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Ecological Relations of Agricultural Populations of Ecdysteroid-Containing Plants *Rhaponticum carthamoides* (Willd.) Iljin and *Serratula coronata* L. with Herbivorous Insects

Report 2. Composition Variability of Phytoecdysteroids in Agrocenoses and Their Role in the Vulnerability of Plants to Phytophagans

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Abstract—The accumulation and variability of ecdysteroids, which are analogs of the insect molting hormones, were studied during ontogeny of agricultural populations of *Rhaponticum carthamoides* (*Leuzea carthamoides* DC.) and *Serratula coronata* with relation to the plant age and cultivation conditions. The physiological role of ecdysteroids in the ecological interactions with pests was evaluated.

It was found that the enhancement of herbivore activity coincided with biochemical changes in the composition of ecdysteroids having different physiological activities and was accompanied by damage to reproductive organs. During ontogenetic (age-related) changes and seasonal development in the vegetation season, the content of the physiologically active ecdysteroid 20-hydroxyecdysone decreased and relatively moderately active inokosterone and weakly active ecdysone were accumulated in reproductive shoots.

Accumulation of the weakly active ecdysteroid ecdysone in phytomass is affected by environmental conditions and anthropogenic factors of cultivation. The factors contributing to the damage potential are environmental microclimate, relief features of population locations, and agrotechnical measures (frequency of phytomass removal, excessive moisture content in the rooting layer).

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Rhaponticum carthamoides (Willd.) Iljin (*Leuzea carthamoides* DC.) and *Serratula coronata* L. are large-herb perennial plants used in medicine and agriculture to obtain various pharmacological preparations and fodder additives [1–8]. The major biologically active substances of the examined species are ecdysteroids, whose concentration in some organs can reach 0.5–1.5% in *R. carthamoides* and 1.2–3.1% in *S. coronata* [1, 9–12]. The major phytoecdysteroids include: 20-hydroxyecdysone (synonyms: ecdysterone, 20-hydroxyecdysone or, in abbreviated form, 20E), inokosterone (or In), ecdysone (or E).

Phytoecdysteroids are identical in chemical structure to the molting hormones of insects (zooecdysteroids) and are necessary at ultralow concentrations for insect development. The ecdysteroid 20-hydroxyecdysone at concentrations of 10^{-8} to 10^{-9} M initiates the transformations occurring in embryogenesis and during larva development with metamorphosis to the adult insect [13, 14]. It is suggested that the concentration of phytoecdysteroids in some plant organs, which reaches very high values (10^{-5} M), is related to the deterrence function and plant protection against insect damage [15].

Laboratory experiments have yielded ambiguous results on the insecticidal activity of ecdysteroids. In some cases, the addition of phytoecdysteroids to an artificial insect-feeding medium led to irreversible changes in insect development: larval death, disruption of molting, and the anomalies reducing the viability of individuals—supernormal number of molts, premature pupation, and the occurrence of larvae with several head capsules [16, 17]. High toxicity of ecdysteroids against aphids was noted—spraying of a 0.05% solution of 20-hydroxyecdysone caused death of 85.4% of individuals [13].

According to other data, ecdysteroid-containing preparations do not exhibit insecticidal activity [18] and have been certified for use in agriculture in Japan since 1994 to synchronize the pupation of the silkworm larvae *Bombyx mori* [19]. According to Dinan [15], more than half of the examined representatives of lepidopterous insects are tolerant to ultrahigh concentrations (reaching 1%) of 20-hydroxyecdysone in the feed ration. Nor did this ecdysteroid have a significant effect on the development of the peach-potato aphid *Myzus persicae* [20]. In other experiments, the physiological effect of phytoecdysteroids depended on the applied dose: at high concentrations (0.3–0.7%), they showed repellent activity [21], and low doses (0.005–0.035%) promoted the survival, growth, and development of larvae [5, 22].

Unfortunately, in the cited experiments, the composition and ratio of the major phytoecdysteroids in the feed ration were not taken into account. It is known that the biological activity of individual compounds differs greatly: in biotests, 20-hydroxyecdysone shows a high activity—7.5×10^{-9} M, inokosterone relatively moderate activity—$1.1–2.0 \times 10^{-7}$ M, and ecdysone weak activity—1.1×10^{-6} M. The other zooecdysteroids circulating in arthropod hemolymph are even less active or inert—$10^{-4}–10^{-6}$ M [23–25]. The results of the first part of our study suggest that the ambiguous deterrent activity of ecdysteroid-containing plants toward insects may be a consequence of variations in their composition during plant development, i.e., biochemical changes in the composition of phytoecdysteroids having different activities [26].

The objectives of the second stage of our research was to study the variability in the accumulation of individual different-activity ecdysteroids in agricultural populations of *R. carthamoides* and *S. coronata* in relation to age and cultivation conditions and to evaluate their physiological role in ecological interactions with insect pests.

RESULTS OF ECOLOGICAL MONITORING OF PHYTOPHAGAN ATTACK IN BIOCENOSES

Vulnerability of *R. carthamoides* and *S. coronata* to herbivores under conditions of European North.[1] In the first part of our research (1989–2005) based on the results of long-term monitoring of ecological interactions of agricultural populations of *R. carthamoides* and *S. coronata* with herbivores, it was concluded that there is no direct relationship between the total concentration of ecdysteroids in plants and their vulnerability to pests [26]. For vegetative shoots, regardless of soil conditions, rates of development, and relatively low level of ecdysteroids, extensive insect damage was not detected in any one of the populations studied.

For foliage organs, there are short periods in the life cycle in which they do not have a pest resistance potential. These periods are limited by: (a) the stage of transition of seedlings from the latent to the juvenile age state (seedling stage), (b) the stage of death of old leaves. In the first case, damage is related to the time when the cotyledonary leaves of the primary primordial shoot are still not capable of independent synthesis of ecdysteroids [27] and consume storage compounds from the reproductive organs (seeds), and in the second case, the true foliage organs have already lost this capability [9]. Isolated cases of point injuries to old and dying leaves of *R. carthamoides* by insects indicate that young and adult foliage organs have a deterrent effect which is observed at an ecdysteroids concentration above 0.1% (2.0×10^{-6} M). The vegetative shoots of juvenile, immature, virginal, reproductive, and senile plants were not damaged in any development stage.

The ecological interactions of reproductive shoots with herbivores have a different pattern. After the onset of the reproduction period, herbivores infestation and damage to reproductive organs and their elements (Fig. 1) was recorded. The frequency and severity of damage correlated not only with the ontogenetic age of plants but also with the vegetative development stage and ecdysteroid content (Fig. 2). In the cenoses, damage was observed on elements that had the highest concentration of ecdysteroids (in *R. carthamoides*, receptacles with a 0.8–1.2% concentration of ecdysteroids and seeds with a 0.57–0.66% concentration; in *S. coronata*, the upper and apical metamers of reproductive shoots with a 1.2–2.8% concentration and seeds with a 0.9–1.2% concentration).

The severity of damage was enhanced by ontogenetic senescence of plants, leading to complete or partial destruction of the seed crop. In *R. carthamoides*, the insect pests were cetoniine beetles (*Oxythyrea funesta*, *Potosia cuprea* ssp. *metallica*) (Coleoptera: Scarabaeidae), and in *S. coronata*, these were aphids (Homoptera: Aphididae) and an unidentified species of gall midges (Diptera: Cecidomyiidae). A concomitant factor is the environmental microclimate (air humidity and temperature), which depends on the relief features of population locations (Table 1). Open and well-heated space with low humidity favored the infestation of *R. carthamoides* by cetoniine beetles, and the closed and unventilated space in the stand of *S. coronata* on loams and peats, coupled with increased humidity, favored mass infestation of reproductive shoots by aphids.

In 2006, only a 17-year-old population on sandy loam was affected by cetoniine beetles, of which 98% were represented by *Oxythyrea funesta* and only 2% by *Potosia cuprea* ssp. *metallica*. The following statistical trend (Table 2) was found: in the plot cultivated in the previous year (2005) with a single removal of phytomass, the population density of herbivores on inflorescences was 3.5 times lower than that in the area cultivated with a double removal (0.68 against 2.40 individuals per inflorescence).

Vulnerability of ecdysteroid-containing plants in other geographical areas. Analysis of the literature

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[1] Characteristics of the objects, natural climatic conditions, and research techniques are considered in the first part of the paper [26].

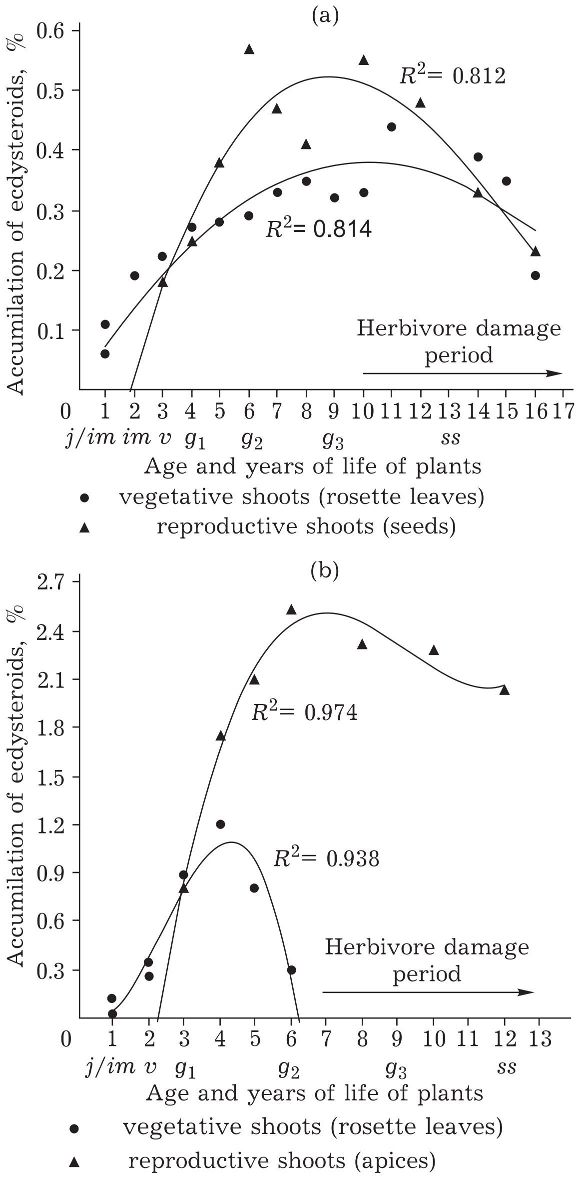

Fig. 1. Colonization of reproductive shoots of *Rhaponticum carthamoides* (a) and *Serratula coronata* (b) by herbivores in ontogeny.

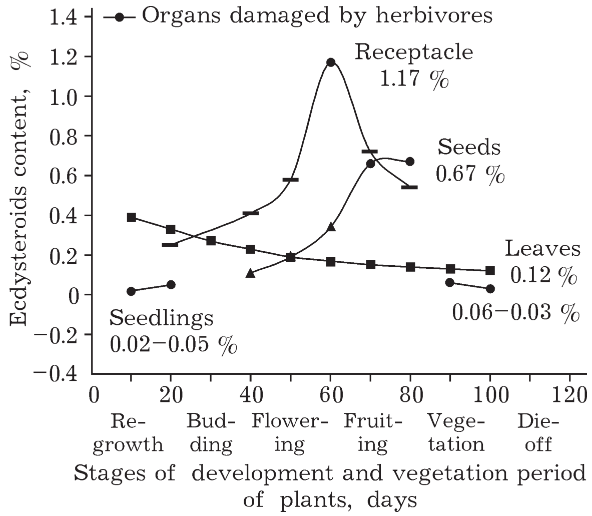

Fig. 2. Herbivore damage to organs of *Rhaponticum carthamoides* at different stages of development.

shows that observations of a positive correlation between high concentrations of active substances and vulnerability of reproductive shoots of ecdysteroid-containing plants to herbivores are not isolated cases. Lomanova and. Kuznetsova, researchers of the Siberian Botanical Garden of Tomsk State University [28], found that an introduced complex of ecdysteroid-containing plants of the genus *Silene* (about 40 species) was damaged each year by 24-spot ladybird *Subcoccinella vigintiquatuorpunctata* (Coleoptera: Coccinellidae). The phytophagan caused the most severe damage to leaves and buds during flowering, which coincided with the maximum ecdysteroid content—0.84% in *S. italica* and 1.21% in *S. viridiflora* [29]. In studying a reproductive agricultural population of *R. carthamoides* for two years (1993–1994), experts of the Institute of Entomology in Czech Republic found that high levels of ecdysteroids did not have a selective deterrent effect on arthropods whose composition (126 species) was similar to the species diversity of insects on plantations of sugar beet (*Beta vulgaris*), which does not synthesize ecdysteroids [30].

Postnikov [31] notes the presence of spotted beetles in inflorescences of *R. carthamoides* and high seed damage (up to 68%) by pests in natural biocenoses. According to data of Shcherbakov [32–33], in Gorny Altai and subalpine meadows of Kuznetsk Alatau, 48% of inflorescences of *R. carthamoides* are infested by flower-fly larvae of two species—*Chaetostomella cylindrica* R. D. and *Acanthiophilus helianthi* Rossi (Diptera; Tephritidae). The flight of herbivorous adults coincided with the flowering stage of *R. carthamoides* (end of June–beginning of July). Kosterin [34] reports that lepidopteran herbivores (Lepidoptera) are common in the geographical ranges of other plant species from the genera *Rhaponticum* and *Serratula.* In Central Altai, *Melitaea scotosia* Butler is found at 1100–1600 m above sea level and *Melitaea phoebe* (Goeze) at 2500 m above sea level. In the Baikal region, Amur River basin, Northeast China, Korea, and Japan, *Melitaea phoebe saturata* Staudinger and *Vanessa cardui* (L.) are described. The flight of butterflies lasts from the middle of June to the end of July–August and coincides with the flowering stage of the ecdysteroid-containing species *R. carthamoides*, *R. uniflorum*, *S. coronata*, and *S. centauroides* [35].

Severe damage to the apical parts of reproductive shoots of *S. coronata* by aphids has been observed in recent years at the laboratory of the Botanical Garden of the Institute of Biology, Komi National Center, Ural Branch of the Russian Academy of Sciences (unpublished data, provided by Professor V. P. Mishurov in

Table 1. Environmental microclimate in grass stand of agricultural populations of *Rhaponticum carthamoides* and *Serratula coronata*

Soil texture	Years of laying*	Relief in adjacent	Micro-climate in stand**	Soil moisture content at various dates, %***			
				25.V–14.VI	18–26.VI	05.VII–15.VIII	25.VIII–20.IX
Sandy loam	$\frac{1989\,(1\text{ ha})}{1991\,(1\text{ ha})}$	Slightly billowy, elevated, well-ventilated	+ / –	16.4–20.6	4.4–8.7	3.0–6.3	11.1–17.5
Sand	$\frac{1995\,(1\text{ ha})}{1996\,(2\text{ ha})}$	Slightly billowy, elevated, open	++ / –	12.7–16.4	3.0–6.3	2.5–5.4	8.6–15.1
Clay loam	$\frac{1995\,(3\text{ ha})}{1995\,(2\text{ ha})}$	Billow, low, semi-ventilated	– / +	24.3–27.4	12.6–14.3	11.4–16.4	17.3–22.1
Peat	$\frac{1994\,(2\text{ ha})}{1994\,(1\text{ ha})}$	Billow, low, confined, unventilated	– – / ++	9.3–13.1	9.2–11.9	4.6–12.1	14.5–18.8

Note: * Data for *Rhaponticum carthamoides* are in the numerator and those for *Serratula coronata* in the denominator; the plot area is in parentheses. ** Relative temperature/humidity of air: elevated (+), high (+ +), reduced (–), low (– –). *** Soil moisture contents are given as average maximum values.

Table 2. Effect of the frequency of phytomass removal on the population density of *Oxythyrea funesta* on reproductive shoots of *Rhaponticum carthamoides*, 2006)

Frequency of removal	Beetle population on shoots (pcs/plant)	Variability coefficient,%	Variability range, pcs./receptacle
Single	0.68 ± 1.18	171.1	0–4
Double	2.40 ± 2.06	85.9	1–8

2000 and 2004). Doctor Alfred Kump from Linz (Austria) told us that seeds of *Rhaponticum scariosum* which he collected in the Alps in 2006 were injured by 15–40% of the examined quantity, and pupae of two unidentified insect species were found in inflorescences. According to Dr. Pierre-Andre Loizeau from the Alpine Botanical Garden in Geneva (Switzerland, 2006), seeds of *Stemmacantha (Rhaponticum) heleniifolium* and *Stemmacantha rhapontica* ssp. *lamarckii* are annually attacked by herbivores.

Thus, irrespective of geographical sites, the reproductive shoots of ecdysteroid-producing plants are subjected to attack and severe damage by phytophagous insects. Ultrahigh concentrations of ecdysteroids ($2–5 \times 10^{-5}$ M) does not deter or protect species from herbivore damage; neither do they cause irreversible changes in the development of insect pests although it is known that their hormonal activity is exhibited at very low concentrations—10^{-8}–10^{-9} M [36]. At the same time, at a concentration 25–40 times lower, foliage organs are not damaged.

The assumption of many researchers that the accumulation of phytoecdysteroids in the apical parts of reproductive shoots of plants is due to the deterrence function and protection against insect feeding [15, 29, 37] is not confirmed under natural conditions. A different view of the ecological role of phytoecdysteroids is taken by a team of authors who believe that ecdysteroids function as allelochemical toxins and antifeedants only for nonadapted insect herbivores [38, 39]. However, the mechanisms involved in the resistance of insects to high concentrations of ecdysteroids in vegetative tissue have not been explained.

VARIABILITY IN THE ECDYSTEROID COMPOSITION IN THE PLANTS STUDIED

Effect of age. First we studied the composition and relations between individual ecdysteroids in the phytomass based on their age both during the life cycle and the growing season. Before reaching the reproductive period, *R. carthamoides* contains only the highly active phytoecdysteroid 20-hydroxyecdysone (Table 3). Moderately active inokosterone is found in vegetative shoots of young reproductive plants, and weakly active ecdysone in middle-aged and old reproductive plants. During development of reproductive organs, the ecdysone content increases sharply—from 1.5 to 4.7% in the budding stage, compared to the vegetation stage, and then to 13.3% in the flowering stage of senescing old plants.

In reproductive shoots, the composition of ecdysteroids varies similarly. During regrowth, stem leaves contain trace amounts of inokosterone (1.6%), and by the onset of flowering, its weight percentage increases to 18.2–20.7%. Ecdysone is not found at the beginning

Table 3. Composition variability of major ecdysteroids in aerial organs of *Rhaponticum carthamoides* during development (sandy loam, peat)

Development stage	Date of vegetation	Biological age	Relative content of ecdysteroid, %			20E, In/E ratio
			20E	In	E	
Rosette leaves:						
vegetation	14.IX	*im*	100	–	–	>1 000
The same	31.VIII	*v*	100	–	–	>1 000
The same	28.VIII	g_1	99.1	0.9	–	990 : 10 : 0
regrowth	26.V	g_2	97.5	2.5	–	975 : 25 : 0
The same	26.V	*ss*	98.0	2	–	980 : 20 : 0
vegetation	31.VIII	g_3	94.3	4.2	1.5	60 : 3 : 1
budding	01.VI	g_2	93.9	1.4	4.7	20 : 0.3 : 1
flowering	18.VI	g_3	84.0	2.7	13.3	6 : 0.2 : 1
Stem leaves:						
regrowth	25.V	g_2	98.4	1.6	–	980 : 16 : 0
flowering	21.VI	g_1	72.7	18.2	9.1	8 : 2 : 1
blossom fall	29.VI	g_1	61.5	20.7	17.8	3 : 1 : 1
fruiting	17.VII	g_1	75.0	6.8	18.7	4 : 0.4 : 1
Seeds:						
fruiting	15.VII	g_1	100	–	–	>1 000
The same	17.VII	g_2	100	–	–	>1 000
The same	18.VII	g_3	97.5	1.9	0.6	160 : 3 : 1
The same	14.VII	*ss*	74.1	22.4	3.5	21 : 6 : 1
flower corolla	21.VI	*ss*	100	–	–	>1 000

Note: Age states: *im*, immature; *v*, virginal; g_1, g_2, g_3, young, adult, and old reproductive; *ss*, subsenile. Phytoecdysteroids: 20E, 20-hydroxyecdysone; In, inokosterone; E, ecdysone; dash denotes the absence or trace amounts of ecdysteroids in samples (abundance content below 0.1%). Notation is identical for all tables and figures.

of the vegetation season, but then its content reaches 9.1% during flowering and 17.8–18.7% during seed filling and fruiting. The composition of ecdysteroids in seeds reflects their age variability patterns in leaves—young reproductive plants contain only 20-hydroxyecdysone, and senescing old plants contain inokosterone and ecdysone. The weight percentage of the last two ecdysteroids increases considerably in old plants in the subsenile age state (from 1.9 to 22.4% for inokosterone and from 0.6 to 3.5% for ecdysone). The ratio of 20-hydroxyecdysone and inokosterone to ecdysone (20E, In/E) varies as follows: in intact plants, it is close to 1000 : 1; at the onset of flowering, it reduces to 20–6 : 1, and at the onset of fruiting, to 3–4 : 1.

In *S. coronata*, the variability in the phytoecdysteroid composition in reproductive shoots is also related to age changes (Table 4). As in *R. carthamoides*, stem leaves of *S. coronata* contain both 20-hydroxyecdysone and inokosterone. Ecdysone appears in the early reproductive age state, and its content increases during the life cycle from 1.7 (in young reproductive plants) to 11.7% (in senescing old plants).

In plants of the same age, ecdysone increases during the vegetation season; for example, from May 25 to June 3 (stage of reproductive-shoot extension), the weight percentage in apical metamers increases from 4.3 to 9.3%. The ratio of 20-hydroxyecdysone and inokosterone to ecdysone changes as follows: in intact plants in the virginal and early reproductive age states, it is close to 1000 : 1; in affected reproductive plants, it decreases to 20–9 : 1 during the shooting and budding stage and then to 9–6 : 1 during the flowering and fruiting period.

Similar patterns of composition changes of phytoecdysteroids with plant development were recorded in *S. coronata* plants grown in the Komi Republic [40]. In the period from regrowth to flowering and then to fruiting, the weight percentage of 20-hydroxyecdysone in foliage organs decreased from 90.3 to 60.0 and 32.7%, and the ecdysone content increased by a factor of 10–from 3.1 to 30.6–32.7% (the 20E, In/E ratio changed from 30 : 1 to 2 : 1).

Ripening seeds are acceptors of phytoecdysteroids from the donor organs in their redistribution from foliage organs through stems [11]. As a consequence, the

Table 4. Variability of composition of major ecdysteroids in reproductive shoots of *Serratula coronata* during development (clay loam, peat)

Development stage	Date of vegetation	Biological age	Ecdysteroids,%			20E, In/E ratio
			20E	In	E	
Apical metamers:						
shooting	11.VII	*v*	84.0	16.0	–	840 : 16 : 0
budding	24.VI	g_1	98.5	1.5	–	985 : 15 : 0
The same	11.VII	g_1	86.3	13.7	–	860 : 14 : 0
The same	24.VI	g_1	90.3	8.0	1.7	53 : 5.0 : 1
shooting	25.V	g_3	85.6	10.1	4.3	20 : 2.4 : 1
The same	27.V	g_3	84.4	10.4	5.2	16 : 2.0 : 1
The same	29.V	g_3	75.4	16.7	7.9	10 : 2.1 : 1
The same	03.VI	g_3	84.4	6.3	9.3	9 : 0.7 : 1
budding	18.VI	g_2	82.3	11.3	6.4	12 : 1.8 : 1
The same	19.VI	g_3	71.5	16.8	11.7	6 : 1.4 : 1
flowering	08.VII	g_3	76.3	14.3	9.4	8 : 1.5 : 1
Seeds	03.IX	g_3	81.3	9.8	8.9	9 : 1.1 : 1

composition of phytoecdysteroids and the ecdysone content in seeds (8.9 wt.%) during fruiting in early September is similar to the composition and ratio of ecdysteroids in reproductive shoots during budding and flowering in June–July.

Thus, during ontogenetic changes and seasonal vegetative development, reproductive shoots have a reduced content of the most active compounds and accumulate weakly active and inactive compounds. The organs of intact adult reproductive plants of *R. carthamoides* contain trace amounts of inokosterone (0.9–2.5 wt.%) and does not contain ecdysone. The herbivore damage to reproductive shoots in ontogeny (Figs. 1 and 2) coincides with their accumulation of inokosterone and ecdysone (Table 3). Similarly to *R. carthamoides*, the herbivore damage to reproductive shoots of *S. coronata* in ontogeny (Fig. 1b) coincides with their accumulation of inokosterone and ecdysone (Table 4).

Effect of cultivation conditions. We studied the effect of cultivation conditions on composition change of ecdysteroids in agricultural populations in which the degree of herbivore damage depended on the relief features (microclimate) of population locations and agrotechnical measures (removal of biomass). In agricultural populations of *R. carthamoides* on peat and sandy soils, double removal (in contrast to single removal) led to accumulation of ecdysone (2.4–2.8 wt.%) in vegetative shoots, and the ratio between highly active 20-hydroxyecdysone and weakly active ecdysone decreased from 1000 : 1 to 40–34–13 : 1 (Table 5). In old plants on sandy soil, the seeds formed after double removal of biomass in the previous cultivation year contained the same weight percentage of ecdysone as in the case of single biomass removal (3.5%), but the weight percentage of 20-hydroxyecdysone was reduced (66.2 against 74.1%) due to increased inokosterone content.

In agricultural populations of *S. coronata* located on low relief features (peatlands) with excessive humidity, the creation of ventilation conditions in the stand led to a decrease in the soil moisture content from 12.1 to 4.6% (Table 1) and a decrease in the ecdysone content in reproductive organs from 9.4 to 5.2%. Similarly, on loamy soils under open ventilation conditions, the mass fraction of weakly active ecdysone decreased from 6.4 to 3.4%.

Thus, the comparison of the cultivation conditions of *R. carthamoides* and *S. coronata* shows that the degree of damage to agricultural populations by herbivores is influenced by the presence and increased accumulation of the weakly active ecdysteroid ecdysone in the phytomass, which, in turn, are due to the anthropogenic and environmental factors in the growth and development of plants during vegetation. In the case of overmoistening and intense mowing, the ratio of the highly active ecdysteroid 20-hydroxyecdysone to inactive ecdysone decreases by a factor of two (8–12 : 1 against 15–23 : 1).

ECOLOGICAL AND BIOCHEMICAL INTERACTIONS BETWEEN HERBIVORES AND ECDYSTEROID-PRODUCING PLANT SPECIES

Ecdysteroid composition in insect pests. Herbivorous insect collected from the apical parts of severely affected plants were investigated (Table 6). In the biomass structure of the spotted cetoniine beetle *Oxythyrea funesta* and *Trichius fasciatus* ecdysteroids were not

Table 5. Influence of cultivation conditions on composition of ecdysteroids in vegetative shoots of *Rhaponticum carthamoides* and reproductive shoots of *Serratula coronata*

Soil texture	Biological age	Intensity of biomass removal	Development stage	Relative content of ecdysteroid, %			20E, In/E ratio
				20E	In	E	
			R. carthamoides				
Peat	g_2	Single	Budding	97.5	2.5	–	975 : 25 : 0
Sand	g_1	Double	The same	97.6	–	2.4	40 : 0 : 1
The same	g_2	Single	The same	97.7	2.3	–	977 : 23 : 0
The same	g_2	Double	The same	94.4	2.8	2.8	34 : 1 : 1
Peat	*v*	Single	Vegetation	99.1	0.9	–	990 : 10 : 0
The same	g_1	Double	The same	92.9	–	7.1	13 : 0 : 1
Sandy loam	*ss*	Single	Seeds	74.1	22.4	3.5	21 : 6 : 1
The same	*ss*	Double	The same	66.2	30.3	3.5	19 : 9 : 1
			S. coronata				
Peat	g_3	Semiventilated	Flowering	79.2	15.6	5.2	15 : 3 : 1
The same	g_3	Unventilated	The same	76.3	14.3	9.4	8 : 1.5 : 1
Clay loam	g_3	Ventilated	The same	78.9	17.7	3.4	23 : 5 : 1
The same	g_3	Unventilated	The same	78.8	14.8	6.4	12 : 2.3 : 1

Table 6. Composition of ecdysteroids in the biomass of the herbivorous insects collected from reproductive of shoots of *Rhaponticum carthamoides* and *Serratula coronata*

Parameter	Date	Biological age	Ecdysteroids concentration	Relative content of ecdysteroid, %			20, In/E ratio
				20E	In	E	
Biomass of herbivorous beetles:							
Oxythyrea funesta	29.VI	*ss*	0.00	0.0	–	–	–
Trichius fasciatus	29.VI	*ss*	0.00	0.0	–	–	–
Potosia cuprea metallica	29.VI	*ss*	0.024	100.0	–	–	>1 000
Damaged shoots of *S. coronata*	28.VII	g_3	0.85	78.8	14.8	6.4	12 : 2.3 : 1
Aphid biomass	28.VII	g_3	0.90	81.4	12.0	6.6	12 : 1.8 : 1
Honeydew after aphids*	28.VII	g_3	0.22	91.0	4.5	4.5	20 : 1 : 1

*After washing consolidated viscous honeydew from shoots with distilled water.

found. In the biomass of *Potosia cuprea* ssp. *metallica*, ecdysteroids were contained in trace amounts (0.024%), probably due to the remnants of undigested food in the gut. Furthermore, ecdysteroids are represented only by 20-hydroxyecdysone (100 wt.%). At the same time, the comparative concentration of ecdysteroids in seeds and receptacles are 20–40 times higher and the ecdysteroid composition is different (74.1% 20-hydroxyecdysone, 22.4% inokosterone, 3.5% ecdysone). This suggests that the phytoecdysteroids coming with food are inactivated in the gut of insects, with 20-hydroxyecdysone being less utilized.

The biomass of aphids is saturated with ecdysteroids, whose concentration at the level of their affected shoots is 0.9% against 0.7–1.0%. The relative concentration of ecdysteroids in intact shoots reached 2.3–2.8%, indicating that they are utilized by insects from the plant sap. A comparison of the composition of phytoecdysteroids from the aphid biomass, honeydew after aphids, and affected plants shows that the composition was changed by herbivores. As in the case of *R. carthamoides*, 20-hydroxyecdysone was the least utilized—91.0 wt.% in the honeydew after aphids, 81.4 wt.% in the insect biomass, and 76.3–79.2 wt.% in the shoots of *S. coronata*.

Estimation of the physiological activity of individual ecdysteroids for insects. There are several important points for understanding the development and inhibition of the physiological activity of ecdysteroids in the insect organism:

1. For the entire diversity of arthropod species, about 50 zooecdysteroids were identified, of which the main one is 20-hydroxyecdysone; ecdysteroids of secondary value are ecdysone, which is transformed to 20-hydroxyecdysone in peripheral tissues, and then makisterone A, makisterone C, 2 deoxy-20-hydroxyecdysone, 3-dehydroecdysone, 3-epi-ecdysone, etc. [41]. According to the database of ecdysteroids present in living systems [42], their activities in biotests differ by six orders of magnitude and vary from 10^{-10} to 10^{-4} M. The physiological activity of 20-hydroxyecdysone is 10^{-8} M. The others zooecdysteroids circulating in arthropod hemolymph are weakly active or inert. From the activity ratio, inokosterone is 15 times and ecdysone 148 times less active than 20-hydroxyecdysone.

2. As can be seen from the results of our studies, the ratio of individual ecdysteroids is not constant but varies during plant development, especially in the reproductive period. During the sequence of phenophases and ontogenetic (age-related) changes, the content of the most active compounds decreases and weakly active compounds are accumulated. In the prereproductive period, *R. carthamoides* contains only the highly active ecdysteroid 20-hydroxyecdysone, and the aerial organs of *S. coronata* contain additionally small amounts of moderately active inokosterone. Middle-aged and old reproductive plants are found to contain inactive ecdysone, whose content increases sharply during formation and development of reproductive organs, especially in senescing old plants in the flowering and fruiting period.

3. For both cultivars studied, there is a relationship between the composition of ecdysteroids and their damage by pests. The herbivore damage to reproductive shoots of *R. carthamoides* and *S. coronata* in ontogeny coincides with their accumulation of inokosterone and ecdysone. The ratio of 20-hydroxyecdysone and inokosterone to ecdysone (20E, In/E) varies as follows: (a) in intact plants of both species, it is close to 1000 : 1; (b) in *R. carthamoides*, it decreases to 20–6 : 1 at the onset of flowering and to 3–4 : 1 at the onset of fruiting; in *S. coronata* at the beginning of active growth of reproductive shoots (the shooting and budding stage) it decreases to 20–9 : 1, and by the beginning of flowering and fruiting, to 9–6 : 1.

From the experimental data and literature analysis, it follows that less active ecdysteroids in insects can partially or completely block the physiological effect of more active compounds. According to Ukrainian researchers, the sum of ecdysteroids isolated from reproductive shoots of *S. coronata* and *S. inermis* under laboratory conditions increased fertility and egg production of insects by a factor of 1.8–2.0 [5, 43]. According to Tanaka [44], in fifth-instar larvae of the silkworm *Bombyx mori* (Lepidoptera: Bombycidae), a dose of 20-hydroxyecdysone equal to 100–300 ppm caused disruption of molting and death if previously, from the fourth instar, the larvae had been reared on a background diet containing 300 ppm 20-hydroxyecdysone. If less active ecdysone was added at 200 ppm to food, the activity of 20-hydroxyecdysone was blocked and remained low even with an increase in its content to 500 ppm (the 20E/E ratio is 2.5 : 1). Similar results were obtained in experiments with the sweet-potato hornworm *Agrius convoluvli* (Lepidoptera: Sphingidae)—in the presence of ecdysone, the larvae could feed on a diet with very high concentrations of 20-hydroxyecdysone (1600 ppm).

According to the results of other Japanese researchers, 95% pure 20-hydroxyecdysone isolated from rhizomes of the Brazilian plant (*Pfaffia iresinoides*) is safe for fifth-instar larvae of the silkworm *Bombyx mori* even if its concentration in food is 2%. At doses differing by a factor of 500 (40–20 000 ppm or 10^{-7} to 10^{-4}M), the larvae did not differ in survival from the control [19]. In this case, the inactive ecdysteroids of *Pfaffia iresinoides* are inactive rubrosterone and pterosterone [45], and the ratio of 20-hydroxyecdysone to them is 19 : 1.

In comparative antifeedant tests of extracts of bugle plants of the genera *Ajuga* against pea aphid *Acyrthosiphon pisum* (Homoptera: Aphididae), high antifeedant activity was found in *Ajuga chamaepitys*, in which the total content of ecdysteroids was 19 times lower than that in *Ajuga reptans*,—0.01 against 0.19% [46]. A feature of *A. chamaepitys* was that its extract contained the highly active compounds 29-norcyasterone and makisterone A (at the level of 20-hydroxyecdysone). The major component of *A. reptans* was the inactive ecdysteroid 28-epi-sengosterone.

Mechanisms of inactivation of phytoecdysteroids in the insect organism. In studying the role of phytoecdysteroids in interactions with insects, it is reasonable to consider the physiological function of herbivore-damaged reproductive organs in plant life. Seeds contain an embryonic shoot and begin the long life cycle of the plant. Before germination under natural conditions, the embryonic new individual is in temporary dormancy, waiting out the adverse fall–winter season under snow cover. During dormancy, the ecdysteroids stored in seeds should be inactivated and water-insoluble lest they should be washed away from the fruits in the humid environment. This is achieved by accumulation of high levels of fatty acids (over 20% in *R. carthamoides* [47]) and formation of esters of these acids with ecdysteroids, which perform the functions of accumulation, inactivation, and safety of the latter [48].

The reverse biotransformation of ecdysteroids to the water-soluble transport form in seeds occurs in early spring, after enzymatic hydrolysis of fatty acids by esterases during germination [49]. The protective role of ecdysteroids in foliage organs requires activation of ecdysteroid receptors (EcR) in insect tissues, where complexes of these compounds with other physiologically active substances play an important role, responsible for the resulting biological activity of the species [42]. Peptides play a central role in the activation and inactivation of ecdysteroids, in particular, through the formation of high- and low-density lipoproteins. Peptides are the major carriers of the biological activity of ecdysteroids and are important for the start of gene transcription mechanisms via cofactors as well as for the protection against inactivation [41]. Low molecular weight peptides stimulate the activation of the phosphatase enzyme responsible for ecdysteroid release and are a factor in the alternative, non-genome-specific, route of activation of hormonal activity compounds in insects through membrane receptors [50].

In addition, it is known that plants are sources of other agents that could interact with the ecdysteroid receptor complex (EcR/USP), activating or repressing its target functions. For example, it has been shown that flavones (rutin and its metabolites) can considerably inhibit EcR-dependent gene transcription [51]. The ligands of the second receptor USP (ultraspiracle protein) are presumably phospholipids [52]. Analogs of this receptor can be activated by some derivatives of chlorophyll, in particular, phytanic acid [53], and carotene derivatives—9-*cis*-retinoic acid [52].

A common feature of the above-stated facts of ecological interaction between insects and ecdysteroid-containing plants is that an increase in herbivore activity coincides with the flowering-fruiting period and is accompanied by damage to reproductive organs which concentrate phytoecdysteroids and lipids. Foliage organs remain intact, all other conditions being the same. Apart from the total concentration and composition of ecdysteroids (ratio of highly active to weakly active compounds), the cause of the different deterrent activities of foliage and reproductive organs should be sought in differences for other components in the chemical composition.

Characterizing the protective mechanisms of plants against herbivores, Ostroumov and Telitchenko [54] note the presence of a wide class of chemical substances which play an important role in the ecological and biochemical interactions between plants and insects. In our case, a comparison of the chemical composition of organs of insect-damaged and undamaged *R. carthamoides* (seeds and leaves) shows that the seeds are 5–6 times richer in lipids than leaves—18–24% [47] against 3.1–4.4% [31]. The foliage organs of *R. carthamoides* synthesize 2.0–3.9% flavonoids and 1.4–1.9% water-soluble derivatives of phenolic acids and contain up to 34% protein substances and 670–1100 mg/kg carotenoids [55, 58]. The content of polyphenolic substances (tannins) reaches 8.6–13.3% [59]. Thus, a combination of the complex composition of ecdysteroids with proteins, flavonoids and polyphenolic and terpenoid compounds is an additional biochemical protective barrier against herbivores.

The food attractiveness of the reproductive organs of *R. carthamoides* and *S. coronata* for insects may be due to the necessity of obtaining some phytoecdysteroids from outside as a source of sterols for the development of nymphs and adult reproduction as is shown, for example, for the ecdysteroid makisterone C in homopterous insects [41]. It is considered likely that the ecdysteroids present in plants in the form of water-soluble compounds [24] serve as a transport form of sterols for delivery from foliage organs to seeds through reproductive stems [47]. Sterol esters differ in a high degree of lipophilicity and are not suitable compounds for movement in plants since they are localized at the sites of their synthesis (including seeds).

It has been shown that inactivation of the absorbed ecdysteroids in the insect organism can occur by various routes: through the gut and hemolymph, by formation of inactive esters with fatty acids (C_{22}-esters; C_3-acetates), through oxidation and epimerization (C_3-α-epimers), phosphorylation (C_2, C_3, C_{22}-phosphates) and C_{26}-ecdysonic acids [41, 48, 60–62]. Another possible inactivation pathway is the microbial transformation resulting from the symbiotic activity of the bacterial microflora of beetles and aphids, providing food digestion in the midgut [63, 64].

In particular, the enzyme ecdysone-oxidase plays a key role in the irreversible transformation of the physiologically active ecdysteroid—20-hydroxyecdysone to inactivated compounds [65]. The activity of the enzyme is found in hemolymph, peripheral tissues, and fat body but it is most pronounced in the midgut, where food absorption occurs [66]. The activity of the enzyme is regulated at the transcription level by excess of the major ecdysteroids from the second instar larval stage; therefore, the hormonally active compounds introduced from outside (20-hydroxyecdysone and their analogs) are subjected to rapid inactivation.

CONCLUSIONS

1. The results of long studies of ecological interactions between agricultural populations of *Rhaponticum carthamoides* and *Serratula coronata* and phytophagans show that the deterrent activity of ecdysteroid-containing plants depends on the composition of individual compounds in phytomass. An increase in herbivore activity coincides with biochemical changes in the composition of ecdysteroids having different physiological activity and is accompanied by damage to reproductive organs.

2. Herbivores damage to reproductive shoots of *R. carthamoides* and *S. coronata* coincides with their accumulation of the ecdysteroids inokosterone and ecdysone. During ontogenetic (age-related) changes and seasonal development in the vegetation season, the content of the physiologically active ecdysteroid 20-hydroxyecdysone in reproductive shoots decreases

and moderately active inokosterone and weakly active ecdysone are accumulated.

3. Less active ecdysteroids in the insect organism can block the physiological action of more active compounds. Plant damage by insects is observed as the ratio of 20-hydroxyecdysone and inokosterone to ecdysone decreases: in intact plants, it is close to 1000 : 1; in affected plants, this parameter decreases to 20–9 : 1 with transition to flowering and then to 3–6 : 1 by the onset of fruiting.

4. The variability of ecdysteroid composition in plants can be influenced by the cultivation conditions leading to the accumulation of inactive ecdysone. Environmental factors (soil moisture content and the temperature and relative humidity of air in the stand) due to the relief features of population locations, and agrotechnical measures (frequency of phytomass removal) can contribute to the damage potential.

5. The food attractiveness of the reproductive organs of *R. carthamoides* and *S. coronata* for insects may be due to the necessity of obtaining some phytoecdysteroids as a source of sterols for the development of nymphs and adult reproduction. Inactivation of the absorbed ecdysteroids in herbivores can occur through the gut and hemolymph, by formation of esters with fatty acids, oxidation and epimerization, phosphorylation and formation of ecdysonic acids, and through the symbiotic activity of bacterial microflora in the midgut.

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