Settings

Test Number	Kind of Food Offered
1	Normal food plant (f.p.) masked with extract from dried <i>Dryopteris filix-mas</i> (D.f.)
2	Normal food plant (f.p.) masked with extract from dried <i>Dryopteris austriaca</i> (D.a.)
3	Normal food plant (f.p.) masked with extract from dried <i>Athyrium filix-femina</i> (A.f.)
4	Dried <i>D. filix-mas</i> masked with extract from the normal food plant
5	Dried <i>D. austriaca</i> masked with extract from the normal food plant
6	Dried <i>A. filix-femina</i> masked with extract from the normal food plant
7	Dried <i>D. filix-mas</i> , swelled in tap water
8	Dried <i>D. austriaca</i> , swelled in tap water
9	Dried <i>A. filix-femina</i> , swelled in tap water
10	Dried normal food plant, swelled in tap water
11	Normal food plant masked with extract from fresh <i>Dryopteris filix-mas</i>
12	Normal food plant masked with extract from fresh <i>Dryopteris austriaca</i>
13	Normal food plant masked with extract from fresh <i>Athyrium filix-femina</i>
14	Fresh <i>D. filix-mas</i> masked with extract from the normal food plant
15	Fresh <i>D. austriaca</i> masked with extract from the normal food plant
16	Fresh <i>A. filix-femina</i> masked with extract from the normal food plant
17	Fresh <i>D. filix-mas</i> , swelled in tap water
18	Fresh <i>D. austriaca</i> , swelled in tap water
19	Fresh <i>A. filix-femina</i> , swelled in tap water
20	Fresh normal food plant, swelled in tap water
21	Fresh <i>D. filix-mas</i> , untreated
22	Fresh <i>D. austriaca</i> , untreated
23	Fresh <i>A. filix-femina</i> , untreated
24	Fresh normal food plant, untreated

For each of the two test series 24 setups were designed. The details are given in Table 1. The test larvae were kept under the described conditions until either all were dead or the first individuals tried to pupate (which can easily be recognised as *Agelastica alni* beetles pupate in the soil. Thus, larvae ready to pupate attempt at digging holes in the gypsum). *Phratora vitellinae* pupate on the

leaves of their food plant, the initiation of their pupation can be observed directly.

We used the Statistical Package for Social Sciences (SPSS) to calculate a logistic regression. Probabilities of error were calculated by Chi<sup>2</sup> or Fisher's exact test (the latter in cases where n was too low for Chi<sup>2</sup>).

**Table 2.** Mortality rates of *Agelastica alni* under test conditions. ■ : traces of feeding on any test day, □ : no traces of feeding on any test day, ⊙ : traces of feeding on some test days only (and to a considerably less degree). *A.f.*: *Athyrium filix-femina*; *D.a.*: *Dryopteris austriaca*; *D.f.*: *Dryopteris filix-mas*; f.p.: normal food plant (*Alnus glutinosa*)

Test No.	Young larvae						Old larvae					
	Day 3		Day 8		Day 10		Day 3		Day 8		Day 10	
	alive	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive	dead
1	18/1	12/7	10/9	16/10	13/2	12/2	■	■	■	■	■	■
2	17/2	14/5	14/5	17/1	13/4	13/4	■	■	■	■	■	■
3	17/2	6/12	5/13	16/1	14/3	13/4	■	■	■	■	■	■
4	1/16			20/1	0/21	13/4	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>
5	19/2			19/1	0/20	13/4	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>
6	14/4			18/0	0/18	13/4	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>	⊙ <sup>3)</sup>
7	12/7	0/19		18/2	0/18	12/2	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>
8	4/17			19/1		12/2	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>
9	9/8			20/0		12/2	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>	⊙ <sup>4)</sup>
10	19/1	2/17	0/19	22/0	18/4	17/5	■	■	■	■	■	■
11	20/0	19/0	19/0	19/1	17/3	17/3	■	■	■	■	■	■
12	19/1	16/3	15/4	17/3	17/3	17/3	■	■	■	■	■	■
13	20/0	17/2	17/2	19/0	17/2	17/2	■	■	■	■	■	■
14	0/20			11/19	0/30	17/2	⊙ <sup>5)</sup>	⊙ <sup>5)</sup>	⊙ <sup>5)</sup>	⊙ <sup>5)</sup>	⊙ <sup>5)</sup>	⊙ <sup>5)</sup>
15	0/20			17/2	0/19	17/3	⊙ <sup>6)</sup>	⊙ <sup>6)</sup>	⊙ <sup>6)</sup>	⊙ <sup>6)</sup>	⊙ <sup>6)</sup>	⊙ <sup>6)</sup>
16	4/16			17/0	0/17	17/3	⊙ <sup>7)</sup>	⊙ <sup>7)</sup>	⊙ <sup>7)</sup>	⊙ <sup>7)</sup>	⊙ <sup>7)</sup>	⊙ <sup>7)</sup>
17	0/18			12/15		17/2	□	□	□	□	□	□
18	0/20			20/0		17/2	□	□	□	□	□	□
19	0/12			19/1		17/2	□	□	□	□	□	□
20	19/0	17/1	15/3	20/0	20/0	20/0	■	■	■	■	■	■
21	9/14	0/23		13/2		20/0	□	□	□	□	□	□
22	3/15			7/8		20/0	□	□	□	□	□	□
23	12/0			10/15		20/0	□	□	□	□	□	□
24	83/0	80/0	79/0	79/0	74/5	74/5	■	■	■	■	■	■

<sup>1)</sup>: Up to day 7 less feeding traces per day, from day 7 through day 10 no more feeding traces at all.

<sup>2)</sup>: Up to day 3 less feeding traces per day, after day 4 no more live larvae.

<sup>3)</sup>: Up to day 3 very few feeding traces per day, after day 3 no more traces, no live larvae from day 5.

<sup>4)</sup>: Few feeding traces on day 1, thereafter no more traces.

<sup>5)</sup>: Up to day 3 few feeding traces, after day 3 no more live larvae

<sup>6)</sup>: Few feeding traces on day 1, thereafter no more traces, no more live larvae after day 4.

<sup>7)</sup>: Only on days 2 and 3 traces of feeding, no more live larvae after day 4.

### 3. RESULTS

#### 3.1. *Agelastica alni*

The results of the feeding experiments are listed in Table 2. Decreasing total numbers of individuals are due to the fact that some larvae managed to escape from the test boxes. The nearly non-existing mortality in the control sample proves that the experimental conditions were appropriate. In addition to the observed numerical results we found that those larvae who had taken up extract from dried or fresh *Athyrium filix-femina* produced conspicuously less defensive secretion when disturbed than those larvae fed on untreated normal food plant or on normal food plant masked with extract of the other two fern species. Moreover, duration of larval stage 2 lasted for 12 days when the larvae were fed on normal food plant masked with extract from dried *Athyrium*

*filix-femina* as compared to nine days in the control group, or 8.5 to 10 days in the other tests with normal but treated food plant.

The logistic regression revealed a significant influence of food (which plant matter was offered) and the masking of the test food (with the exception of *Dryopteris felix-mas*, the extract of which had no significant influence), whereas the treatment of the test food and of the masking (dried and swelled or fresh) did not lead to significant differences.

In Table 3 we present the results of comparisons between different experimental settings, for young and old larvae. Due to the high mortality in some tests, only those data are presented completely where enough larvae survived up to day 10.

**Table 3.** Comparison of the mortality rates under different test conditions against control (test no. 24). Probabilities of error calculated with Chi<sup>2</sup> or Fisher's Exact

#### 3a: Young larvae

Test No.		Probability of Error
1	(F.p. masked with dried <i>D.f.</i> ) on day 10 > control	p < 0.001
2	(F.p. masked with dried <i>D.a.</i> ) on day 10 > control	p < 0.001
3	(F.p. masked with dried <i>A.f.</i> ) on day 10 > control	p < 0.001
4	(Dried <i>D.f.</i> masked with f.p.) on day 3 > control	p < 0.001
5	(Dried <i>D.a.</i> masked with f.p.) on day 3 > control	p < 0.040
6	(Dried <i>A.f.</i> masked with f.p.) on day 3 > control	p < 0.001
7	(Dried <i>D.f.</i> swelled) on day 3 > control	p < 0.001
8	(Dried <i>D.a.</i> swelled) on day 3 > control	p < 0.001
9	(Dried <i>A.f.</i> swelled) on day 3 > control	p < 0.001
10	(Dried f.p. swelled) on day 10 > control	p < 0.001
11	(F.p. masked with fresh <i>D.f.</i> ) on day 10 > control	n.s.
12	(F.p. masked with fresh <i>D.a.</i> ) on day 10 > control	p < 0.002
13	(F.p. masked with fresh <i>A.f.</i> ) on day 10 > control	p < 0.040
14	(Fresh <i>D.f.</i> masked with f.p.) on day 3 > control	p < 0.001
15	(Fresh <i>D.a.</i> masked with f.p.) on day 3 > control	p < 0.001
16	(Fresh <i>A.f.</i> masked with f.p.) on day 3 > control	p < 0.001
17	(Fresh <i>D.f.</i> swelled) on day 3 > control	p < 0.001
18	(Fresh <i>D.a.</i> swelled) on day 3 > control	p < 0.001
19	(Fresh <i>A.f.</i> swelled) on day 3 > control	p < 0.001
20	(Fresh f.p. swelled)	no difference to control
21	(Fresh <i>D.f.</i> untreated) on day 3 > control	p < 0.001
22	(Fresh <i>D.a.</i> untreated) on day 3 > contro	p < 0.001
23	(Fresh <i>A.f.</i> untreated) on day 3 > control	n.s.

## 3b: Old larvae

Test No.		Probability of Error
1,2,3	1 (f.p. masked with dried <i>D.f.</i> ), 2 (f.p. masked with dried <i>D.a.</i> , 3 (f.p. masked with dried <i>A.f.</i> ) on day 10	no differences to control
4,5,6	4 (Dried <i>D.f.</i> masked with f.p.), 5 (Dried <i>D.a.</i> masked with f.p.), 6 (Dried <i>A.f.</i> masked with f.p.) on day 3	no differences to control
7	(Dried <i>D.f.</i> swelled) on day 3 > control	p < 0.040
8	(Dried <i>D.a.</i> swelled), 9 (Dried <i>A.f.</i> swelled) on day 3	no differences to control
10	(Dried f.p. swelled) on day 10 > control	p < 0.040
11	(F.p. masked with fresh <i>D.f.</i> ), 12 (F.p. masked with fresh <i>D.a.</i> ), 13 (F.p. masked with fresh <i>A.f.</i> ) on day 10:	no differences to control
14	(Fresh <i>D.f.</i> masked with f.p.) on day 3 > control	p < 0.001
15	(Fresh <i>D.a.</i> masked with f.p.) on day 3 > control	p < 0.040
16	(Fresh <i>A.f.</i> masked with f.p.) on day 3 > control	n.s.
17	(Fresh <i>D.f.</i> swelled) on day 3 > control	p < 0.001
18	(Fresh <i>D.a.</i> swelled), 19 (Fresh <i>A.f.</i> swelled) on day 3:	no differences to control
20	(Fresh f.p. swelled) on day 10 > control	n.s.
21	(Fresh <i>D.f.</i> untreated) on day 3 > control	p < 0.030
22	(Fresh <i>D.a.</i> untreated) on day 3 > control	p < 0.001
23	(Fresh <i>A.f.</i> untreated) on day 3 > control	p < 0.001

**Table 4.** Comparison of the mortality rates of young and old larvae under different test conditions. Probabilities of error calculated with Chi<sup>2</sup> or Fisher's Exact

Test No.		Probability of Error
1	(F.p. masked with dried <i>D.f.</i> ) young > old on day 10	p < 0.050
2	(F.p. masked with dried <i>D.a.</i> ) young > old on day 10	n.s.
3	(F.p. masked with dried <i>A.f.</i> ) young > old on day 10	p < 0.010
4	(Dried <i>D.f.</i> masked with f.p.) young > old on day 3	p < 0.010
5	(Dried <i>D.a.</i> masked with f.p.) young > old on day 3	n.s.
6	(Dried <i>A.f.</i> masked with f.p.) young > old on day 3	n.s.
7	(Dried <i>D.f.</i> swelled) young > old on day 3	p < 0.050
8	(Dried <i>D.a.</i> swelled) young > old on day 3	p < 0.001
9	(Dried <i>A.f.</i> swelled) young > old on day 3	p < 0.002
10	(Dried f.p. swelled) young > old on day 10	p < 0.010
11	(F.p. masked with fresh <i>D.f.</i> )	no difference between young and old on day 10
12	(F.p. masked with fresh <i>D.a.</i> )	no difference between young and old on day 10
13	(F.p. masked with fresh <i>A.f.</i> )	no difference between young and old on day 10
14	(Fresh <i>D.f.</i> masked with f.p.) young > old on day 3	p < 0.001
15	(Fresh <i>D.a.</i> masked with f.p.) young > old on day 3	p < 0.001
16	(Fresh <i>A.f.</i> masked with f.p.) young > old on day 3	p < 0.001
17	(Fresh <i>D.f.</i> swelled) young > old on day 3	p < 0.001
18	(Fresh <i>D.a.</i> swelled) young > old on day 3	p < 0.001
19	(Fresh <i>A.f.</i> swelled) young > old on day 3	p < 0.001
20	(Fresh f.p. swelled) young > old on day 10	n.s.
21	(Fresh <i>D.f.</i> untreated) young > old on day 3	p < 0.010
22	(Fresh <i>D.a.</i> untreated) young > old on day 3	n.s.
23	(Fresh <i>A.f.</i> untreated) young > old on day 3	n.s.
24	(Fresh f.p. untreated) young > old on day 10	p < 0.030

**Table 5.** Comparison of mortality rates on the three fern species. Probabilities of error calculated with Chi<sup>2</sup> or Fisher's Exact**5a: Young larvae**

Test No.		Probability of Error
1	(F.p. masked with dried <i>D.f.</i> ) > 2 (F.p. masked with dried <i>D.a.</i> ) on day 10	n.s.
3	(F.p. masked with dried <i>A.f.</i> ) > 1 (F.p. masked with dried <i>D.f.</i> ) on day 10	n.s.
3	(F.p. masked with dried <i>A.f.</i> ) > 2 (F.p. masked with dried <i>D.a.</i> ) on day 10	n.s.
4	(Dried <i>D.f.</i> masked with f.p.) > 5 (Dried <i>D.a.</i> masked with f.p.) on day 3	p < 0.001
4	(Dried <i>D.f.</i> masked with f.p.) > 6 (Dried <i>A.f.</i> masked with f.p.) on day 3	p < 0.001
6	(Dried <i>A.f.</i> masked with f.p.) > 5 (Dried <i>D.a.</i> masked with f.p.) on day 3	n.s.
8	(Dried <i>D.a.</i> swelled) > 7 (Dried <i>D.f.</i> swelled) on day 3	p < 0.010
9	(Dried <i>A.f.</i> swelled) > 7 (Dried <i>D.f.</i> swelled) on day 3	p > 0.050
8	(Dried <i>D.a.</i> swelled) > 9 (Dried <i>A.f.</i> swelled) on day 3	p < 0.050
22	(Fresh <i>D.a.</i> untreated) > 21 (Fresh <i>D.f.</i> untreated) on day 3	n.s.
21	(Fresh <i>D.f.</i> untreated) > 23 (Fresh <i>A.f.</i> untreated) on day 3	p < 0.001
22	(Fresh <i>D.a.</i> untreated) > 23 (Fresh <i>A.f.</i> untreated) on day 3	p < 0.001

**5b: Old larvae**

Test No.		Probability of Error
1	(F.p. masked with dried <i>D.f.</i> ) > 2 (F.p. masked with dried <i>D.a.</i> ) on day 10	n.s.
3	(F.p. masked with dried <i>A.f.</i> ) > 1 (F.p. masked with dried <i>D.f.</i> ) on day 10	n.s.
3	(F.p. masked with dried <i>A.f.</i> ) > 2 (F.p. masked with dried <i>D.a.</i> ) on day 10	p < 0.050
5	(Dried <i>D.a.</i> masked with f.p.) > 4 (Dried <i>D.f.</i> masked with f.p.) on day 3	p < 0.050
4	(Dried <i>D.f.</i> masked with f.p.) > 6 (Dried <i>A.f.</i> masked with f.p.) on day 3	n.s.
5	(Dried <i>D.a.</i> masked with f.p.) > 6 (Dried <i>A.f.</i> masked with f.p.) on day 3	n.s.
14	(Fresh <i>D.f.</i> masked with f.p.) > 15 (Fresh <i>D.a.</i> masked with f.p.) on day 3	p < 0.020
14	(Fresh <i>D.f.</i> masked with f.p.) > 16 (Fresh <i>A.f.</i> masked with f.p.) on day 3	p < 0.010
15	(Fresh <i>D.a.</i> masked with f.p.) > 16 (Fresh <i>A.f.</i> masked with f.p.) on day 3	n.s.
17	(Fresh <i>D.f.</i> swelled) > 18 (Fresh <i>D.a.</i> swelled) on day 3	p < 0.001
17	(Fresh <i>D.f.</i> swelled) > 19 (Fresh <i>A.f.</i> swelled) on day 3	p < 0.001
19	(Fresh <i>A.f.</i> swelled) > 18 (Fresh <i>D.a.</i> swelled) on day 3	n.s.
22	(Fresh <i>D.a.</i> untreated) > 21 (Fresh <i>D.f.</i> untreated) on day 3	p < 0.050
22	(Fresh <i>D.a.</i> untreated) > 23 (Fresh <i>A.f.</i> untreated) on day 3	n.s.
23	(Fresh <i>A.f.</i> untreated) > 21 (Fresh <i>D.f.</i> untreated) on day 3	n.s.

Comparisons of mortality of young and old larvae are given in Table 4. In some tests, it did not differ significantly, as long as only numbers of surviving larvae are counted. It was, however, evident that young larvae died more frequently from starvation because they refused to feed on certain food at all. Old larvae took up leaf mat-

ter in several tests but died afterwards, either due to intoxication or from starvation after refusal of further feeding.

There was no significant difference in mortality rates when tests with dried against fresh fern leaves were

compared. Mortality was significantly higher on dried as compared to fresh food plant, and on normal food plant masked with extract from dried as compared to fresh *Dryopteris filix-mas* and *Athyrium filix-femina*.

In order to reveal possible differences between the three fern species, the respective settings have been compared. The results are given in Table 5. All comparisons not listed in Table 5 did not yield significant differences between the fern species.

### 3.2. *Phratora vitellinae*

As with *Agelastica alni*, those larvae who had taken up extract from dried or fresh *Athyrium filix-femina* produced conspicuously less defensive secretion when disturbed than those larvae fed on untreated normal food plant or on normal food plant masked with extract of the other two fern species. This applied especially to the first larval instar.

In addition, there occurred severe distortions of moulting, elytron formation and melanisation when the larvae were fed on willow leaves masked with extract from *Dryopteris filix-mas* (Fig. 1).



Fig. 1. Adult *Phratora vitellinae* showing massive wing deformation after having fed on willow leaves masked with extract of *Dryopteris-filix-mas*.

## 4. DISCUSSION

### 4.1. *Agelastica alni*

Young and old larvae fed on alder leaves even when they were covered with fern extract. This result is at odds with the generally held assumption that the foraging behaviour of herbivore insects is guided by olfactory and gustatory stimuli of the plants (e.g., DETHIER 1970; FRAENKEL 1959; HARBOURNE 1982; HSIAO 1969; SCHOONHOVEN 1972, 1973). JERMY (1966) stated that oligophagy is characterised rather by the avoidance of

repellent substances than by search for attractants. This is rather unlikely since in our experiments the larvae avoided untreated fern leaves and accepted masked alder leaves as well as masked fern leaves which they should have despised according to JERMY'S statement. Obviously, feeding was triggered by the presence of feeding stimulants from alder leaves.

Mortality after feeding on alder leaves masked with extract from dry fern leaves demonstrates that at least toxic substances – and most probably also possible repellents and attractants – were in the aqueous extract at effective concentrations.

Of course, the mortality of young larvae fed on fresh or soaked fern leaves (see Table 4) could be caused by starvation. This definitively does not apply to the old larvae feeding on fern leaves masked with extract from leaves of the normal food plant (test nos. 4, 5, 6), who fed on leaves of all three fern species. The dramatic mortality (all test larvae were dead after five days) must be due to incompatible or toxic compounds.

The significantly higher mortality of old larvae in the control situation (test no. 24) is most probably due to the higher damage of the leaves they fed on as a consequence of their chewing. The partly damaged leaves dried faster than those the young larvae fed on.

Masking alder leaves with extract from fresh fern leaves (tests no. 11, 12, 13) did not lead to significant differences of mortality rates in young and old larvae. Thus, the extract cannot contain effective repellents and the toxic components of the extract do not affect young and old larvae to different extents. However, since in tests no. 12 & 13 with young larvae mortality was significantly higher than in the control, some substance in the aqueous extract of *Dryopteris austriaca* and *Athyrium filix-femina* must have had an effect on the young larvae.

The most interesting results are those of tests 14, 15, and 16 (fresh fern leaves masked with extract from normal food plant): young larvae did not feed on these leaves at all, old larvae ingested leaf material, on day 3 mortality was significantly higher on *Dryopteris filix-mas*, while after seven days no larva had survived. This is clear evidence that (1) some stimulus from the normal food plant caused the larvae to feed on fern leaves, and (2) that the ingestion of fern material causes death either by starvation or through toxic components. This explanation is supported by the results of tests no. 21, 22, and 23, where untreated fern leaves – with one exception – have not been ingested at all. In test no. 21, young larvae took up material of *D.f.*-leaves, however at decreasing amounts per day until on day 4 no larva had survived. This strange behaviour was most probably caused by two factors, in contrast to the other two fern species: the glandular

trichomes are not exposed on the surface in *D.f.* and the larvae could, therefore, not that easily detect repellent or toxic substances from these glands, and young larvae possess probably less chemoreceptors than old ones. As SWAIN (1977) reports, the larvae of phytophagous insect are equipped with a lower number of chemoreceptors than adults. It seems plausible that the number of chemoreceptors increases during larval development. Since eggs are normally deposited on the larval food plant, and since first instar larvae are much less mobile than older ones, we can expect that young larvae are less choosy with respect to food plants than older ones.

#### 4.2. *Phratora vitellinae*

Only a few larvae were available, so the results of the feeding experiments do not allow for statistical analyses. The obvious result that adult beetles from those larvae who ingested components of *Dryopteris filix-mas* showed elytral deformations points in the same direction as the results of *A. alni*: *D. filix-mas* either contains more or more toxic substances than the other two ferns tested, or these substances reach higher quantities in the aqueous extract.

The small sample size does not permit sound generalisations from these results. However, there is hardly any other explanation for the distorted moulting than that the aqueous extract of *D. filix-mas* contained effective amounts of phytoecdysone. This is surprising since the study of SELVARAJ et al. (2005) revealed that steroids were only in the chloroform and ethanol extracts and in the hexane fraction, but not in the aqueous fraction of *Pteridium aquilinum*.

#### 4.3. Conclusions

The larvae in our experiments fed on ferns only if these leaves were covered with an aqueous extract of the original food plant, *Alnus glutinosa* or *Salix* spp., respectively (with the one mentioned exception of young *Agelastica alni* larvae ingesting – and dying – from untreated leaves of *Dryopteris filix-mas*). Whenever larvae took up fern leaf material, they suffered conspicuously, by dying, by less production of defensive secretion, or by wing deformation during pupal development. This means that (1) phytoecdysones contained in the fern leaves are not effective as repellents but as toxins. They can, therefore, indeed act as defence against phytophagous insects, however not unless these insects have ingested leaf material. Thus, both points of view apply – that phytoecdysteroids do not deter insects from feeding on a fern plant (JONES & FIRN 1978) and that phytoecdysones form an effective protection against attacks of phytophagous insects (e.g., RUSSELL 1977). (2) Feeding activity on a certain plant is most probably triggered by the presence of feeding stimulants rather

than prevented by the presence of repellents. (3) The differences found between the effectiveness of *Dryopteris filix-mas* and the other two fern species provides a further caveat against uncritical generalisations of results from studies with bracken (*Pteridium aquilinum*), which dominate the literature on fern-insect interaction.

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