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THE ROLE OF SESQUITERPENE LACTONES AND PHENOLICS IN THE CHEMICAL DEFENCE OF THE CHICORY PLANT

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Key Word Index—*Cichorium intybus*; Compositae; chicory; 8-deoxylactucin; lactupicrin; cichoriin; *Schistocerca gregaria*; antifeedants; plant defence.

Abstract—Amounts of the sesquiterpene lactones and the major phenolics were determined in the chicory plant at different times during the growing season. The levels of the sesquiterpene lactones (lactucin, lactupicrin and 8-deoxylactucin) and the hydroxycoumarin cichoriin were found to be highest in the most actively growing regions of the plant. In two-choice and no-choice feeding experiments with borosilicate discs, 8-deoxylactucin, lactupicrin and cichoriin significantly reduced feeding of *Schistocerca gregaria* at levels comparable to those present in the plant. Cichoriin was still significantly antifeedant at 0.006% dry wt, while aesculin, aesculetin and the caffeic acid ester, chicoric acid were inactive. We conclude that the three sesquiterpene lactones secreted in the latex provide a significant barrier to herbivory in chicory, although the phenolics and notably cichoriin also protect the plant from insect feeding.

INTRODUCTION

One of the tenets of the modern theory of plant–insect coevolution, first proposed by Ehrlich and Raven [1] and later elaborated by other authors [cf. 2], is that the majority of plants are likely to be defended from herbivory by their secondary chemistry. Most of the evidence supporting this theory is circumstantial. Direct experimental data are difficult to obtain, since it is necessary to establish which chemicals, of many that may be present, are important feeding deterrents and to determine the degree of protection these chemicals may provide against different insects. In order to obtain further support for the idea that secondary compounds have an ecological role in plants, we set out to examine the secondary chemistry of a chosen plant species, chicory, to see whether we could establish which of its chemicals were likely to be important in defence.

The chicory plant, *Cichorium intybus* L., was chosen because it has lush green leaves and few obvious physical defences; yet it is noticeably free from herbivore attack, both in natural populations and when cultivated. It is also known to be distasteful to some insects; Jermy [3] reported the leaves as being completely unpalatable to three coleoptera and one lepidopteran tested. Furthermore, chicory is well known as a source of two sesquiterpene lactones, lactucin and lactupicrin [4], and several other sesquiterpene lactones have been implicated as feeding deterrents or allelopathic agents in members of the Compositae [5, 6].

Additionally, the chicory plant, particularly the fresh root, is extremely bitter-tasting to humans. Although chicory is cultivated, the effect of cultivation has had little effect on its secondary chemistry. Indeed, chicory root, when dried and roasted, is used as a coffee substitute because it imparts a bitter flavour. The other major constituents of chicory are phenolic in nature [4], but their value to the plant in protecting it from herbivory has never been investigated.

We therefore decided to isolate and identify the major secondary metabolites of chicory and then to investigate their properties as insect antifeedants. The levels of some of the main components were investigated throughout the growing season in the different plant parts. The results obtained are discussed here in terms of their role in the chemical defence of the chicory plant.

RESULTS

The secondary chemistry of chicory

Identification of constituents. The first stage in the investigation was the identification of the major secondary constituents in root and leaf of chicory. A total of 15 compounds were recognized in the ethanolic extracts (Table 1). Most of these compounds have been identified as chicory constituents in earlier work [4].

According to the literature [7], the two major sesquiterpene lactones of the root are the guaianolides lactucin **1** and its *p*-hydroxyphenylacetic acid ester lactupicrin **2**. Our examination of both root and leaves revealed that the two major lactones, in fact, are lactupicrin and 8-deoxylactucin **3**, while lactucin itself is always present in relatively minor amount. 8-Deoxylactucin was earlier isolated from a related species, *Lactuca serriola* [8] so that its presence in chicory is expectable. It was identified by IR and MS measurements. The occurrence of **3** in chicory has been confirmed independently by Pyrek [9], who identified it by direct comparison with an authentic specimen.

From HPLC analyses of methanolic extracts of root and leaf (see Experimental), no other sesquiterpene lactones were detected. The three compounds are clearly

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Table 1. Secondary constituents of the chicory plant

Sesquiterpene lactones	Caffeic acid derivatives
1 Lactucin	**13** Caffeic acid
2 Lactupicrin	**14** Chicoric acid
3 8-Deoxylactucin	**15** Chlorogenic acid
Flavonoids	Unidentified phenolics *
4 Luteolin 7-glucuronide	**16** blue → light blue
5 Quercetin 3-galactoside	**17** blue → light blue
6 Quercetin 3-glucuronide	**18** blue → light blue
7 Kaempferol 3-glucoside	**19** pink → yellow
8 Kaempferol 3-glucuronide	**20** blue fluorescence
9 Isorhamnetin 3-glucuronide	**21** dark → yellow
Coumarins	**22** dark → yellow
10 Aesculetin	**23** yellow fluorescence
11 Cichoriin	**24** green fluorescence
12 Aesculin	

*Colours in UV light without and with ammonia recorded; positions on a 2D-chromatogram are shown in Fig. 1.

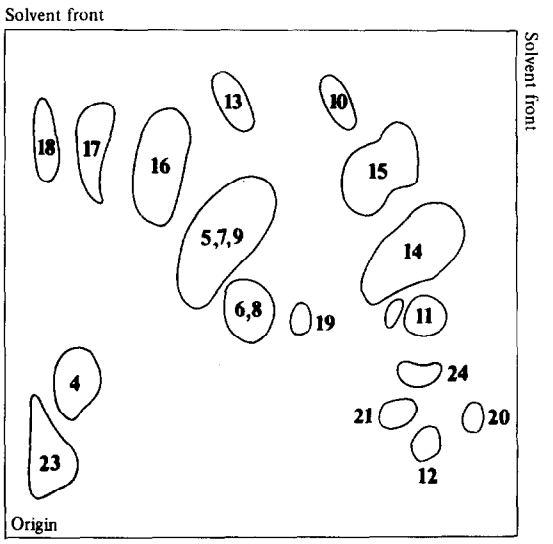

Fig. 1. Two-dimensional chromatogram of phenolics of chicory. Solvents BAW in 1st direction (bottom to top) and 15% HOAc (left to right). For key to phenolics separated, see Table 1.

located in the free state in the latex of root and leaf. Chloroform leaf washings do not contain any lactone and attempts to release glycosidically-bound lactone from root or leaf also failed (cf. the occurrence of sesquiterpene lactone glycosides in *Taraxacum officinale* roots [10]). All three lactones **1–3** were tested for their bitterness and they were equally bitter to human tasting.

Earlier work on the flavonoids of chicory leaves [11] has revealed the presence of apigenin 7-arabinoside, luteolin 7-glucoside, quercetin 3-rhamnoside and quercetin 3-galactoside. As described elsewhere [12], we have only been able to confirm the presence of the latter compound. Our detailed studies have shown that the 3-glucuronides of kaempferol, quercetin and isorhamnetin are present, together with kaempferol 3-glucoside. Small amounts of other flavonoids are also present. The three components **21**, **22** and **23** on the 2D-chromatograms of the phenolic constituents (Fig. 1) may be flavonoid in nature. On the basis of colour reactions and relative mobilities, **21** and **22** are possibly flavone *C*-glycosides while **23** may be a flavonol 5-glycoside. Since none of the flavonoids identified had pronounced taste properties, their antifeedant activities were not investigated.

Six simpler phenolics were readily recognized as major constituents from 2D-chromatograms (Fig. 1). These are aesculetin (6,7-dihydroxycoumarin), cichoriin (the 7-glucoside), aesculin (the 6-glucoside), caffeic acid, chlorogenic acid and chicoric acid (dicaffeoyltartaric acid). These are all known components of chicory and of several related members of the Cichorieae [4]. Six other unidentified phenolics were detected (compounds **16–20**, **24** in Table 1) but these were not further investigated because of their low concentrations.

Two triterpenes, α- and β-lactucerol, and the sugar alcohol, mannitol, have been reported in minor amount in chicory but these constituents were not re-examined during the present work. Alkaloids and polyacetylenes are reported to be absent from the plant and we could find no evidence for their presence in any quantity.

Distribution within the plant. Effective defence chemicals would be expected to occur widely within a plant and to be present at all stages of plant growth. Therefore the occurrences of the various secondary constituents were determined at monthly intervals during the growing season in root and leaf (Table 2). As will be seen, the most consistent constituents are the three sesquiterpene lactones which are present in root and leaf throughout this period. They are also present in quantity in dormant roots during the winter. Of the phenolics, chicoric acid is the only compound so regularly present. Nevertheless, cichoriin and aesculin, while absent from the roots, are usually present at every stage of growth in the leaves.

There are other noticeable differences in chemistry between root and leaf. The roots also lack the flavonoids, which are prominent in the leaf; again while chicoric acid occurs in the roots, chlorogenic acid does not. The occasional occurrence of the three unidentified root components **16–18** in the foliage is of interest. During the early part of the growing season they are absent, but **16** appears in June, while all three appear in the basal leaves in July and in mid- and top-stem leaves in September. Their presence in the leaves appears to be associated with physiological changes, e.g. the onset of senescence, so that a role in defence would appear to be most unlikely.

The phenolic profile of the petals differs markedly from that of both root and leaf. There are fewer components than in the leaf. Additionally, there are anthocyanins, two malonated delphinidin 3,5-diglucosides [13], which are not present elsewhere in the plant. The leaf, if it produces anthocyanin, synthesizes cyanidin 3-(6-malonylglucoside) [14], but this pigment only occurs in a few cultivars and was not encountered during our investigations of wild species. The absence of sesquiterpene lactones from the petals suggests that they are not defended from herbivory. Since chicory petals have a very short life (24 hr), such defence is probably not necessary.

The concentrations of secondary constituents. The maintenance of significant levels of a defence chemical is crucial to its providing protection from herbivory. The amounts of the three sesquiterpene lactones **1–3** were measured by HPLC in chicory roots and leaves during the growing season. Since some losses were inevitable during extraction and purification, the figures obtained (Table 3) are

Table 2. The occurrence of secondary components in different parts of the chicory plant

Organ	Sampling date	Presence/absence of 1	2	3	4	5–9	10	11	12	13	14	15	16	17	18	19	20	21–22	23	24
Roots	March	+	+	+	−	−	−	−	−	+	+	−	+	−	−	−	−	−	−	+
	April	+	+	+	−	−	tr	−	−	+	+	−	+	+	+	−	−	−	−	+
	May 2	+	+	+	−	−	−	−	−	+	+	−	+	+	−	−	−	−	−	+
	May 28	+	+	+	−	−	−	−	−	−	+	−	+	+	+	−	−	−	−	+
	July	+	+	+	−	−	−	−	−	+	+	−	+	+	+	−	−	−	−	+
	August	+	+	+	−	−	−	−	−	+	+	−	+	+	+	−	−	−	−	+
	Sept.	+	+	+	−	−	−	−	−	+	+	−	+	+	−	−	−	−	−	−
Base leaves	March	+	+	+	−	+	+	+	+	+	+	+	−	−	−	+	+	+	−	+
	April	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	−	−
	May 2	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	−	−
	May 28	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	−	+	−	+
	June	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	−	−
	July	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	−
	August	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
	Sept.	+	+	+	+	+	−	+	−	+	+	+	+	−	−	+	+	−	−	−
Mid-stem leaves	May 28	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−
	June	+	+	+	+	−	+	+	−	+	+	+	+	−	−	+	+	+	−	−
	July	+	+	+	+	+	+	+	+	−	+	+	−	−	−	+	+	+	−	−
	August	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	−
	Sept.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Top-stem leaves	June	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	−
	July	+	+	+	−	+	−	+	+	+	+	+	+	+	−	+	+	+	−	−
	August	+	+	+	+	+	tr	+	+	tr	+	+	tr	−	−	+	+	+	−	−
	Sept.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Petals	August	−	−	−	+	+	+	+	−	+	−	−	−	−	−	−	+	−	−	−

For key to compounds, see Table 1.

Table 3. % presence as dry weight of individual and total sesquiterpene lactone content

		8-deoxylactucin	lactupicrin	lactucin	Total
Roots	March	0.11	0.07	0.04	0.22
	April	0.19	0.16	0.03	0.38
	May 2	0.24	0.22	0.07	0.52
	May 28	0.29	0.39	0.14	0.81
	July	0.04	0.04	0.03	0.11
	August	0.05	0.14	0.05	0.25
	Sept.	0.11	0.36	0.20	0.68
Base leaves	March	0.04	0.03	—	0.06
	April	0.06	0.04	—	0.10
	May 2	0.10	0.08	0.03	0.21
	May 28	0.15	0.17	0.02	0.34
	June	0.15	0.24	0.01	0.40
	July	0.07	0.08	0.02	0.17
	August	0.08	0.14	0.03	0.25
	Sept.	0.09	0.12	0.02	0.22
Mid-stem leaves	May 28	0.12	0.15	0.02	0.27
	June	0.11	0.18	0.03	0.32
	July	0.11	0.12	—	0.22
	August	0.07	0.08	—	0.15
	Sept.	0.06	0.18	0.07	0.32
Top-stem leaves	June	0.09	0.14	0.01	0.24
	July	0.15	0.26	0.04	0.45
	August	0.08	0.23	0.07	0.38
	Sept.	0.12	0.12	0.04	0.28

minimal values. As will be seen, the total sesquiterpene lactone levels vary from 0.11 to 0.81% dry wt in the root and from 0.06 to 0.45% dry wt in the leaves. Average values are 0.42% in the root and 0.26% in the leaf. The proportions of the individual lactones do not vary much during the season, the lactucin levels being consistently lower than the levels of the other two constituents.

As can be seen from Table 3, the levels of lactone in the root in the Spring are higher than in the foliage, but as flowering begins, the reverse is true and the concentration in the root decreases at the expense of the concentration in the upper foliage. The situation is reversed again in August and September, when the plant reaches senescence, with higher levels in the root than in the leaf. While the concentrations of sesquiterpene lactones do vary according to the stage of growth of the plant, they only change within certain limits. Significant concentrations are maintained in both root and leaf throughout the life of the plant.

Measurements on two of the main phenolics of chicory, cichoriin and chicoric acid, during the growing season again show that consistent levels are maintained in the plant from March to August (Table 4). There are very high levels of cichoriin in the flowers, but within the leaf, the amounts do not differ much from base to top stem. These values for cichoriin, 0.04% in leaf and 1.75% in flower, can be compared with the earlier data of Gorecki and Mrugasiewicz [15], who reported 2.4% total coumarin in short leaves, 1.22% in long leaves and 4.05% in flowers. These authors values are several times higher, because they were also measuring aesculin and aesculetin content as well.

Table 4. % presence as dry weight of cichoriin and chicoric acid

		Cichoriin	Chicoric acid
Roots	March	—	0.06
	April	—	0.34
	May 2	—	0.06
	May 28	—	0.03
	July	—	0.12
	August	—	0.20
	September	—	0.07
Base leaves	March	0.08	—
	April	0.04	0.14
	May 2	0.04	0.82
	May 28	0.06	0.43
	June	0.04	0.66
	July	0.02	0.41
	August	0.05	0.54
	September	0.02	0.41
Mid-stem leaves	May 28	0.03	0.50
	June	0.03	0.78
	July	0.04	0.16
	August	0.03	0.38
	September	0.04	0.60
Top-stem leaves	June	0.04	0.52
	July	0.05	0.35
	August	0.07	0.23
	September	0.04	0.42
Petals	August	1.75	—

By contrast with cichoriin, chicoric acid occurs in major amounts in both root and leaf. Average values in the root are 0.13% dry wt and in the leaf 0.46% dry wt. Our leaf data (Table 4) are close to those of Scarpati and Oriente [16] who reported yields of 0.5–1.1% dry wt in chicory leaf and of Wöldecke and Herrmann [17] who obtained 0.15% dry wt from endive leaf.

Locust antifeedant studies

Locusts were chosen for antifeedant studies, because of their polyphagy, the ease of breeding in captivity and short generation time and the fact that many inhibitory tests have already been conducted on these insects [18]. *Schistocerca gregaria* was selected in preference to *Locusta migratoria*, since in feeding studies with different plant leaves, *S. gregaria* has been shown to eat 75% of Compositae species provided, whereas *Locusta* would only eat 20% and then with reluctance [19].

The results of antifeedant activity of four of the major secondary substances in chicory are given in Table 5, together with an indication of their significance from paired *t*-test analysis. In the two-choice experiments, ten 2–3 day old adults were separately provided with a borosilicate disc treated with 5% dry wt glucose (control) and a disc treated with 5% glucose and added chemical at a given concentration (treatment). When at least half of one disc had been consumed (2–12 hr), the experiment was stopped and the remaining areas of disc determined. Feeding deterrency (as %) can then be calculated as 2 × (50 − % treatment eaten).

Both the sesquiterpene lactones tested exert a similar effect on feeding: there is a fairly sharp threshold at a concentration of 0.2% dry wt and this levels off at a higher concentration to maintain a deterrency of about 70%. Equivalent levels of lactone occur in the chicory plant (see Table 3) so that it is a reasonable assumption that these compounds are capable *in vivo* of repelling locust feeding. Indeed, in other feeding experiments with discs of powdered leaf material, *Schistocerca gregaria* adults were deterred from feeding, particularly when top stem leaf samples were compared with basal leaf samples which have less lactone present. On a molar basis, lactupicrin is significantly deterrent at about half the concentration of 8-deoxylactucin (Table 4), so that the presence of the *p*-hydroxyphenylacetic acid group appears to enhance deterrency. Insufficient material was available to test the third chicory lactone, lactucin.

The results for chicoric acid reveal that *Schistocerca* is only significantly deterred from eating at a concentration of 1.1% dry wt. Since this level is unlikely to be present in the plant (Table 4), it would appear that this compound is not a deterrent. By contrast, cichoriin is a deterrent at levels as low as 0.02% dry wt. The low point at this value may be accounted for by the fact that the locusts in this experiment ate relatively small amounts of the total and that they may not have tried to eat the treatment disc at all. Even so, at the higher concentration of 0.17%, cichoriin is still a very active feeding deterrent.

The possibility that the pink fluorescence in the ultraviolet of cichoriin might have been detected in the treated discs by the locusts and thus have effected the experiment was tested by repeating an experiment in the dark.

Table 5. Feeding responses of *Schistocerca gregaria* to different concentrations of sesquiterpene lactones and phenolics in two-choice experiments

	Concn. % dry wt*	% treatment eaten		Concn. % dry wt*	% treatment eaten
Lactupicrin	0.11	45.6	Cichoriin	0.001	48.9
	0.21	22.5†		0.006	33.8‡
	0.35	16.7†		0.011	33.9‡
	0.49	12.9†		0.021	17.1†
	0.68	16.5†		0.042	30.4‡
	1.00	11.0†		0.084	33.1†
				0.168	3.7†
8-Deoxylactucin	0.12	45.6			
	0.17	49.8	Chicoric acid	0.30	40.8
	0.22	53.7		0.48	43.4
	0.24	24.5†		0.83	27.7
	0.36	14.1†		1.06	7.4†
	0.49	19.4†			
			Coumarin	0.1	51.9
			Aesculetin	0.1	42.3
			Aesculin	0.1	49.9

*Compounds applied at these concentrations to borosilicate discs impregnated with glucose at 5% dry wt concn.
†Indicates that the data are significantly different at $P = 0.01$.
‡Indicates that the control and treatment data are significantly different at $P = 0.1$.

However, at a concentration of 0.084% dry wt cichoriin, similar deterrency was observed as in the light.

The unexpected repellency of cichoriin in locust feeding experiments suggested that other related coumarins should be tested. However, neither the related 6-glucoside, aesculin, or the aglycone aesculetin had any effect on feeding at 0.1% concentration. Coumarin, which has a bitter taste, was also ineffective at the same concentration.

In order to investigate further the inhibitory effects of cichoriin, a single choice experiment was set up. This was done in order to see whether in the two-choice situation, locusts were avoiding the cichoriin-treated disc only because they preferred the untreated disc. Results, however, showed that the discs treated with 0.17% dry wt cichoriin were not eaten over the course of 22 hr, while the consumption of untreated discs increased at about the rate of 6 cm^2 per hr over the same time scale. Hunger did not increase the rate of feeding on treated discs, so that cichoriin at this level caused more than a ten-fold decrease in feeding activity. Although cichoriin is present in chicory leaf at a lower level than the sesquiterpene lactones (cf. Tables 3 and 4), it is an active deterrent at such low levels that it could well be a significant antifeedant in the plant.

DISCUSSION

Three secondary components found in the leaves and two in the roots of *Cichorium intybus* have been shown to be feeding deterrents towards the polyphagous acridid, *Schistocerca gregaria*. Although present throughout the plant, the highest levels of these substances are to be found in those parts of the plant which are of greatest importance at any particular time, the most actively growing tissues. It has been shown that a chemical difference between the base and top stem leaves at the time of flowering renders the top stem far less palatable, and this is likely to be due to the additive effect of several feeding deterrents. Such a mechanism involving a combination of deterrents is more beneficial to the plant than a single deterrent, since compounds may lose their effectiveness through habituation; *S. gregaria* has been shown to respond to the very powerful antifeedant, the triterpenoid azadirachtin in this way [20].

The actual effectiveness of these three deterrents may be modified in the plant due to two further factors. First, the level of phagostimulants present may counteract the effects of deterrents and these again may vary in the plant according to the part examined and the time of harvest. Second, the location of the sesquiterpene lactones in the latex may significantly increase their repellency. Some kinds of insect feeders such as lepidopterous leaf miners may avoid feeding altogether on laticiferous plants because the latex prevents them from burrowing within the leaf. Indeed, such leaf miners are known to specifically avoid feeding on the members of the Cichorieae for this very reason [Keward, L., private communication]. The combination of a sticky latex with bitter-tasting lactones could be highly effective in arresting the feeding of a range of potential insect predators.

Previous studies of the effects of sesquiterpene lactones on insects have rarely indicated such significant antifeedant activity as found here for 8-deoxylactucin and lactupicrin. The main effect of lactones generally on lepidopterous larvae seems to be in reducing larval growth [21], although antifeedant effects on *Spodoptera exempta* and *Epilachna varivestris* has been recorded for certain germacranolides [22]. In the case of beetle feeding, antifeedant properties have been noted for alantolactone in *Tribolium confusum* [6] and for desacetoxymatricarin in the Colorado beetle [23]. Some *Vernonia* sesquiterpene lactones have been shown to be antifeedant to *Locusta* [Bernays, E. A., unpublished results] but our studies appear to provide the first evidence of antifeedant activity at low concentrations against *Schistocerca*.

The most unexpected finding of this investigation is that cichoriin is a feeding repellent. The activity is

specifically linked to structure, since closely related molecules, e.g. the isomeric 6-glucoside, are inactive. Simple hydroxycoumarins as a class, when tested have rarely shown harmful effects on insects. Coumarin itself in relatively high concentration inhibits feeding of some insect species. For example, Adams and Bernays [24] tested coumarin at a level 0.4% dry wt against fifth instar *Locusta migratoria* in a no-choice situation and found feeding activity diminished by 50%. Tests of various hydroxycoumarins or their derivatives on insects have, however, normally proved negative [23]. Furanocoumarins, by contrast, are well known to be toxic, particularly to Lepidoptera [25].

These present findings explain in part the recognized pest resistance of the chicory plant. Only very few insects are known to be able to survive on this species. The major chemical defence would seem to lie in the presence of cichoriin in the leaf and of lactupicrin, lactucin and 8-deoxylactucin in both leaf and root. All four compounds occur in the plant in sufficient concentration to be effective. They have all been detected as major constituents in a variety of cultivated and wild material of *C. intybus* and thus appear to be universally present in all plant populations. Some of the other secondary constituents, namely chicoric acid and aesculin, do not appear to contribute directly to deterrence. They may conceivably reinforce the effectiveness of the four main deterrents, but this has yet to be established experimentally. Other minor components of chicory remain to be identified and tested as potential antifeedants.

EXPERIMENTAL

Plant material. A range of wild and cultivated forms of *Cichorium intybus* L. were grown from seed. Roots of the cvs. Magdeburg and Bataille were used for large scale extraction of the secondary constituents. Seed of a wild accession supplied by the Botanic Garden of the University of Marburg were sown to provide plants for the quantitative analysis.

Sesquiterpene lactones. These were isolated in quantity from the root, using the method of Holzer and Zinke [7]. Final separation was achieved by column chromatography on silica gel eluted with EtOAc, and by semi-preparative HPLC on a Partisil Column eluted with $CHCl_3$–MeOH (19:1) and monitored at 254 nm. TLC was carried out on silica gel plates in (1) $CHCl_3$–C_6H_6–MeOH (9:9:2), (2) $CHCl_3$–MeOH (24:1) and (3) EtOAc. Lactupicrin had mp 133–137°; λ_{max}^{EtOH} 254 nm; MS: molec. ion-*p*-hydroxyphenylacetic acid at *m/z* 258 and fragment at 152 (ester group); R_f 0.27 (1), 0.38 (2) and 0.74 (3). 8-Deoxylactucin had λ_{max}^{EtOH} 255.5 nm, MS molec. ion at *m/z* 260, R_f 0.38 (1), 0.43 (2) and 0.62 (3). Lactucin had λ_{max}^{EtOH} 255 nm, MS molec. ion at *m/z* 276, R_f 0.23 (1), 0.30 (2) and 0.48 (3). MS fragmentation patterns and IR measurements agreed with lit. data [7–9].

Quantitative analyses were conducted on weighed amounts of fresh root and dried leaf. The tissues were blended in excess MeOH, extracted at 100° for 1 hr and the MeOH eventually removed *in vacuo*. An equal vol. of EtOH was added to the aq. residue and the soln left at 6° overnight to ppt the fructan. The supernatant, after washing with petrol, was made up to a known volume.

The lactones were then extracted into $CHCl_3$ (×2) and the $CHCl_3$ extracts taken to dryness and redissolved in $CHCl_3$–MeOH (19:1). The lactones were then analysed by HPLC on a Partisil column in the same solvent, the concns being determined by peak area measurement, based on ε values of Pyrek [8].

Phenolics. Methods of isolating and identifying the flavonoid components of chicory are described elsewhere [12]. The other phenolics were isolated following a similar extraction and purification process as indicated above. The final aq. residue was used for phenolic analysis. For survey purposes, 2D-PC was carried out in *n*-BuOH–HOAc–H_2O (4:1:5, top) and 15% HOAc. The phenolics were isolated on a preparative scale by repeated banding on Whatman No. 3 paper in the standard solvent systems [26]. They were characterized by spectral and R_f measurements and identified by direct comparison with authentic markers.

For quantitative analysis, weighed samples were extracted and purified as for sesquiterpene lactones. Aliquots of the final aq. residue were separated by 2D-chromatography on Whatman No. 3 paper in BAW and 15% HOAc. The appropriate spots were cut out, eluted with 80% MeOH, made up to a standard volume and the concentration determined spectrophotometrically.

Locust experiments. Schistocerca gregaria were reared in metal cages, lit by a 40 W bulb with a 16 hr day, 8 hr night cycle at a constant temp. of 26°. All experiments were conducted on 2–3 day old adults, which had been fed during the previous 24 hr with fresh barley or grass. Males and females were used in equal numbers and no insect was used more than once. All feeding experiments were conducted at 26° in clear plastic boxes 27 × 15 × 10 cm, one locust per box. Locusts were given the choice of a control glass fibre disc (4.25 cm diam.) treated with 0.4 ml of a 0.025 M sucrose soln in 80% EtOH and a disc further treated with a known concentration of chemical in 80% EtOH (phenolics) or in $CHCl_3$–MeOH (19:1) (sesquiterpene lactones), and then dried. Each experiment ran until at least half of one disc had been eaten (2–12 hr). The remaining areas of the two discs were measured with an Li-Car electronic area measuring device and the area later calculated, the area of an intact disc being 14.2 cm^2. The amount of treatment disc eaten as a percentage of the total amount eaten = treatment/control + treatment (× 100). If no deterrence occurred, this value would be 50%. A mean of ten replicates was calculated and a paired *t*-test carried out to check true differences between control and treatment. Detailed results for each insect experiment are available [27].

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