

# Ecological Relations of Agricultural Populations of Ecdysteroid-Containing Plants *Rhaponticum carthamoides* (Willd.) Iljin and *Serratula coronata* L. to Herbivorous Insects. Report 1

N. P. Timofeev

Scientific-Production Enterprise Farm "BIO", Koryazhma, Russia

E-mail: timfbio@atnet.ru

**Abstract**—Accumulation of phytoecdysteroids in agricultural populations of *R. carthamoides* (Willd.) Iljin and *S. coronata* L. and their resistance to insect herbivores in different ontogenetic periods were investigated for 16 years. As a result of the investigations, species-specific features of ecdysteroid accumulation in vegetative and reproductive plant organs were determined; relationships between the distribution of phytoecdysteroids in the structure of plants and ecological interactions with insects have been revealed; the factors contributing to insect infestation have been studied; and the pest damage has been evaluated.

**DOI:** 10.1134/S1995425509050166

**Key words:** *Rhaponticum carthamoides*, *Serratula coronata*, medicinal plants, agricultural populations, ecdysteroids, herbivorous insects

*Rhaponticum carthamoides* (Willd.) Iljin (syn. *Leuzea carthamoides* (Willd.) DC) and *Serratula coronata* L. are species capable of synthesizing ultrahigh ecdysteroid levels—up to 0.4–1.5% in *R. carthamoides* and 1.2–2.3% in *S. coronata* [1–3]. Based on the results of long-term botanical studies, they are recommended for cultivation as new medicinal plants in Siberia and northern Europe [4–8].

Successful commercial cultivation of these species depends largely on their ability to withstand pest attacks. Zooecdysteroids are known to play an important role in the arthropod development. Periodic molting and metamorphoses are caused by peaks of the ecdysteroids produced by the prothoracic glands under the action of brain neuropeptides [9].

The identity of phytoecdysteroids synthesized by plants and the arthropod molt hormone suggests that increased ecdysteroid concentrations in separate plant organs (4–5 orders of magnitude higher than that in the insect hemolymph) can have the biological function of protecting against pests.

Laboratory tests of the activity of phytoecdysteroids as deterrents and regulators of insect growth and development have given ambiguous results [10–12], and the experimental techniques used in such studies were imperfect: a small number of species, primarily of the order Lepidoptera, were tested; the larvae did not have alternative food sources, unlike individuals in natural habitats; different plant organs with different ecdysteroid levels were not tested simultaneously; the expo-

sure methods (treatment of fodder with chemically purified ecdysteroids, immersion of insects in aqueous and alcoholic extracts, injections) were not identical to the natural feeding conditions.

In addition, it was not taken into account that chemically isolated ecdysteroids in nutritious forage, ground leaves, and extracts were unstable under high-humidity conditions, in the presence of microflora [13–15], and in ultraviolet light [16] and could be inactivated in a short time. This might have been one of the reasons that a significant repellent effect of the phytoecdysteroids of *S. coronata* was observed at very high concentrations, 0.3–0.7% [12], whereas the physiological effect of lower doses promoted the survival, growth, and development of larvae and was called biostimulating [17] or adaptogenic [18].

As noted in a review by Dinan [19], despite numerous laboratory biotests, progress in this area is hampered by a lack of conclusive evidence for a significant protective role of ecdysteroids on intact plants. Important data on the antifeedant activity of phytoecdysteroids and their contribution to interactions in the plant–insect system can be obtained by comparative studies of the insect pest infestation and resistance of plants from agroecosystems with different ecdysteroid levels.

The purpose of the present study was to estimate the accumulation of phytoecdysteroids and resistances of agricultural populations of *R. carthamoides* and *S. coronata* L. to herbivores at different ontogenetic stages;

to study the relationship between the distribution of ecdysteroids in the structure of plants and ecological interactions with insects; to identify factors that enhance the insect pest damage or protect the species from insect attack; and to estimate the pest damage to plants.

## OBJECTS AND METHODS

Characteristics of agricultural populations. The studies were performed during 1989–2005, in the southeast of Arkhangelsk oblast (61–62°N), which includes Kotlas raion and the Koryazhma River. The research objects were 12 uneven-aged seed-origin agricultural populations of *R. carthamoides* and *S. coronata*, each of 1–3 hectares. The areas are occupied by communities with a monoedificator. Depending on soil conditions and ontogenetic age, the population density (thousand individuals per hectare) of *R. carthamoides*/*S. coronata* was respectively: 23–32/15–56 for virginial individuals; 12–24/10–27 for young individuals, 4–24/7–21 for old reproductive individuals, and 1–16/5–20 for subsenile individuals. The populations were cultivated in rows 70 cm apart, and the crop was harvested once in the fruiting stage, except on clay loams, where both species were cultivated without harvesting the aerial biomass throughout the cultivation. Mineral fertilizers were applied in a dose (NPK)<sub>60–90</sub> in the first three years of life.

The agricultural populations were located on the main types of natural soil:

(a) sandy soddy medium-podzolic soil on sandy glaciofluvial deposits;

(b) sandy soddy medium-podzolic soil underlain by medium loams;

(c) peaty gley-podzol on two-component deposits with an admixture of sand at the top and heavy loam at the bottom;

(d) surface-gley loamy soddy cryptopodzol. The cultivated land is drained by canals spaced 40–45 m apart (except on sandy soil).

According to the nature of the divide, the topographical position of the populations is low (on peats and clay loams) or high (on sands and sandy loams). The areas with sandy soils are characterized by the best warming and air humidity deficit in the daytime. The agroecosystems on loamy and peaty soils have a cooler microclimate and increased ambient humidity due to excessive soil moisture.

As the drainage canals are overgrown with forest plantation cultures, the environment of the populations is confined and un aerated (isolated on three sides by deciduous species 10–12 m high), semi-open (intermittent isolation on two sides) or open (at a distance of 50–200 m from the forest margin). From late June to early July, semi-open aerated conditions were created on part of the agricultural peatlands by mowing neighboring areas of perennial grasses for fodder purposes.

Natural-climatic conditions. The research territory belongs to the middle taiga subzone and is part of the European–West-Siberian taiga-forest bioclimatic area, which is characterized by a short frost-free period, considerable cloudiness, insufficient sunlight in the ultraviolet region, and overmoistening. Permanent snow cover occurs on November, 11–16 and persists until April, 17–19. The growing season lasts 165–186 days, including frost-free 105 days (77–139). The mean annual temperature sum is 911°C (54–57 days) for temperatures above 15°C; 1577°C (107–110 days) for temperatures above 10°C; and 1936°C (153 days) for temperatures above 5°C. The mean temperature in July is +17.4°C. The absolute temperature gradients reach +35°C in the shade and –51°C on the soil surface.

Air temperature transition through +5°C and the beginning of the growing season of perennial cultures occur in late April–early May. Soil frost of –5...–7°C and cold spell with repeated snowfalls retard the growth and development of plants until the beginning of the second–third decade of May. Spring frosts cease at the beginning of the first decade of June, and autumn spells begin in late August–early September. Autumn temperature transition through +5°C with the end of the growing season is observed in late September–early October.

The region is characterized by a long photoperiod. At the beginning of the growing season, the daytime length is 16 h, and during the flowering of *R. carthamoides* (the end of the second decade—the beginning of the third decade of June), it is 20 h. At the onset of flowering of *S. coronata* (the middle of July), the daytime length is 18 h, and in the second half of the flowering period (the middle of August), it is 15 h.

The zonal humidity factor (the ratio of precipitation to evaporation) is close to 1.5. The yearly amount of precipitation is 495–538 mm, including 367–387 mm during the warm period. The mid-decade relative air humidity at noon is 54–62%. In some summer drought periods, the afternoon humidity drops to 25–35% or below; in the night and morning hours, atmospheric water vapor is condensed in the form of dew.

A characteristic feature of the region is a frequent change of air masses. The Arctic air masses enter the area to cause air and soil frost. In summer, cyclones from the Atlantic Ocean and the Barents Sea often cause cold snaps and drizzling and frequently heavy rains. The wind speed averages 4.2 m/s. Wind speed of 15 m/s or more is rare (up to nine days per year). Southerly winds prevail in winter, and north-westerly winds [20], in summer.

Methods. Biological periodization of the life cycle was performed following Rabotnov [21] and Zaugol'nova et al. [22]. The continuous ontogenetic process of the agricultural populations was divided into the virginial (prereproductive), reproductive, and senile (post-reproductive) periods. Chronological age was counted from the time of seedling emergence, and biological age is given for the dominant group in the structure of

the age spectrum. The age states and their distinctive features were determined by studying the following qualitative characters of individuals during field phenological observations and stationary morphological analyses: the ratio of the individual proportions of the reproductive and vegetative shoots in the aerial biomass, reproductive parameters, the fruiting rate and quality, biomorphological features of the rhizome structure, etc.

In the prereproductive period, the indices of age states were: the development of an embryonic rosette shoot with a caudex for the juvenile state, tillering of vegetative shoots and rhizome development for the immature state, and the development of a system of vegetative shoots and no fruiting for the virginal state. Distinctive features of young, middle-aged, and old reproductive plants were determined from the absolute and relative indices of vegetative, reproductive, and immature shoots (number and share), real seed yield, and seed quality (from the average weight of 1000 seeds and the proportion of filled and shriveled fractions).

The age states of the reproductive period were identified using the following criteria: development of reproductive shoots, poor fruiting, and no rhizome death for the young reproductive state; relative maximum of reproductive shoots, rapid growth and fruiting, a balance between new growth and dieback for the adult reproductive state; a sharp decrease in the proportion of reproductive shoots, impaired growth, imperfect and irregular fruiting, and the prevalence of necrosis on the rhizome branches for the old reproductive state. In the postreproductive period, the subsenile age state was identified by the absence of reproductive shoots in most individuals, greatly reduced quality of fruiting, impaired ability to form regeneration buds, and particulation of rhizomes.

Field and phenological observations of the plant development stages in the growing season were performed according to Methodological Instructions [23]. The following phases were noted: shoot regrowth, budding, early flowering, peak flowering, fruiting, shoot necrosis, and rest. Interactions between plants and insects were studied by phenomenological monitoring of plant growth and development during the plant life cycle. The observations were performed three times from the moment of regrowth to the bud stage, two times during flowering, and once a week during fruiting. When some plant organs were attacked by herbivorous insects, observations were performed daily or every other day.

Relative humidity and air temperature in the agroecosystems were measured by digital portable devices; data were taken during the 1996–2004 growing seasons. The measurements were made at three levels: in the grass stand, at the level of buds and inflorescences, and above the grass stand (at 60–70, 140–160, and 220 cm, respectively). Measurements were performed three times each for 3–5 min from 13 to 17 hours. Dur-

ing the hours of darkness, data were taken using the programmable storage function of the digital devices.

During sampling of individuals for studies of biological age, morphological structure, ecdysteroid contents, and insect damage to plants in the agricultural populations, 60–80 m<sup>2</sup> experimental plots were laid out at 6–9 points along two diagonals of the field.

For studies of the phytomass structure, 6–10 typical individuals were randomly selected from each of the plots. The aerial part was cut off at the soil surface, and the underground part was dug out, shaken off from the root soil, and washed out in flowing tap water. The aerial parts were separated into morphologically different organs: (a) vegetative shoots consisting of rosette leaves of different ages (young, adult, and old); (b) reproductive shoots comprising stems, stem leaves (upper young, middle adult, and lower old), and inflorescences. The reproductive shoots, in addition, were differentiated into apical, upper, middle, and lower parts, and the inflorescences into the receptacle and seeds. In the underground part, the following organs were distinguished: the rhizome consisting of regeneration buds and secondary roots; the tap root branching into lateral roots.

The dynamics of phytoecdysteroids during the ontogeny of the agricultural populations was studied according to the age periods and age states of the life cycle for *R. carthamoides* on sandy clay soil and for *S. coronata* on sandy clay and peaty soils. Plant samples of the juvenile, immature, and virginal age states were identified and analyzed in the prereproductive period; young and middle and old reproductive plants in the reproductive period; and subsenile plants in the postreproductive period. Samples were taken of the following ecdysteroid-containing biomass components: leaf blades of vegetative tillers in *R. carthamoides* and reproductive shoot apices in *S. coronata*. The sampling was timed to coincide with the period of the maximum content of the target substances—budding and flowering stages [24, 25]. Samples of vegetative shoots were collected from 20–25 plants (two rosette leaves 35–45 cm long from each plant), and samples of reproductive shoots from 12–15 individuals (seven–eight metamers 25–30 cm long).

The plant material was dried in the shade at a variable temperature of 23–25°C to 35–40°C and a relative air humidity of 25–40%. The samples were placed in 2–3 cm layers on racks located at a height of 40–60–150 cm from the floor level. The dried material was one-piece, except for the largest flower-bearing stems which were cut into 5–7 cm segments. The residual humidity of the air-dry raw material determined by accelerated drying at 130°C was 10–12%. Air-dry raw material samples for phytoecdysteroid analysis were prepared by quartering. Before analysis, they were stored in plastic bags at room temperature.

The ecdysteroid concentration in various plant organs and biomass components were investigated by re-

versed-phase high-performance liquid chromatography RP-HPLC) with computer data processing using the internal standard method<sup>1</sup> [26]. Chromatography was carried out on a Milikhrom-5-3 microcolumn liquid chromatograph with a 80 × 2 mm column using a Nucleosil C18 sorbent with a particle size of 5 μm (Medikant company, Orel, Russia) and a (75 : 24.2 : 0.8) water–ethanol–butanol mixture as eluent. The elution rate was 100 μl/min. The UF detector had λ = 242 nm. The paper gives averaged values of two biological and three analytical replications. The total content of phytoecdysteroids is given in percentage of the air-dry material.

The susceptibility of the species to insect attacks—the individual infestation of shoots of *R. carthamoides* by insect herbivores (cetoniine beetles, shield bugs) was determined by visual counting of the number of individuals per inflorescence; the population infestation was determined on experimental plots, each containing 250–330 plant individuals. In estimating the susceptibility of *S. coronata* to aphid attack, we counted the number of attacked shoots and noted the length of the infested part and the average number of insects on the perimeter of a 1-cm-long region. The attacked plants on the plots were tagged to trace the consequences of their infestation by pest insects.

Herbivore damage was determined after manual harvesting and threshing of the seed crop (separately from sporadically, moderately, and severely infested and uninfested plants). The dependence of the fruiting quality on the degree of herbivore damage was estimated from the reproduction level (weight and number of inflorescences and percentage seed yield) per shoot and individual. The real seed productivity of *R. carthamoides* was determined from the inflorescence yield using samples consisting each of 110–970 flower heads. The samples of *S. coronata* consisted of 550–1100 heads including the sum of the terminal and lateral inflorescences collected from 15–25 individuals. After threshing, the inflorescence seed yield was determined by weighing on a laboratory balance. Seed quality was determined from the average weight of 1000 seeds and from the proportion of filled and shriveled seeds; pest damage was noted. Seed quality was assessed by making transverse cuts (indentations) with a sharp object on the lateral faces of the seeds.

## RESULTS AND DISCUSSION

Specific features of development. According to the life form, both examined species are polycarpic herbaceous perennials. In subalpine meadows, the ontogeny of *R. carthamoides* lasts 50–75 years. The average rela-

tive age of individuals is 25–35 years. In the reproductive period, plants aged 6–9 to 30–48 years are noted. Senile individuals are most often absent in natural ce-noses [27, 28]. In culture, the life cycle of plants can last 12–15 years or more [29, 30].

In agricultural populations, adult individuals of *R. carthamoides* consist mainly of vegetative shoots (17–50 pieces) which form a tuft 50–110 cm in diameter. The reproductive shoots are few in number: on the average, 0.2–0.8 pieces per individual in the young age state, 0.7–1.1 in the adult state, 0.2–0.5 in the old state, and 0.03–0.13 in the subsenile age state. In northern Europe, the phytomass of the species is represented mainly by rosette leaves of vegetative shoots: 100% in the immature age state, 90–95% in the virginal age state, and 84–91% in the reproductive state [31]. The rosette leaves are petiolate and large, 60–80 to 100–120 cm long, with a leaf blade 10–25 to 35–43 cm wide [6, 28]. The stem leaves are much smaller: the lower leaves are petiolate and 15–24 cm long; the upper leaves are sessile and 2–5 cm long.

The development of the reproductive shoots of *R. carthamoides* to the bud stage takes 15–23 days, to the flowering stage 48–56 days, and to fruiting 72–77 days. Their growth ceases with the onset of flowering. Seed formation and development is related to the functioning of the floral receptacle, whose tissues provide transport of nutrients from the leaf organs to the reproductive shoot apices. The reproductive shoots are 150–180 cm high; a single inflorescence—a head 4–7 cm in diameter—is produced at the upper part of the hollow stem. The flowering is synchronous and falls on June, 11–26, depending on age and soil conditions, and the fruiting stage falls on July, 10–17. After maturing, the seeds are dispersed by the wind and are in induced dormancy under a snow cover until spring germination. After fruiting, the reproductive shoots die, and the rosette shoots continue to grow, gradually decreasing in size and number, until persistent frosts.

The ontogeny of *S. coronata* is poorly studied. For plants cultivated in northern Europe, its duration is not less than 11 years according to Savinovskaya [32] and over 14 years according to Timofeev [30]. Young individuals consist of 3–7 rosette shoots and reach 35–40 cm in height. Adult individuals form a tuft 100–150 cm in diameter and 140–190 cm high, which consists mainly of upright or inclined stems of reproductive shoots. Their number varies from 12 to 34 pieces per individual in adult reproductive-age plants and from 5 to 14 in old reproductive-age plants. The share of vegetative shoots in the phytomass is insignificant—1–8 or 3–12 (15% in mass fraction) [31]. The rosette leaves are 32–74 cm long; the stem leaves gradually decrease in size from base to apex from 40–20 to 7–3 cm along the length and from 18–10 to 3–2 cm along the width.

At the onset of flowering, the growth and development of the reproductive shoots of *S. coronata* (unlike *R. carthamoides*) do not cease but continue for about

<sup>1</sup> Biochemical analyses of plant samples were performed at the Laboratory of Plant Biochemistry and Biotechnology (1992–2000) and the Biochemical Laboratory of the Botanical Garden (2001–2005), Institute of Biology, Komi Scientific Center of the Russian Academy of Sciences (Syktyvkar).

1.5–2.0 months—from middle July to late August—early September, because of branching and the formation of a system of lower-order shoots. Generally, the development of *S. coronata* to the bud stage takes 33–40 days, to flowering 65–90 days, and to fruiting 105–120 days. Vegetative shoots develop up to the bud stage, and gradually die at the onset of flowering.

The axial structure of the reproductive shoots of *S. coronata* includes, on the average, 13–15 (9–18) metamers, which in the vertical direction can be divided into four zones: lower, middle, upper, and apical. On the unbranched part of the stem, the lower metamers nos. 1–3 are each 8–20 cm long and, unlike the others, have petiolate leaves; the middle metamer nos. 4–7 (5–16 cm along the axis) have sessile leaves. In the upper metamers nos. 8–11 (3–9 cm along the axis), second-order lateral shoots 70 cm long develop from the bases of the stem leaves. These shoots produce third-order shoots with buds, of which only part reaches flowering. The apical metamers nos. 12–15 (each 2–7 cm) have lateral shoots 5–20 cm long, which flower after the leading shoot and produce filled seeds. The total number of inflorescences on an individual plant varies from 15 to 70.

The underground part of *R. carthamoides* plants is located in a soil layer 0–30 cm deep, consists of a rhizome and a tap root with numerous hard and thin secondary and lateral roots colored gray and dark and branched out into 5–7 orders 3–7 to 25–40 cm long and 0.05 to 2–3 (5–8) mm in diameter [6, 33]. The tap root system is not well developed and is significant only in the early phases of plant development, before rhizome formation. The rhizome is woody; in its upper part, regeneration buds germinate at the base of the growing basal parts of rosette shoots and then develop into dicyclic and polycyclic vegetative shoots, which, after 2–5 seasons of functioning, become reproductive shoots and flower.

In adult plants of *S. coronata*, the rhizome is ligneous and horizontal with dicyclic regeneration buds in its upper part, which expand into vegetative shoots in the first year of development and into reproductive shoots in the second year. The middle and lower parts of the rhizome sprout into numerous yellowish-grayish cord-like secondary roots 10–25 cm long and 1–2 mm in diameter. The tap root system is not well developed and functions only until the beginning of the reproductive period.

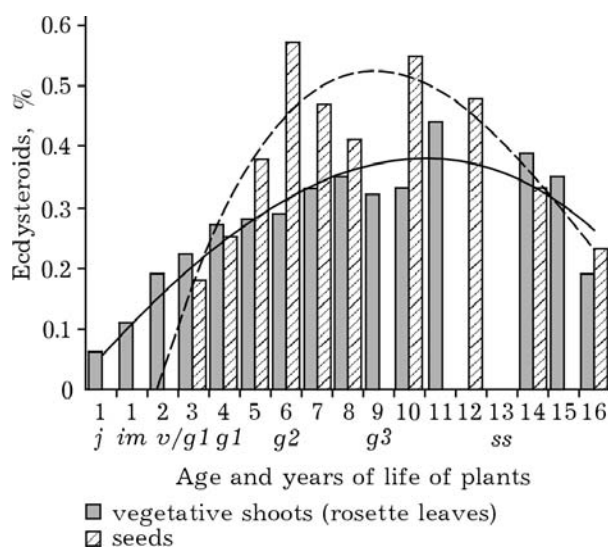
Dynamics of phytoecdysteroids during ontogeny of *R. carthamoides*. It is known that phytoecdysteroids are characterized by high mobility and the capability of being redistributed and accumulated within the age elements after biosynthesis [34, 35]. Redistribution between the growing old and developing organs through structural phytomass components can depend on both the biomorphological features of the species and differences in ontogeny.

Literature data on ecdysteroid contents in the aerial parts of plants are fragmentary and do not clarify the pattern of ontogenetic variability; reproductive plants of *R. carthamoides* were studied in the first, third, fourth, and sixth year of growth [24, 36], and reproductive plants of *S. coronata* in the third and fourth years of life [25, 37–39]. In *R. carthamoides*, the synthesizing and donor organs are the adult leaves of vegetative (rosette) shoots, and accepting organs are the rapidly growing apical parts and the developing seeds of reproductive shoots.

Under the conditions studied, the full life cycle of *R. carthamoides* lasts 16 or more years. The length of the reproductive period was from the third to ninth year of life on clay loams, from the fourth to eleventh year on peats, from the fourth to twelfth year on sandy loams, and from the sixth to eleventh year of life on sands. Old populations in the subsenile age state were noted on clay loams in the tenth and eleventh year of cultivation and on sandy loams in the thirteenth to sixteenth years. The agricultural populations on sands and peats cultivated for twelve years were still in the middle-reproductive age state.

According to our studies, the phytoecdysteroid content in the aerial organs depended directly or indirectly on the growth processes due to the long-term development of species in ontogeny. In adult leaves of vegetative shoots, the phytoecdysteroid level is minimal in the first year of development and increases with age, reaching a stable level at the onset of the reproductive period (Fig. 1). In old plants, it gradually decreases with time to the levels of the prereproductive period.

The ecdysteroid content in vegetative shoots is 0.06–0.11% in the juvenile age state 25–40 days after



**Fig. 1.** Dynamics of phytoecdysteroids in the ontogeny of *Rhaiponticum carthamoides*. Curves in Figs. 1 and 2 are smoothed curves of approximation of experimental data (Arkhangelsk oblast, middle taiga subzone of northern Europe; 1990–2005). For the notation of the age of plants see in section Methods.

seedling emergence, 0.17–0.19% in the immature age (first and second years of life), 0.22% in the virginal age (third year), and 0.27–0.28% in the young reproductive age (fourth and fifth years of life). Adult, old-reproductive, and subsenile individuals have almost the same ecdysteroid content. In adult reproductive plants, it is 0.33–0.35% (seventh and eighth years of life). In old reproductive plants (ninth to twelfth years of life), the variation over years is 0.32 to 0.44%. In subsenile plants of the fourteenth and fifteenth years of life, the content was 0.39–0.35%, and in the sixteenth year, it was reduced to the level of virginal plants, 0.19%.

In the reproductive shoots of *R. carthamoides*, the apical parts accumulating ecdysteroids are represented by inflorescence receptacles in the bud stage and by seeds in the fruiting stage. The ontogenetic variation of the phytoecdysteroid level in fruits is similar to its dynamics in rosette leaves. In the prereproductive period of the agricultural populations, the seeds of single fruiting plants contain insignificant amounts of ecdysteroids, 0.19%. In the reproductive period, the concentration increases considerably, to 0.57%, and in the senile period, it decreases to 0.33–0.23%.

In the seeds of plants of different age states, the ecdysteroid content varied as follows, %: (a) in young reproductive plants, 0.25 in the fourth year and 0.38 in the fifth year; (b) in middle-aged plants, 0.57 in the sixth year, 0.47 in the seventh year, and 0.41 in the eighth year; (c) in senescing plants, 0.55 in the tenth year and 0.48 in the twelfth year. The reduced ecdysteroid level observed in middle-aged plants during the peak fruiting season (from 0.57 to 0.41%), was almost restored to the former level (0.53%) after a one-year break in reproduction. This may indicate the existence of stress in the distribution of phytoecdysteroids in the donor-acceptor relations between the vegetative and reproductive shoots.

**Dynamics of phytoecdysteroids in the ontogeny of *S. coronata*.** In our studies, the ontogeny of *S. coronata* lasted more than 14 years. A distinctive feature of this species is an early transition to reproductive development—from the second or third year of life. The duration of the reproductive period is: from the third to tenth year of life on clay loams, from the third to twelfth year on peats, and from the third to twelfth year on sandy loam. Senescing reproductive plants in the age of eight to ten years are noted on clay loams and in the age of nine to twelve years on peats and sandy loams; old reproductive plants in the age of thirteen to fourteen years are noted on sandy loam. On sandy soils, because of a delay in the development due to drought conditions, six- to ten-year old agricultural populations were still in the young reproductive age state.

In the vegetative shoots of *S. coronata*, unlike in *R. carthamoides*, the ecdysteroid content increases only to the young reproductive age state (Fig. 2), and during budding, it amounts to 0.03–0.05% in juvenile plants (in 1–2 months after seedling emergence), 0.12–0.25%

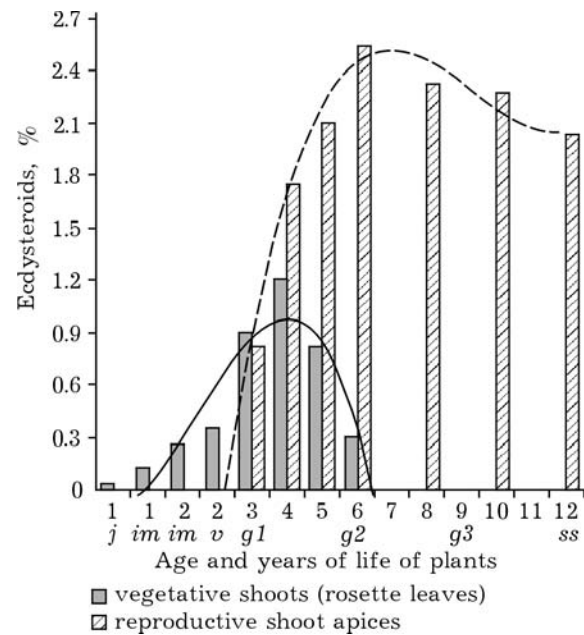


Fig. 2. Dynamics of phytoecdysteroids in the ontogeny of *Serratula coronata*.

in immature plants (first and second years of life), 0.34% in virginal plants (second year), and 0.89–1.20% in young reproductive plants (third and fourth years of life). Subsequently, with the dominance of reproductive shoots in the phytomass, the share of vegetative shoots in adult leaves decreases sharply: first to 0.81 (fifth year) and then to 0.30% (sixth year).

In the reproductive shoot apices of young reproductive plants of *S. coronata*, the phytoecdysteroid concentration increases consistently with age and is 0.81, 1.75, and 2.10% in the third, fourth, and fifth year, respectively. The maximum parameters are characteristic of the adult reproductive age state, 2.53% (sixth year of life), but during the peak fruiting season, they consistently decreased to 2.32–2.27% (eighth to tenth years). In subsenile plants (twelfth year), the ecdysteroid content was close to that in young reproductive plants—2.03%.

**Distribution of ecdysteroids in the phytomass structure of plants.** In the growing season, maximum ecdysteroid concentrations in both species are observed during budding and early flowering. In vegetative shoots, it is higher in the physiologically younger elements: 0.35% in young, 0.28% in adults, and 0.10% in old dying rosette leaves (Table 1). The same is true for *S. coronata*: 1.2–0.8–0.2% in rosette leaves and 1.8–1.3–0.2% in stem leaves, respectively. In reproductive shoots, the concentration gradient (%) increases from the lower to apical metamers; during budding in *R. carthamoides*: 0.10 in the lower part of the stem, 0.16 in the middle, 0.25 in the upper part, and 0.58 in the apical part; in *S. coronata*: 0.1 in the lower part, 0.4 in the mid-

**Table 1.** Phytoecdysteroid concentration and damage to organs and structural elements of *R. carthamoides* and *S. coronata* by herbivores

Organs of plant	<i>R. carthamoides</i>			<i>S. coronata</i>		
	Ecdysteroids, %		Damage by herbivores	Ecdysteroids, %		Damage by herbivores
	Budding	Flowering		Budding	Flowering	
<i>Vegetative shoots</i>						
Leaves:						
young	0.35	0.31	—	1.2	0.7	—
adult	0.28	0.19	—	0.8	0.3	—
old dying	0.10	0.03	—	0.2	0.07	—
<i>Reproductive shoots</i>						
Stem, part:						
apical	0.58	0.11	—	<b>2.8</b>	<b>0.9</b>	+++
upper	0.25	0.03	—	<b>2.3</b>	<b>0.7</b>	++
middle	0.16	0.02	—	0.4	0.1	—
lower	0.10	0.01	—	0.1	0.03	—
Inflorescence:						
receptacle	0.72	<b>1.17</b>	+...++	1.7	<b>2.0</b>	+...+++
seeds	—	0.57	—	—	<b>1.2</b>	+
Stem leaves:						
young upper	0.28	—	—	1.8	1.1	—
adult middle	0.28	0.03	—	1.3	0.4	—
old lower	0.28	0.02	—	0.20	0.05	—
Root system						
Rhizome	0.18	0.07	—	0.4	0.3	—
Regeneration buds	—	0.1–0.3	—	—	0.1–0.2	—
Tap root	0.13	0.12	—	0.2	0.1	—
Secondary, lateral roots	0.15	0.11	—	0.3	0.2	—

Note: Estimates of damage incidence: —, absent; +, rare, occasional; ++, frequent; +++, very frequent. The phytoecdysteroid concentration in the damaged parts of plants is in bold.

dle, 0.7–2.3 in the upper part, and 2.0–2.8 in the apical zone. During flowering, maximum phytoecdysteroid levels are observed in the rapidly developing organs (for *R. carthamoides* and *S. coronata*, respectively): 1.17/2.0% in inflorescence receptacles and 0.57/1.2% in seeds. In the underground organs, the concentration (%) is rather low: for *R. carthamoides*, it is 0.07 in rhizomes (basal parts of shoots), 0.11–0.12 in roots, and 0.1–0.3 in regeneration buds; for the root and rhizome phytomass of *S. coronata*, 0.1–0.3.

It has been suggested that the redistribution and accumulation of phytoecdysteroids in the developing and rapidly growing apical parts of plants are related to the deterrence function and protection against insect feeding [19]. The same hypothesis (explanation of the physiological meaning of ecdysteroid accumulation in reproductive organs) was put forward by Chadin with coauthors [25] for *S. coronata* plants grown in the Komi Republic and by Zibareva [40] for Caryophyllaceae

plants introduced in the Botanical Garden of the Tomsk State University.

Interactions between *R. carthamoides* and herbivores. In the prereproductive period, regardless of soil conditions and rates of development and in spite of the low level of ecdysteroids (0.1–0.2%), insect damage to the plant organs was not observed in any of the populations. The vegetative shoots represented by leaves and stalks, as well as regeneration buds, were not damaged in any of the age states of both the reproductive and senile ontogenetic periods and seasonal phases (the total phytoecdysteroid level varied from 0.10 to 0.75%). The underground organs (roots and rhizomes containing 0.07–0.18% ecdysteroids) also remained intact.

There were isolated cases of insignificant damage to the soft tissue of old and dying vegetative leaves of reproductive-age plants by unidentified lepidopteran larvae (Lepidoptera). Point window-shaped injuries of size 2–3 to 10–15 mm were observed after the fruiting

of the plants (in August–September). The ecdysteroid concentration was very low (0.06%) and close to that in dying leaf tissues (0.03%).

Similarly, in the first year of life, point injuries to seedlings (in the phase of six leaves) were found in 15 of the examined 1014 plants. In 80% of the cases, injuries were observed on the physiologically old first leaf and less often (in 20% of the cases) on the second or third leaves. The ecdysteroid concentration in these leaves was 0.026%. It is likely that the isolated cases of point injuries to old leaves, against the background of intact young and adult leaves, suggest a deterrent effect in the latter, which is observed at ecdysteroid concentrations over 0.1%.

The interaction between reproductive shoots and herbivores follows a different pattern. In the young reproductive age state (fourth to fifth years of life on sandy loams, 1993–1994; ninth to eleventh years of life on sands, 2003–2005), *R. carthamoides* inflorescences in the bud stage were attacked by coleopterans (Coleoptera: Curculionidae) and hemipterans (Heteroptera: Pentatomidae). Representatives of weevils (the stem-boring weevil *Apin seniculus* and the pear leaf-eating weevil *Phyllobius pyri*) and shield bugs (green shield bug *Palomena prasina* and the pentatomid bug *Dolycoris baccarum*) gathered within the inflorescence bracts (2–3 to 12–15 individuals per inflorescence) in the daytime during 7 to 10 days (the buds contained 0.25–0.29% phytoecdysteroids). The presence of the insects did not have a negative effect on seed development and quality.

As the reproductive shoots of separate plants reached the adult reproductive state, they were attacked by cetonine beetles (Cetoniinae) (Coleoptera: Scarabaeidae), which gnawed inflorescences, fed on the sap, and damaged receptacles. The insects were on the inflorescences for 24 hours. According to long-term observations, the severity of damage changed with age and was enhanced by ageing processes in ontogeny (Table 2). With chronological age, the infestation incidence (%) increased annually: 0 individuals in the agricultural population were infested by herbivores in the sixth–eighth years of life, 0.46 in the ninth year, 0.72 in the tenth year, 5.57 in the eleventh year, 40 in the thirteenth year, and 98 in the fourteenth–fifteenth years. Simultaneously, the number of herbivores increased: in plants of the middle reproductive age state, 1–3 beetles were simultaneously present in each inflorescence, and in subsenile plants, 2–6 (13th year) to 3–20 beetles (14–15th years) beetles. In shoots infested by 15–20 herbivores, the apical parts showed signs of wilting.

The species diversity structure of cetonine beetles is as a rule 98–99% represented by *Oxythyrea funesta*. A few individuals of *Potosia cuprea* ssp. *metallica* were found; only in 2005 in old plantations (16th year of life) did its share increase to 8.1%. Sometimes, the bee beetle *Trichius fasciatus* (Scarabaeidae: Trichiinae) was found, on the average, one individual per 250–500 inflorescences. During the fruiting period of *R. carthamoides*, which coincides with the flowering period of *S. coronata*, isolated cases of infestation of the reproductive-stem apices by aphids (*Aphididae*) from the or-

**Table 2.** Factors characterizing damage to *R. carthamoides* by cetonine (*Oxythyrea funesta*)

Type of soil	Space	Air humidity at 1–5 p.m., %	Age of plants		Damaged parts of plants		Shoot infestation	
			in ontogeny*	years of life	Organs	Biomass elements	Damaged inflorescences, %	Herbivores, specimen/shoot
Sand	Open	9–21	g2	6–8	Undamaged		–	–
The same	The same	9–21	g2	9	Reproductive shoots	Receptacle	0.46	1–2
The same	The same	9–21	g2	10	The same	The same	0.72	1–3
The same	The same	9–21	g2	11	The same	The same	5.57	1–3
Sandy loam	Semi-open	16–33	g2–g3	6–11	Undamaged		–	–
The same	The same	16–33	ss	13	Reproductive shoots	Receptacle	40.00	2–6
The same	The same	16–33	ss	14–15	The same	The same	98.00	3–20
The same	The same	16–33	ss	16	The same	The same	52.94	1–12
Peats	The same	38–53	g2	11	The same	The same	2.78	1–2
The same	Closed	43–69	g2	11	Undamaged		–	–
Clay loam	Half-closed	37–45	g2–g3	6–9	The same		–	–

Note: \*The age states: g2 and g3, adult and old reproductive states; ss, subsenile. Notation is the same for all tables and indicated for the dominant group in the structure of the age spectrum which characterizes the biological age of the agricultural population.



der Homoptera were observed. Aphid nymphs settled on the shadow, northern side of the shoots; their development was retarded, and after the drying of the stem, they died after without having reached the adult phase.

Results of biochemical analyses of the phytomass showed that damage was caused to the parts of plants that had the highest ecdysteroid levels and the organs that had lower levels remained intact (Table 1). Damage was observed on the reproductive shoot apices represented by inflorescence receptacles, which contained 0.54–0.72–1.17% ecdysteroids. A comparison of the habitat conditions of the agricultural populations on different soils revealed (Table 2) that a concomitant factor of damage was the low relative air humidity in the stand (9–33%). This is associated with increased air temperature in the daytime (34–39°C) and is due to the population location in well-heated areas with sandy soils.

The populations on clay loams (aged 6–9 years) and peats (aged 7–10 years), characterized by higher relative humidity in the stand (up to 43–69%) and moderate temperature (26–31°C), were not damaged by herbivores. In 2005, an examination of an 11-year-old population on peat revealed that in the warmer and better aerated area of the location, 2.78% of the inflorescences of *R. carthamoides* were infested by the cetonine beetle *Oxythyrea funesta*. The wetter closed zone of the agroecosystem occupying about 80% of the area is free of insects.

Thus, the primary factors influencing the insect damage to agricultural populations of *R. carthamoides* are biological age and ageing processes, which are apparently accompanied by structural changes in the ratio of major and minor ecdysteroids, which can differ in activity by several orders of magnitude [41]. An additional factor stimulating the infestation of reproductive organs by herbivores is the low relative humidity of the environment. Increased humidity, combined with a cool microclimate, hampers insect attack.

The economic damage to reproductive shoots by pests was manifested as a decrease in inflorescence weight and seed quantity and quality (Table 3). According to the results of separate collection, the seed yield

was 16.5% for severely infested plants and 30.8% for slightly infested plants (against 47.8% for uninfested plants). Because of the insect damage to receptacles, the seeds were underdeveloped and the fruiting quality was low and was characterized by the presence of a significant amount of shriveled fruits (49.1–54.4% against 15.1% in uninfested plants). The total number of full (developed) nuts per inflorescence was 39.8 and 60.6, respectively (191.6 in uninfested plants). The weight of 1000 seeds is 9.2 and 11.1 g, respectively (against 14.4).

Interactions between *S. coronata* and herbivores. In the prereproductive period of *S. coronata*, herbivores were not detected on vegetative shoots (ecdysteroid content 0.25–0.34%). In all years of observation, no damage to rosette and stem leaves was observed in reproductive and senile plants (ecdysteroid concentration 0.3–1.1%). On reproductive shoots, pests were found beginning from the adult reproductive age state, and extensive damage to the populations was observed after their transition to the old reproductive age state (Table 4). In this case, the herbivores were aphids (Aphididae).

Colonies of these insects occupy the apical and upper parts of reproductive stems beginning from the second half of the bud stage or at the onset of flowering, when the total content of ecdysteroids reaches 2.0–2.8% in the apical metamers and 1.6–2.3% in the upper metamers (Table 1). No cases of mass or individual death of aphids were noted although the surface of their body was in direct contact with the plant sap released and spreading over the stem.

The plants which were retarded in development and had not reach flowering were intact. The population on sandy loam located in an open windy area remained intact for 14 years: in summertime, aphids were not found on young, adult or senescing and old plants (Table 4).

An analysis of the factors characterizing the aphid damage to *S. coronata* revealed the great significance of ontogenetic age and flowering in the growing season. In an agricultural population of middle-aged reproductive plants (sixth–eighth years of life) located on

**Table 3.** Fruiting quality of *R. carthamoides* versus degree of herbivore damage (sandy loam, subsenile age, cetonine beetles *Oxythyrea funesta*)

Degree of damage	Reproduction per shoot			Seed quality		Number of seeds per inflorescence, ps.	
	Influorescence weight, g	Weight of seeds, g	Seed yield, %	Shriveled, %	Mass of 1000 ps., g	Total	Filled
Undamaged	6.8	3.25	47.8	15.1	14.4	225.7	191.6
Damaged:							
severely	4.4	0.72	16.5	49.1	9.2	78.3	39.8
soderately	5.4	1.66	30.8	54.4	11.1	132.9	60.6
single	6.4	2.80	43.7	27.7	13.2	211.8	157.4

**Table 4.** Factors characterizing damage to *S. coronata* by herbivores (Aphididae)

Type of soil	Type of space	Air humidity at 1–5 p.m., %	Age of plants		Damaged parts of plants		Infestation by aphids		
			in ontogeny*	years of life	Organs	Biomass elements	Percentage of shoots, %	Shoot length	Number per cm
Sand	Open	9–21	<i>g1</i>	9	Not damaged		–	–	–
Sandy loam	The same	16–33	<i>g2–g3</i>	6–14	The same		–	–	–
Peat	Closed	43–69	<i>g1</i>	3–5	The same		–	–	–
The same	The same	43–69	<i>g2</i>	6–8	Reproductive shoots	Apical and upper metamers	30–40	20–30	25–40
The same	The same	43–69	<i>g3, ss</i>	9–13	The same	The same	70–100	50–70	40–60
Peat <sup>1</sup>	Semi-open	33–47	<i>g2–g3</i>	7–10	The same	Apical metamers	5–10	2–10	8–15
Clay loam <sup>2</sup>	The same	37–45	<i>g3</i>	8	The same	The same	0–10	5–15	7–10

Note: Cultivation regimes: <sup>1</sup> mowing of marginal zones and neighboring sites, <sup>2</sup> without removal in all years.

peats, about one-third of the shoots of *S. coronata* was occupied by aphid colonies. In senescing plants (ninth–tenth years), the shoot infestation increased from 30–40 to 70%, and in old plants (13th year) to nearly 100%. The upper and apical parts of reproductive shoots were infested by aphids for 50–70 cm; each centimeter of the length on the stem perimeter was occupied by 40–60 aphid nymphs.

A concomitant factor of damage was the high air humidity due to the location of agricultural populations. The closed and draft-proof space around a population on peat favored mass infestation of reproductive shoots by herbivores, and open location on sand and sandy loam prevented it. On peats, the temperature in the evening decreased from 26–29 to 12–15°C, and dew fell, which persisted in the stand from 9–10 p.m. to 10 a.m. This produced increased humidity—80–95% in the evening and morning hours and 43–69% in the daytime. The air temperature in the stand of the populations on sandy soils reached 39–41°C; dew fell late at night and evaporated early in the morning. In the daytime, there was atmospheric moisture deficit (9–21%).

The mowing of the boundary zones and neighboring areas of peatland populations in the early the bud stage to create semi-open aerated space reduced the shoot infestation incidence from 30–40 to 5–10% and the total number of insects on the infested shoots by a factor of 5–15. In addition, the old reproductive agricultural population (eighth–ninth years of life) located on clay loams with a humid microclimate but cultivated without removal of biomass was also slightly infested by aphids (for 0–10%).

In late autumn in areas with a humid microclimate, small red larvae, presumably midges (Diptera: Itonididae) appeared in inflorescences of *S. coronata*. They developed in the receptacle tissue and feed on seeds beginning from the milky-wax stage of ripeness to the pe-

riod of profound rest in late autumn (phytoecdysteroid concentration 0.9–1.2%).

There is a direct relationship between the severity of damage by herbivores and the reproduction quality of *S. coronata* (Table 5). In damaged plants, the apical and upper metamers of the leading shoots cease to develop and dry up, and seeds do not develop or remain underdeveloped. Severely and moderately infested plants are characterized by a smaller number and weight of the developed inflorescences (4.9–6.0 inflorescences per shoot against 10.6 in uninfested plants; and 0.86–2.21 g per inflorescence against 4.58 g).

The fruiting indices are low: in the case of severe damage, the seed yield from one inflorescence is 5.4% against 26.6% in uninfested plants. The seeds are underdeveloped and are characterized by an increased percentage of shriveled seeds compared to uninfested plants (28–32% against 7.8%). The weight of 1000 seeds is equal to 2.61 g against 4.38 g in uninfested plants. The damage caused to the seed crop by pests is significant: the weight of seeds from the plants attacked by herbivores is 30–40 smaller than that from uninfested plants (0.22 g against 6.08 g/individual).

## CONCLUSIONS

1. During the long-term studies of *R. carthamoides* and *S. coronata* in northern Europe, it was established that the full life cycle of the species in the agricultural populations lasted 16 years or more. In *R. carthamoides*, the concentration of the main active substances—phytoecdysteroids—in vegetative shoots was minimal in the first year of development (0.06–0.11%), increased with time, and was relatively stabilized upon reaching the reproductive age (0.33–0.44% in the 7–12th years of life). In old plants, it gradually decreased to the levels of the prereproductive period (0.39–

**Table 5.** Fruiting quality of *S. coronata* versus degree of herbivore damage

Degree of damage	Reproduction					Fruiting parameters			
	per shoot		per inflorescence			Seed quality		Weight of seeds	
	number of inflorescences	weight of inflorescences, g	weight of one inflorescence, g	weight of seeds, g	seed yield, g	shriveled, %	weight of 1000 ps, g	per shoot, mg	per individual, g
Undamaged:									
flowering	4.9	1.98	0.39	–	–	–	–	–	–
fruiting	10.6	4.58	0.43	114.3	26.6	7.8	4.38	1216.1	6.08
Damaged:									
severely	4.9	0.86	0.18	4.8	5.4	28.4	2.61	23.9	0.22
moderately	6.0	2.21	0.37	88.1	23.8	32.2	3.78	526.8	2.30
weakly	8.4	3.44	0.41	84.3	20.5	50.9	3.76	704.7	4.11

0.35–0.19% in the 14–15–16th years). In the apical parts of reproductive shoots (seeds) the ecdysteroid content in young reproductive plants was insignificant (0.25–0.38% in the 4th–5th years) and maximal in the middle-aged and senescing plants (0.41–0.57% in the 6–12th years); in senile plants, it was close to the level of young reproductive (0.33–0.23% in the 14–16th years).

2. In the vegetative shoots of *S. coronata*, the ecdysteroid content increased only to the young reproductive age state (0.89–1.20% in the 3rd–4th years of life). In mature reproductive plants, the concentration decreased sharply (to 0.81–0.30% in the 5th–6th years). In the apical parts of reproductive shoots, the concentration consistently increased from the minimum (0.81% in the third year) to the maximum level in adult reproductive plants (2.53–2.27% in the 6th–10th years). In old subsenile plants, the ecdysteroid content was close to that in young reproductive plants (2.03% in the 12th year).

3. In the growing season, the maximum concentration of ecdysteroids in both species was observed during budding and early flowering. In vegetative shoots, it was higher in physiologically younger leaves. In reproductive shoots, the ecdysteroid concentration gradient increased from the lower to apical metamers. Maximum levels were characteristic of the rapidly developing organs (for *R. carthamoides*/*S. coronata*, respectively): 1.17/2.0% in inflorescence receptacles and 0.57/1.2% in seeds.

4. There is no direct relationship between the total concentration of phytoecdysteroids and pest damage to plants. Vegetative shoots in ontogeny were not damaged by herbivores. During flowering, the reproductive shoot apices of senescing plants of *R. carthamoides* were severely attacked by cetonine beetles (Coleoptera: Scarabaeidae) and *S. coronata* by aphids (Aphididae). Damage was caused to the structural elements of the phytomass with the highest ecdysteroid concentration (receptacles and seeds in *R. carthamoides* and the upper and apical metamers in *S. coronata*). The se-

verity of damage was aggravated by ontogenetic ageing of plants.

5. It is suggested that the susceptibility of the reproductive organs in senescing and old plants to insect attack is caused by biochemical changes in the composition of ecdysteroids having different activity. A concomitant factor is the environmental microclimate (humidity and air temperature), which depends on the location of agricultural populations. The open and well-heated space with low humidity favored the infestation of *R. carthamoides* by cetonine beetles. In contrast, the closed and un-aerated space around populations of *S. coronata*, coupled with increased humidity, favored the mass infestation by aphids and open space prevented it.

6. There is a direct relationship between the severity of damage to plants by herbivores and the reproduction quality. Due to insect damage, the seeds remained underdeveloped and the fruiting quality was low and was characterized by an increased percentage of shriveled seeds. In severely infested plants of *R. carthamoides*, the seed yield was 16.5% (against 47.8% in uninfested plants) and the number of full nuts was 39.8 (against 191.6 in uninfested plants). The weight of 1000 of seeds was equal to 9.2 g against 14.4 in uninfested plants. The pest damage to the seed crop of *S. coronata* was also significant: the seed weight from herbivore-infested plants was 30–40 lower than that from uninfested plants (0.22 against 6.08 g/individual).

This work was supported by the Russian Foundation for Basic Research and the Administration of the Arkhangelsk oblast of the Russian Federation (grants 08-04-98840 and 03-04-96147). The author expresses gratitude to A. L. Lobanov (Candidate in Biology), leading researcher of the Laboratory of Systematization of Insects, Zoological Institute, Russian Academy of Sciences (St. Petersburg) for help in identification of insects and to V. V. Punegov (Candidate in Chemistry), senior researcher of the Biochemical Laboratory of the Botanical Garden, Institute of Biology, Komi Scientific Center, Ural Branch of the Russian Academy of Sci-

ences (Syktyvkar) for help in high-performance liquid chromatography analysis of ecdysteroids.

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