Eur. J. Entomol. **94**: 183–198, 1997
ISSN 1210–5759

Hormonally mediated insect-plant relationships: Arthropod populations associated with ecdysteroid-containing plant, *Leuzea carthamoides* (Asteraceae)

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Insecta, ecdysteroids, phytoecdysones, hormonal effects, secondary plant chemicals, host-plant selection, population dynamics, *Leuzea carthamoides*, Asteraceae

Abstract. An extensive zoocenotic study of arthropod populations was performed on a Siberian plant *Leuzea carthamoides*, which was recently introduced to Central Europe as a medicinal crop. Because it contains a very high concentration of insect hormones (ecdysteroids) (300–1,000 ppm of 20-hydroxyecdysone equivalents in the leaves), the plant was thought to be resistant to attack by non-adapted arthropod herbivores (effective concentrations 25–100 ppm in insect diet). Two consecutive seasons of analysis revealed that, in spite of the high ecdysteroid content, the crops of *L. carthamoides* contained a well-established and consolidated arthropod fauna. 126 species of arthropods were observed on these plants during 1993 and 1994. Of this number, 74 were feeding on the leaves, and 33 of these could complete their adult development on the plants without apparent difficulties. There were also 52 parasitoid and predatory arthropod species of secondary importance.

The results revealed the following four observations that are relevant to the possible resistance of *L. carthamoides* to phytophagous arthropods: (a) the plant is by no means universally resistant to all phytophagous arthropods; (b) the most abundant and, perhaps, most resistant herbivores that can complete their life cycle on the plant are from groups in which ecdysteroid action is little known or unknown, e.g., spider mites, collembolans, thysanopterans, psocopterans or Exopterygota with sucking mouthparts; (c) Endopterygote insect pests, which are the most sensitive to ecdysteroid-containing diet, were represented by only a few species and relatively low abundances; and, (d) all arthropods colonizing this plant belonged to polyphagous or oligophagous species with a relatively wide range of different host plants. Composition of arthropod fauna within plantations of *L. carthamoides* was similar to that observed in studies of the sugar beet (*Beta vulgaris*), which is also an introduced plant. Physiological conditions associated with possible resistance of insects to dietary ecdysteroids are discussed in terms of the following two points of virtual insensitivity: (a) use of molecules other than the plant-contained ecdysteroid as the endogenous hormone, and (b) elimination of the exogenous dietary ecdysteroid by excretion during feeding period.

INTRODUCTION

Before chemical structures of insect hormones became known, Sláma & Williams (1965a) discovered compounds with insect juvenile hormone (JH) activity in American paper products. The origin of the compounds was traced down to the pulp trees, namely to the wood of the Canadian balsam fir (*Abies balsamea*). Sláma & Williams assumed that these hormonally active materials in plants "may be effective in the selective destruction of at least certain of these pests, as well as any other insects which show the same hormonal sensitivities". They also suggested that, due to acquired synthesis of insect hormones, certain plants could possibly "evolve an incredibly sophisticated self-defense against insect predation" (Sláma & Williams, 1965b).

The possibility of hormonally based insect-plant interactions was augmented when phytochemists discovered bioanalogues of another insect hormone, the moulting hormone-ecdysone (Nakanishi et al., 1966; Takemoto et al., 1967). Certain species of plants accumulated these phytoecdysones in quantities exceeding one million-fold the concentrations that were usually encountered in the insect body (Jizba et al., 1967). Subsequently, it was found that experimental induction of growth disorders and lethality can be induced in insects by ingestion of certain phytoecdysones. This led to the assumption that plants evolved the ability to produce insect hormones for their own protection against herbivores by natural selection. This assumption was widely accepted (see reviews by Sláma, 1969, 1979; Williams, 1967, 1970; Rees, 1971). At present, the protective role against herbivores is still the most widely-used explanation for the presence of ecdysteroid insect hormones (phytoecdysones) in plants (Sláma, 1993b; Adler & Grebenok, 1995).

Despite these discoveries, the validity of the protective role of ecdysteroids mentioned above has been questioned often, mainly by ecologists who argued that there was no direct experimental evidence to support the theory (see Beck & Reese, 1976). Two model systems were used in support of the indicated hormonal theory. One was associated with the virtual absence of insect herbivores on the fern *Polypodium vulgare* (Polypodiaceae), which contains substantial amounts of the hormone 20-hydroxyecdysone (up to 3%, Jizba et al., 1967). The second model was associated with an absence of pyrrhocorid bugs (Hemiptera: Pyrrhocoridae) in the forests containing balsam fir. The fir produces juvabione with exclusive JH action for the members of the family Pyrrhocoridae.

Direct experimental proof of an ecologically significant, hormonally based, insect-plant interaction has not yet been presented (cf. Sláma, 1993b). Coevolution of insects and plants has produced many cases in which a particular adapted insect species can consume a food plant which is toxic to most other insect species (see Rosenthal & Berenbaum, 1991 for review and references). We are convinced that this evolutionary feature also applies to structurally diversified secondary plant substances, such as plant-borne bioanalogues of arthropod hormones. Long ago, Williams (1956) reasoned that it is unlikely that insects would develop resistance against their own hormones. Today, the widespread occurrence of all kinds of hormonal bioanalogues in plants is known. It is reasonable to assume that insects, with their enormous diversity of species and developmental forms, may have evolved protective mechanisms against destructive plant chemicals by natural selection, including insect hormone mimics.

For several decades, studies of hormonally based insect-plant interactions did not progress because of the lack of a reliable experimental model system (Sláma, 1993b). Usually, entomocenotic studies of plants that contain insect hormones are complicated by the scattered distribution of the plants among other vegetation. A new experimental opportunity for these studies was presented recently by the cultivation of an Asiatic medicinal plant *Leuzea carthamoides* (Willd.) DC. (Asteraceae) (syn. *Rhaponticum carthamoides*; *Stemmacantha carthamoides*) as a crop in Central Europe. The roots, leaves and seeds of this plant contain large amounts of 20-hydroxyecdysone, together with several other biologically active ecdysteroids (Baltaev & Abubakirov, 1987; Baltaev, 1994). Concentrations of ecdysteroids in *L. carthamoides* greatly exceed the maximum tolerable dietary dosages for nonadapted insects (see Discussion). Due to a high ecdysteroid content and low insect infestation, the plant was thought to be highly resistant to insects (Sláma, 1993a,b). Thus, *L. carthamoides* appears to be well-suited for testing the previous discussed hypothesis regarding the protective role of ecdysteroids in plants. The relatively recent introduction of this Siberian plant to Central Europe has been associated with further advantages represented by possible absence of evolutionarily highly adapted and resistant, endemic Asiatic species. In the remainder of this manuscript, we report the results of an extensive zoocenotic investigation of arthropod fauna associated with plantations of *L. carthamoides* in the Czech Republic.

MATERIAL AND METHODS

All investigations took place in on the fields of *L. carthamoides* that were located in agricultural property managed by the company ADAVO, Velký Osek, Czech Republic. The principal experimental area consisted of 2- to 4-year-old plantations that composed an area of approximately 6 ha. Arthropod samples were collected during the growing season of the plant, which occurred from late April until October. Faunistic samples were collected every two weeks during the active growing season and once a month at the beginning and end of the season. The following two principal sampling methods were used: (a) sweeping for a period of 1 h, and (b) sampling of all arthropod fauna on 200 randomly selected leaves.

Usually, relative abundance of the most common species or groups of arthropods was evaluated directly in the field. Unidentified material was preserved in 80% ethanol for later analysis. Special attention was focused on alterations in species diversity, dominance of individual species at different periods and specific interactions among food chains.

Encountered variations in the population density of individual arthropod species were compared with seasonal changes in the development of the plant. These variations were also evaluated with respect to abiotic factors (Walter's climate diagrams) and the possible significance of individual species in the agroecosystem complexity.

In order to introduce certain quantitative measures into this study, the abundance of the selected dominant species was related to a more or less constant area determined by the surface of 200 randomly selected, medium-size *L. carthamoides* leaves. In the case of extremely mobile insects (leafhoppers, for instance), values corresponding to 200 standard leaves were obtained indirectly. In this case, the abundance for certain mobile species was calculated as the number of swept specimens multiplied by a coefficient, which was determined by the ratio of mean abundance of less mobile species (thysanopteran *Thrips nigropilosus*, for instance) on model leaves to the mean number of specimens of this species that were obtained by sweeping.

Determination of ecdysteroid content in leaves of *L. carthamoides* was accopmlished by means of a standardized biological assay, using ligatured larvae of the greater wax moth, *Galleria mellonella* (Pyralidae) as an assay animal. These values are expressed in 20-hydroxyecdysone equivalents, which correspond to biological activity of the sum of all biologically active ecdysteroids present in the leaves (see Sláma, 1995; Sláma et al., 1974). Essential ecological and physiological information regarding growth and development of *L. carthamoides* is available in a recent review by Golovko et al. (1996).

RESULTS

Phenology of *L. carthamoides*

In its natural biotope in Siberia, *L. carthamoides* grows in alpine or subalpine meadows with continental climatic conditions (Golovko et al., 1996). Correspondingly, the phenology of 3- to 5-year old plants in Velký Osek exhibit also a relatively short, typically continental, annual vegetation period. Formation of the first spring leaf rosettes, regenerating from winter roots, occurs in early April. Emergence is followed by extremely rapid growth and development of the shoot, with flower primordia and button formation occurring

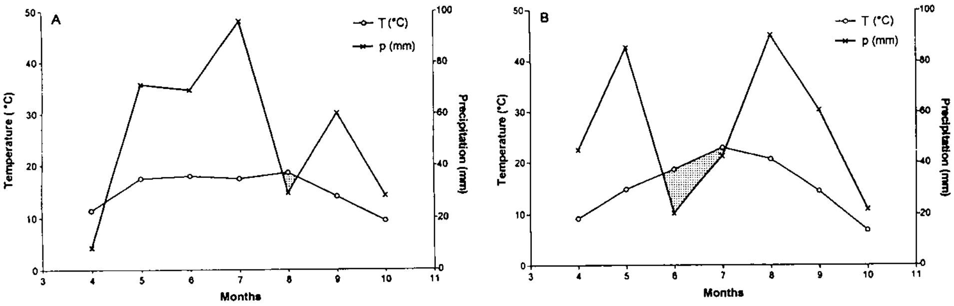

Fig. 1. Temperature and rainfall characteristics (Walter's climate diagrams) for investigated fields of *L. carthamoides* in Velký Osek, during 1993 (A) and 1994 (B).

before the end of May. The flowering period begins in May and terminates in early June. At the time of inflorescence the plant attains its maximum height, which is nearly 1 m. Seeds mature during June–July and, at this time, the flower stems degenerate along with withering and degeneration of the primary spring leaves. This period is followed by growth and development of secondary leaf rosettes (between July and August). In September, these leaves die and the well-developed storage roots overwinter. Vegetation period is terminated during October–November, depending on seasonal conditions (see Bajer, 1991).

Walter's climate diagrams for 1993 and 1994 in Velký Osek (Fig. 1) show maximum average monthly temperatures of 19°C in August of 1993, and 21°C in July of 1994. Two peaks in precipitation occurred in each of the two years; these were 50 mm in July and 30 mm in September of 1993; 43 mm in May and 46 mm in August of 1994. The hatched areas in Walter's climate diagrams provided in Fig. 1 indicate areas characterized by water deficiency.

Ecdysteroid content in the leaves

Table 1 provides levels of ecdysteroid content in both primary (first spring rosettes) and secondary (new rosettes developing in summer) leaves of *L. carthamoides*. The values are the sum of all biologically active ecdysteroids and are related to 20-hydroxyecdysone standard. The highest content of ecdysteroid (17.5 mg/g of dry leaves) was documented in the primary young leaves that developed from the buds of overwintering root stocks. During growth of the primary leaf rosette, the ecdysteroid content decreased sharply to almost undetectable levels in degenerating, yellow leaves present during flowering and ripening of the seeds. The content of ecdysteroid in young, secondary leaves was also initially high and diminished with advancement of the growing.

The data in Table 1 show that a small ecdysteroid content (at least 3 to 5 mg of 20-hydroxyecdysone in 1 g of dry leaves; equivalents are 300 to 500 ppm in the diet of fresh leaves) is present during most part of the growing season. This minimum average concentration of ecdysteroid in *L. carthamoides* leaves is above the threshold that is normally considered tolerable by non-adapted insect species (25–100 ppm, see Discussion).

Table 1. Ecdysteroid content in the primary and secondary leaf rosettes of 4-year-old *L. carthamoides* during 1992 growing season. Values are indicated in mg of 20-hydroxyecdysone equivalents per g of dry leaves.

Month/day	Primary leaves (mg/g)	Secondary leaves (mg/g)
April, 7	17.5	
April, 28	8.27	
May, 26	2.5	5.8
June, 16	1.1	4.8
July, 14	0	3.7
August, 18		3.7
September, 8		3.0
October, 10		1.9

Arthropod herbivores on *L. carthamoides*

In this study, an arthropod species that tolerated the presence of 3 mg/g (dry leaves) or 300 ppm of ecdysteroid when feeding on *L. carthamoides*, was considered resistant against the dietary supply of the hormone. The aim of the study was to identify these resistant arthropod species, in particular those that complete their life-cycle on *L. carthamoides* without apparent developmental defects. Principal defects caused by ecdysteroids are heterochronic deviations of development and are generally known as prothetelies. They are indicated by precocious induction of moults, premature formation of epidermal patterns and profound pathophysiological defects called hyperecdysonism. Limited attention was directed at predatory arthropods, which could eventually ingest exogenous ecdysteroid along with contaminated prey.

Leaves of *L. carthamoides* are regularly eaten by molluscs, especially the snail *Helix pomatia* L. (Gastropoda). Snails appear to feed on this plant in mixed vegetation in several different localities. The developmental fate of snails was not investigated in this study, however, observations of feeding preferences suggest that snails may be resistant to ecdysteroid.

Among Arthropoda, two groups showed a very high degree of resistance to secondary substances produced by *L. carthamoides*; these are spider mites and sminthurids. These animals developed and reproduced normally, even on early green leaves that contained ecdysteroid concentrations above 1,500 ppm (fresh leaves).

During the two consecutive seasons of this study, the data reveal that, despite high ecdysteroid content, plantations of *L. carthamoides* contained a well-established and diverse arthropod fauna. Table 2 provides a list of 126 species of arthropods that were documented in association with the plant during the 1993 and 1994 growing seasons. Of the total number, 74 species were feeding on the leaves. Moreover, 33 species of the latter group could complete their life cycles on the plant without apparent difficulties (these are indicated by # in Table 2).

A more detailed inspection of the data in Table 2 reveals that the most abundant insects on *L. carthamoides* belong to Collembola, Thysanoptera and Psocoptera, groups that have received little attention in current research on ecdysteroids. In addition, a well established fauna of the exopterygote sucking insects such as aphids, leafhoppers and true bugs were present. In contrast, endopterygote insects, which are generally more sensitive to ecdysteroids, were far less abundant. This group was predominantly represented by common polyphagous lepidopteran species, the noctuid *Autographa gamma*, and the oligophagous pyralid *Sitochroa verticalis*. This clearly suggests that *L. carthamoides* is not a good diet for endopterygote insects or, in other words, that the plant is resistant to a number of nonadapted, ecdysteroid intolerant species. Nonetheless, it is evident that a number of other arthropod groups or species have adapted and are able to tolerate high ecdysteroid content. In April, at the beginning of the growing season when ecdysteroid content in fresh leaves is above 2,000 ppm, overwintering adults of some phytophagous species begin infestation of the plant. These include the spider mite *Tetranychus urticae*, the collembolan *Deuterosminthurus repandus*, and the thysanopteran *Thrips nigropilosus*, and may be the species that are most resistant and, perhaps, best adapted to very high ecdysteroid content.

TABLE 2. List of identified arthropod species collected in fields of *L. carthamoides* in Velký Osek, during 1993 and 1994 growing seasons.

No.	Name	Food	Number of individuals				Freq.
			1993	1993	1994	1994	
			200 leaves	sweeping	200 leaves	sweeping	
	COLLEMBOLA						
1	*Bourletiella hortensis* (Fitch)	P #		2			*
2	*Deuterosminthurus repandus* (Agren)	P F	15,137	18,250	22,423	46,667	***
3	*Entomobrya marginata* (Tullberg)	P		2			*
4	*Entomobrya multifasciata* (Tullberg)	P F	9	77	40	42	**
5	*Fasciasminthurus quinquefasciatus* (Krausbauer)	P		1			*
6	*Lepidocyrtus ruber* Schott	P		1			*
7	*Sminthurus multipunctatus* (Schaffer)	P F #	9	26	39	131	**
8	*Sminthurus nigromaculatus* Tullberg	P F	3	16	*	*	*
9	*Xynylla grisea* Axelson	P F		12	*	*	*
	ORTHOPTERA						
10	*Chorthippus albomarginatus* (De Geer)	P	2	**	*	**	**
	DERMAPTERA						
11	*Forficula auricularia* L.	P C		**	*	7	***
	PSOCOPTERA						
12	*Lachesilla bernardi* Badonnel	P F #	104	404	2	125	***
13	*Lachesilla tanaidana* Roesler	P F #	13	21		29	**
	HETEROPTERA						
14	*Adelphocoris lineolatus* (Goeze)	P #		11	*	29	*
15	*Anthocoris nemorum* (L.)	C	4				*
16	*Dolycoris baccarum* (L.)	P	1	6	1	8	*
17	*Eurydema oleracea* L.	P #	1	6			*
18	*Halticus apterus* (L.)	P #			**	43	**
19	*Lygus rugulipennis* Poppius	P #	71	138	18	53	**
20	*Nabis ferus* (L.)	C			*		*
21	*Nabis rugosus* (L.)	C		11			*
22	*Nysius senecionis* (Schilling)	P #	4	12	*	17	**
23	*Orius majusculus* (Reuter)	C	*	*	*	*	*
24	*Orius minutus* (L.)	C	2	16	4	10	*

25	*Orius niger* Wolff	C	*	*	*	*	*
26	*Palomena prasina* L.	P	1	1			*
27	*Rhyparochromus pini* (L.)	P #		7	*	101	*
28	*Tingis cardui* (L.)	P	3				*
	AUCHENORRHYNCHA – Cicadelloidea						
29	*Aceratagallia ribauti* (Ossiannilsson)	P	3	3			*
30	*Aphrodes makarovi* Zachvatkin	P #	67	62	50	492	**
31	*Chlorita paolii* (Ossiannilsson)	P	3	4			*
32	*Empoasca decipiens* Paoli	P #	1,832	482	788	2,478	***
33	*Empoasca solani* (Curtis)	P #	**	96	58	181	**
34	*Eupteryx atropunctata* (Goeze)	P #	463	307	68	61	**
35	*Euscelis incisus* (Kirschbaum)	P	2	2			*
36	*Graphocraerus ventralis* (Fallén)	P	1	1			*
37	*Macrosteles laevis* (Ribaut)	P #	211	38	91	12	**
38	*Neoaliturus guttulatus* Kirschbaum	P #	18	77	**	83	**
39	*Ophiola decumana* Kontkanen	P	1	1			*
40	*Philaenus spumarius* (L.)	P #	15	5	87	27	**
41	*Psammotettix* sp.	P	2	1			*
42	*Streptanus aemulans* (Kirschbaum)	P	9		*	16	*
	STERNORRHYNCHA – Aphidoidea						
43	*Aphis fabae* Scopoli	P #	22	*	5	*	*
44	*Aulacorthum solani* Kaltenbach	P # G					**
45	*Coloradoa rufomaculata* Wilson	P			661	**	**
46	*Macrosiphum euphorbiae* Thomas	P # G					**
47	*Metopolophium dirhodum* (Walker)	P	2	*	*	*	*
48	*Myzus persicae* Sulzer	P # G					***
49	*Neomyzus circumflexus* (Buckton)	P # G					*
	THYSANOPTERA						
50	*Aeolothrips fasciatus* (L.)	C	*		*		*
51	*Aeolothrips intermedius* Bagnall	C	5	27	**	15	**
52	*Hercinothrips femoralis* Reuter	P G #					***
53	*Thrips flavus* Schrank	P	*	10	*	*	*
54	*Thrips major* Uzel	P	**	43	*	*	*
55	*Thrips nigropilosus* Uzel	P #	1,462	274	12,203	413	***
56	*Thrips tabaci* Lindeman	P #	**	48	3,599	127	***
	NEUROPTERA						
57	*Chrysopa commata* Kis et Ujhelyi	C		13		10	*
58	*Chrysopa phyllochroma* Wesmael	C		1		2	*
59	*Chrysoperla carnea* Stephens	C	3	55	19	25	**
	LEPIDOPTERA						
60	*Agrochloa litura* (L.)	P #		1		1	*
61	*Argyrotaenia ljungiana* (Thunberg)	P	1				*
62	*Autographa gamma* (L.)	P #	13	17	24	46	**
63	*Cnephasia asseclana* (Denis et Schiffermüller)	P	1				*
64	*Cnephasia pumicana* (Zeller)	P	1				*
65	*Cnephasia stephensiana* (Doubleday)	P	1				*

66	*Olethreutes lacunana* (Denis et Schiffermüller)	P	1				*
67	*Ostrinia nubilalis* (Hübner)	P #			7	9	***
68	*Sitochroa verticalis* (L.)	P #	64	61	184	14	***
	COLEOPTERA						
69	*Adalia bipunctata* (L.)	C	3		2		*
70	*Cantharis fusca* L.	C	1		1	4	*
71	*Cantharis lateralis* L.	C				6	*
72	*Cantharis livida* L.	C				8	*
73	*Cassida vibex* L.	P #	2	6		6	*
74	*Cassida rubiginosa* (L.)	P #		1			*
75	*Cetonia aurata* (L.)	P			2		*
76	*Ceutorrhynchus floralis* (Paykull)	P		6		4	*
77	*Coccinella quinquepunctata* L.	C				1	*
78	*Coccinella septempunctata* L.	C	2	15	2	14	***
79	*Malachius aeneus* (L.)	C	2		1		*
80	*Malachius bipustulatus* (L.)	C				4	*
81	*Notoxus monoceros* (L.)	P			1	7	*
82	*Oedemera lurida* (Marsham)	P				2	*
83	*Oedemera podagraria* (L.)	P				1	*
84	*Oedemera virescens* (L.)	P		**		41	**
85	*Phyllobius vespertinus* (F.)	P		1		6	*
86	*Phylloperta horticola* (L.)	P				5	*
87	*Propylea quatuordecimpunctata* (L.)	C	1		1	1	**
88	*Psylobora vigintiduopunctata* (L.)	P F			1	4	*
	HYMENOPTERA						
89	*Apis mellifera* L.	P	***	***	***	***	***
90	*Bombus hortorum* (L.)	P	*	*	**	*	*
91	Braconidae spp.	C		***		***	***
92	Chalcididae spp.	C		***		***	***
93	Figitidae spp.	C		**		**	**
94	Ichneumonidae spp.	C		***		***	***
95	Mymaridae spp.	C		**		**	**
96	Proctotrupidae spp.	C		***		***	***
97	Pteromalidae spp.	C		**		**	**
98	Trichogrammatidae spp.	C		**		**	**
	DIPTERA						
99	Agromyzidae spp.	P #	14	**	**	**	**
100	Chloropidae spp.	P	**	**		**	**
101	*Mycodiplosis* sp.	F	82				**
102	*Therodiplosis* sp.	C	*	*		*	*
	ARANEA						
103	*Araeoncus humilis* (Blackwall)	C	*	*		2	*
104	*Dictyna arundinacea* (L.)	C	*	*		2	*
105	*Erigone atra* (Balckwall)	C	*	*		2	*
106	*Erigone dentipalpis* (Wider)	C	*	*		3	*
107	*Enoplognatha ovata* (Clerck)	C	*	**	*	12	**
108	*Evarcha acuata* (Clerck)	C				1	*
109	*Gongylidiellum murcidum* Simon	C				1	*

110 *Lepthyphantes flavipes* (Blackwall)	C				1	*
111 *Mangora acalypha* (Walckenauer)	C	*	***	**	27	**
112 *Meioneta rurestris* (C. L. Koch)	C	*	*		4	*
113 *Microlinyphia pusilla* (Sundevall)	C				1	*
114 *Oedothorax apicatus* (Blackwall)	C	*	*		2	*
115 *Philodromus aureolus* (Clerck)	C				1	*
116 *Pisaura mirabilis* (Clerck)	C				1	*
117 *Porrhoma microphthalmum* (O.P.-Cambridge)	C	*	*		3	*
118 *Silometopus elegans* (O.P.-Cambridge)	C	*	*		2	*
119 *Synageles venator* (Lucas)	C				1	*
120 *Tetragnatha pinicola* L. Koch	C				1	*
121 *Theridion bimaculatum* (L.)	C	*	**	*		
122 *Theridion impressum* L. Koch	C	*	**	*	8	*
123 *Xysticus ulmi* (Hahn)	C				3	*
ACARINA						
124 *Amblyseius* spp.	C	336	**	2,576	**	***
125 *Anystis* spp.	C	**	**	55	**	**
126 *Tetranychus urticae* Koch	P G #	84	*	76	*	***

ABBREVIATIONS: P – phytophagous species; C – carnivorous species; F – fungivorous species; # – species that could complete their development on *L. carthamoides*; G – species was found on *L. carthamoides* in greenhouse conditions. Frequency: *** – common species (> 100 specimens collected during two seasons); ** – occasional species (> 10 and < 100 specimens collected during two seasons); * – rare species (< 10 specimens collected during two seasons).

With respect to feeding mechanisms, phytophagous species encountered on *L. carthamoides* encompassed insects with all types of mouthparts. These included the following: sucking insects that ingest sap from phloem (aphids) and xylem (some leafhoppers), insects that feed on leaf parenchyma (thysanopterans and some leafhoppers), mining insects (larvae of agromyzid flies or small pyralid caterpillars), larvae that consume whole leaves (*Autographa gamma* and *Sitochroa verticalis*), and bees that collect pollen and nectar. In regards to nutritional requirements, most species in Table 2 are polyphages, and some are serious agricultural pests.

Predators on agrocenosis of *L. carthamoides*

Herbivore communities are often characterized by special predator and parasitoid fauna that forms essential links in food-chains. Table 2 identifies 52 entomophagous and 9 mycophagous species (e.g., common cecidomyiid fly, *Mycodiplosis* sp., feeding on *Erysiphe* moulds on old leaves) that were found within plantations of *L. carthamoides* in Velký Osek. The list includes many polyphagous predators; exceptions are an oligophagous dipteran *Therodiplosis*, which feeds exclusively on the spider mites, the predatory mite *Amblyseius*, which regulates the population density of its prey – thysanopterans *Thrips nigropilosus* and *T. tabaci*, and parasites with generally small host-specificity. Changes in the population densities of these predators were clearly correlated with those of their prey.

Arachnids are represented in Table 2 by 21 opportunistic, polyphagous species that commonly reside on agricultural crops or inhabit margins of forests. The spider fauna in this study did not indicate any special dependence on the study crop.

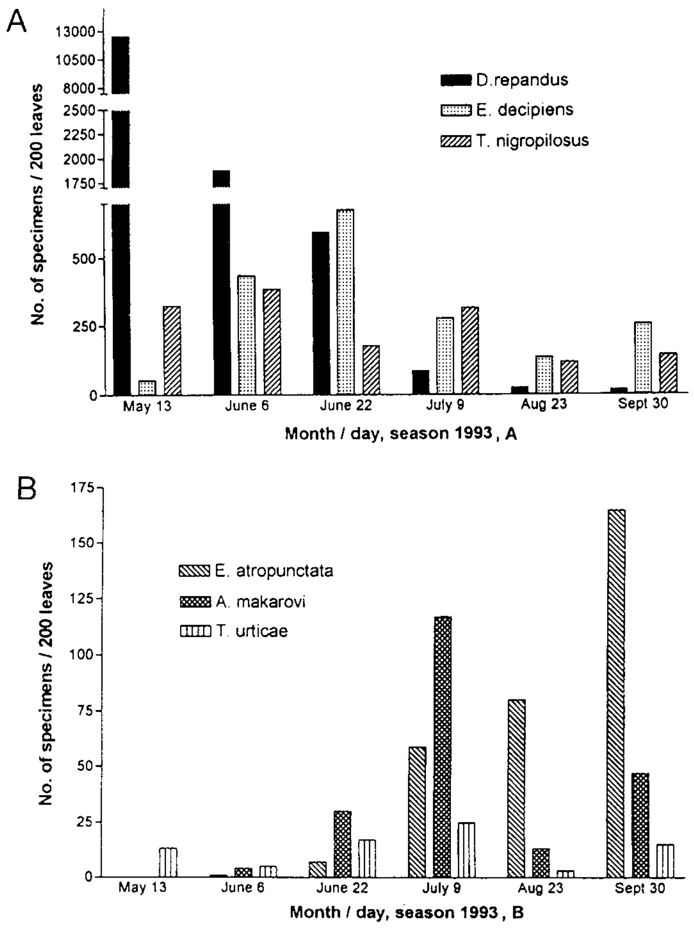

Fig. 2. Selected population dynamic characteristics of the most abundant phytophagous species collected on *L. carthamoides* during 1993. A – *Deuterosminthurus repandus*; *Empoasca decipiens*; *Thrips nigropilosus*. B – *Eupteryx atropunctata*; *Aphrodes makarovi*; *Tetranychus urticae*.

Seasonal changes in the abundance of dominant species

In order to obtain detailed information regarding possible correlations between population density and the concentration of ecdysteroids within the plant, changes in the relative abundance of dominant arthropod species were determined throughout the growing season. Fig. 2A illustrates the relative abundance of the collembolan *D. repandus*, the homopteran *E. decipiens*, and the thysanopteran *T. nigropilosus* during the 1993 growing season. The abundance of collembolans was greatest during spring, when the concentration of ecdysteroids in the leaves attained its maximum. Similar positive correlations between maximum abundance and the highest ecdysteroid content are evident in Fig. 2A for the thysanopteran species *T. nigropilosus*. Evidently, these two species can tolerate maximum

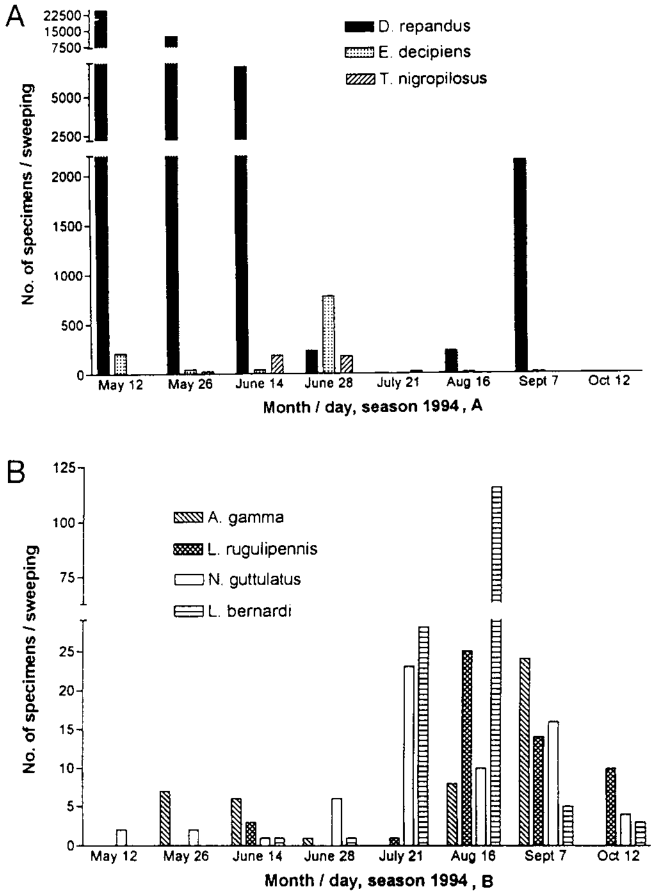

Fig. 3. Selected population dynamic characteristics of the most abundant phytophagous species collected on *L. carthamoides* during 1994. A – *Deuterosminthurus repandus*; *Empoasca decipiens*; *Thrips nigropilosus*. B – *Autographa gamma*; *Lygus rugulipennis*; *Neoaliturus guttulatus*; *Lachesilla bernardi*.

ecdysteroid concentrations and are, theoretically, the most resistant of the species observed in this study. The third species shown in Fig. 2A, the cicadelloid *E. decipiens*, is most abundant in June and July, when the concentration of ecdysteroid has dropped to low levels. This type of reciprocal correlation also applies to two other cicadelloid species and the spider mite, *T. urticae*, which are included in Fig. 2B. This pattern, however, is commonly observed in relative abundance studies, and may be a natural developmental cycle that is normal for herbivores of agricultural crops.

Comparison of the data shown in Figs 2A and 3A reveals that the three dominant species, i.e., *D. repandus*, *E. decipiens* and *T. nigropilosus*, show similar variations in relative abundance on *L. carthamoides* during the course of two consecutive seasons. Population density of *D. repandus* was higher in 1994 than in 1993, whereas population densities of *E. decipiens* and *T. nigropilosus* decreased in 1994. It is possible that, because there were

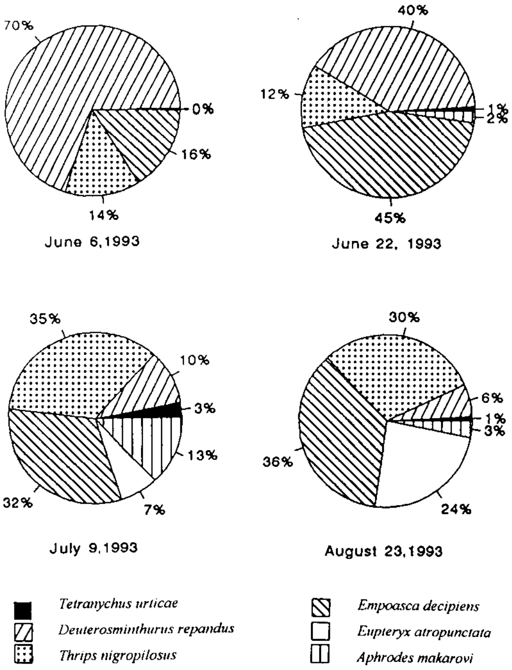

Fig. 4. Comparison of changes in relative abundance of the most common phytophagous species on *L. carthamoides* during 1993.

heavier rains in 1994, the 1994 samples did not adequately assess the population densities of the latter two species. Less abundant species collected in 1994 (see Fig. 3B) also peaked in abundance during the summer months of July, August and September, when ecdysteroid content was relatively low. Some of these species, like *A. gamma*, are bivoltine and exhibit a clearly bi-phasic pattern. Changes in the relative abundance of the six dominant species investigated in 1993 are summarized in Fig. 4.

DISCUSSION

According to previous preliminary observations (Chernish & Lukhtanov, 1981; Lafont et al., 1991; Sláma, 1993a,b; Vokáč & Harmatha, 1993), European plantations of *L. carthamoides* were almost lacking phytophagous insects. Opletal et al. (1993) cited only one curculionid beetle, *Phyllobius maculicornis*, whose larvae fed on roots and whose adults fed on the leaves of *L. carthamoides*. These findings were in agreement with general assumptions that plants with a high ecdysteroid content are protected from extensive attack by arthropod herbivores (Sláma, 1969, 1979; Williams, 1967, 1970; Lafont et al., 1991). However, the results of this study demonstrate that, in spite of rather high ecdysteroid content, *L. carthamoides* are inhabited by a complex entomocenosis composed of local Central European species.

The documentation of 74 herbivorous and 52 carnivorous species of arthropods on this plant has the following implications that are relevant to the possible resistance of *L. carthamoides* against phytophagous insects: (a) the high content of 20-hydroxyecdysone does not ensure universal resistance to all phytophagous arthropods; (b) the most resistant herbivores that can complete their life cycle on this plant are found within arthropod groups in which the action of ecdysteroid hormones is still unknown (e.g., Acari, Apterygota or Exopterygota with sucking mouthparts); (c) the economically important and most physiologically ecdysteroid-sensitive group, endopterygote insects, were represented by only a few noctuid species at relatively low abundances; and (d) most European arthropods that colonize this Siberian plant are polyphagous species that inhabit a diverse range of host plants.

The precise physiological conditions associated with insect resistance to exogenous ecdysteroids are unknown. Unlike JH analogues, which can enter the body through the integument, ecdysteroids only enter the body orally along with food. It should be noted that the endogenous action of ecdysone is strictly limited to the non-feeding periods of insect life. Indeed, there is hardly a species or stage in which ecdysone could act during the feeding period (Sláma, 1978). Feeding larval stages of certain adapted insects have evolved profound resistance to dietary supplies of ecdysteroids, which are rapidly excreted or converted into inactive metabolites (see Rees, 1995 for a review). This adaptation constitutes the first part of an anti-ecdysteroid resistance mechanism in insects (see Sláma, 1978). This mechanism may be exemplified by the most abundant of the herbivores found on *L. carthamoides*, namely spider mites, collembollans and thysanopterans. It is possible that these small, terrestrial, phytophagous arthropods are not just insensitive to 20-hydroxyecdysone of *L. carthamoides*, but are insensitive to dietary ecdysteroids in general.

The second part of the above indicated anti-ecdysteroid mechanism depends on selective changes in structure-activity relationships, i.e., endogenous receptors of the hormone are tuned to recognize a molecule that is different from ecdysteroid mimics present in common food plants. This mechanism may be exemplified by another group of herbivores that frequent *L. carthamoides*, namely sucking Homoptera and Hemiptera. These insects are resistant to 20-hydroxyecdysone, because they use another molecule, makisterone A, as endogenous hormone (Kelly et al., 1984). A similar example of insect resistance based on selectivity of ecdysteroid action is provided by 11,20-dihydroxyecdysone (turkesterone) from *Ajuga turkestanica*, which is enormously active in dipteran insects, but virtually inactive in lepidopterans (Sláma et al., 1993). Conversely, an ecdysteroid with the lactone ring in the side chain, cyasterone, is the most active in Lepidoptera while being very little active in Diptera (Sláma et al., 1993).

In terms of group specificity and seasonal abundance of dominant arthropod species, the composition of entomocenosis that we found on *L. carthamoides* is very similar to that of the sugar beet, *Beta vulgaris* (Chenopodiaceae) (Benada et al., 1985), which is also an introduced plant to Central Europe. The following species occur on both *L. carthamoides* and *B. vulgaris*: *Dolycoris baccarum*, *Eurydema oleracea*, *Lygus rugulipennis*, *Eupteryx atropunctata*, *Macrosteles laevis*, *Philaenus spumarius*, *Aphis fabae*, *Myzus persicae*, *Thrips tabaci*, *Agrochloa litura*, *Autographa gamma*, *Ostrinia nubilalis* and *Tetranychus urticae*. The family Chenopodiaceae contains a number of species that are relatively rich in ecdysteroids (Bathory et al., 1987; Dinan, 1995), although the contents are never as high as those found in *L. carthamoides*. According to a recent review of Chenopodiaceae (Adler & Grebenok, 1995), the leaves of the sugar beet contain only 0.14 μg/g (fresh weight) ecdysteroid, and spinach contains 35–69 μg/g, both of which are far below an effective concentration range.

Although attention has been focused on ecdysteroids, the fact that *L. carthamoides* also contains a number of other classes of physiologically active secondary substances cannot be overlooked. These include, for example, anthocyanins, saponins, phenolics, polyines, flavonol-glycosides and other flavonoids, thiophene acetylenes and various other compounds (Varga et al., 1982, 1986; Szendrei et al., 1984). Thus, the nature of this plant's possible resistance to insects is very complicated.

It has often been questioned whether actual concentrations of ecdysteroids are high enough to interfere with herbivores. Numerous families and species of plants exist in which minimum effective concentrations of ecdysteroids are never reached (Adler & Grebenok, 1995). For example, the silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae) feeds on mulberry leaves, which contain small amounts of 20-hydroxyecdysone. The wide, random distribution of low ecdysteroid levels in plants has led some researchers to conclude that ecdysteroids have biological functions in plants other than secondary substances for defense against insect herbivores (Sláma, 1979, 1993b). Actual dietary concentrations of ecdysteroids that produce disturbances in development are well known. These concentrations usually range between 25 and 250 ppm (fresh leaves, artificial diet). These levels were clearly established in larvae of Diptera (Singh et al., 1982) and Lepidoptera (Kubo et al., 1983; Arnault & Sláma, 1986). The leaves, roots and seeds of *L. carthamoides* contain high concentrations of ecdysteroids (300–1,000 ppm) that are well above these mentioned permissible dietary limits. It is logical to conclude that a non-adapted species that is sensitive to 25–100 ppm of dietary ecdysteroid would, theoretically, be unable to complete development and/or reproduce on *L. carthamoides*.

Recapitulation of the results indicates that the old hormonal hypothesis of Sláma & Williams (1965a) regarding the protective functions of insect hormones in plants should now be accepted only with serious precautions and further investigations. It may be valid for some nonadapted, sensitive endopterygote species, and for species which can synchronize their abundance with lowest ecdysteroid levels (Figs 2B and 3B). In contrast to this latter group, the most abundant species in this study that were considered the most resistant, occurred in greatest abundance during the time of highest ecdysteroid concentrations in the leaves (Figs 2A, 3A). The possibility that these species or groups may be completely immune against exogenous ecdysteroid needs further investigation.

Acknowledgements. We wish to thank the following for cooperation in the determination of collected species of arthropods: J. Jaroš (Lepidoptera), V. Novotný (Auchenorrhyncha), J. Pelikán (Thysanoptera), J. Rusek (Collembola), V. Růžička (Arachnoidea), O. Nedvěd and J. Vilímová (Heteroptera), J. Strejček (Coleoptera). Our gratitude is also expressed to J. Bajer, ADAVO Co., Velký Osek, for facilitation of our work within fields of *L. carthamoides* and to N.D. Springate for valuable comments and criticisms of the manuscript. The work was supported by grant No. 204/93/1053 from Granting Agency of the Czech Republic.

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Received March 12, 1996; accepted November 11, 1996