AGRICULTURAL BIOLOGY, 2023, V. 58, Iss. 1, pp. 114-141
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]
ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 633.8:58.144:58.01:57.017.6:574.45:581.192
doi: 10.15389/agrobiology.2023.1.114eng
doi: 10.15389/agrobiology.2023.1.114rus

EXPERIENCE OF *Rhaponticum carthamoides* (Willd.) Iliin CULTIVATION AS A NATURAL SOURCE OF ECDYSTERONE UNDER THE CONDITIONS OF THE ARKHANGELSK REGION

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The author declares no conflict of interests
Final revision received May 15, 2022
Accepted July 7, 2022

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Abstract

In 2021, the global market for ecdysterone-containing substances amounted to $100 billion and is expected to grow significantly in the next 5 years. The aboveground and underground parts of *Rhaponticum carthamoides* (Willd.) Iliin are primarily suitable to obtain ecdysterone-containing products of pharmaceutical quality. However, both in Russia and abroad, common technologies for *R. carthamoides* cultivation and study are not focused on the quality of the medicinal raw materials. In this paper, we have implemented for the first time an alternative technology for the production of ecdysterone-containing substance from the leaf parts of *R. carthamoides*. The technology is simple and can be scaled up in agropopulations of the Northern European Russia and meets the key requirements for industrial raw materials and end ecdysterone products set by international experts. Our goal was to summarize 32 years of experience in growing *R. carthamoides* on a plantation located in the European Northeast of Russia (Arkhangelsk Province). We assessed the potential for longevity and productivity of the agricultural population by life cycle stages and age, the regularities of ecdysterone accumulation in annually harvested aboveground plant parts, and the quality of obtained plant raw material for the content of standardized substances. The study was performed in the southeast of the Arkhangelsk Province (the Middle Taiga subzone, Kotlass District; 61°20″N, 47°E) in an agropopulation of *R. carthamoides* (the field area of 1 ha) in 1989-2022. The seeds obtained from the Botanical Garden of the Komi Scientific Center, UB RAS (Syktyvkar) were initially originated from the Altai natural population (first collected in 1956). Autumn sowing was performed in mid-October after the beginning of autumn frosts (row spacing of 70 cm, seed embedding depth of 2-3 cm, seed rate of 2.7 kg/ha; 58 % field germination of seeds). Mineral fertilizers ($NPK_{60\text{-}90}$) were applied during the first three years after sowing followed by organic farming without use of mineral and organic fertilizers, chemical pesticides, and plant growth regulators. The aboveground parts were annually harvested during budding. A set of population, agrochemical, morphoanatomical, biochemical and statistical methods were used to asses parameters of the plant population (age states in ontogenesis, population density, gross production of above-ground and under-ground organs, seed yield) and individual plants (growth, development, morphological structure, productivity of roots, leaves, and seeds). Samples were collected at optimal phases of plant development, the aboveground phytomass during budding (I-II decade of June), rhizomes in autumn, after the end of vegetation (October), or in early spring, before the beginning of vegetation (April). Shortened vegetative (rosette) and stem generative (reproductive) shoots with inflorescences were morphologically heterogeneous organs separated in the aboveground part. Industrial harvesting of plant raw materials was carried out in late May–early June, during budding. Plant material (organs, elements and fractions) was dried at 23-25 to 35-40 °C and relative humidity of 25-40 % in accordance with the procedure for harvesting and drying medicinal raw materials. The samples of air-dry raw materials for further determination of the content of primary and secondary metabolites were formed by quartering method. The amount of ecdysterone in dry samples was determined by reverse phase high performance liquid chromatography with the internal standards (a liquid microcolumn chromatograph Milichrom-5, column 80×2 mm, Nucleosil C18 sorbent with a particle size of 5 μm; LLC «Medicant», Russia). Potentially dangerous substances were also assessed. Duration of agropopulation ontogenesis was close to parameters of natural populations in subalpine meadows and is more than 30 years without transition to senile age on the 33rd year of life, plant density reached optimum values of 28-23 thousand pcs/ha, starting from the 3-4 years of life. Diseases and pests did not affect vegetative aboveground mass and roots with rhizomes. The average root mass with rhizomes during the period of relatively stable mass production (from the 5th to the 32nd year of life) was 246.3 g/plant, aboveground mass was 223.4 g/plant. Calculated annual productivity of the agropopulation during the same period averaged approximately 5300 kg/ha for aboveground parts, and approximately 6100 kg/ha for underground parts. Vegetative type of reproduction was most pronounced for subsenile age state (from the 13th to the 32nd year and onwards), when seed production was extremely low, 1.3 kg/ha. The seed type of reproduction was characteristic of the generative period from years 4-5 (8-30 kg/ha) with a peak during years 6-7 (108 and 78 kg/ha). In general, the percent of generative shoots in total plant biomass was insignificant throughout the life cycle. Ecdysterone biosynthesis and accumulation in the leaves of rosellate shoots were directly related to vegetative reproduction, namely to the aboveground mass value (R^2 = 0,768, or close to 80 %). More than 90 % of annually synthesized ecdysterone (22 kg/ha) was concentrated in the aboveground part of plants at optimal harvesting age (from year 5 to year 32), or about 600 kg of ecdysterone for 27 years of operation. Qualitative indicators of medicinal raw materials from leaves of *R. carthamoides* were high and met the requirements for the manufacture of pharmaceuticals with a relative purity of ecdysterone 97 %. The plant material met all the regulatory requirements of the supervisory authorities. The levels of heavy metals (Hg, Cd, As, Zn; Ni, Cu, Cr) did no exceeded the permissible level, there were no prohibited organochlorine and phosphorus compounds, the content of radionuclides ^{90}Sr and ^{137}Cs and nitrites were below the permissible limit. Ecdysterone from the dried flour of the *R. carthamoides* leaves was well extracted into aqueous and alcohol solutions and was well preserved (up to 93-98 % within 24 hours) without preservatives. Its use significantly improved animal health (a 1.6-2.5-fold decrease in mortality), had an anabolic and economic effects (i.e., a 24-33 % higher daily weight gain and a decrease in feed consumption by 11-17 %).

Keywords: phytoecdysteroids, 20-hydroxyecdysone, *Rhaponticum carthamoides*, Leuzea safflower, maral root, feed additives, anabolic substances

Leuzea safflower *Rhaponticum carthamoides* (Willd.) Iliin, 1933 is a perennial plant of the Asteraceae family (genus *Rhaponticum*, subgenus *Fornicium*) [1, 2], synthesizing the biologically active substance ecdysterone (syn.: 20-hydroxyecdysone, 20E) of ecdysteroids (ES). This is the only ecdysterone-containing adaptogen plant (syn.: *Leuzea carthamoides* DC; *Stemmacantha carthamoides*, raponticum safflower, maral grass, maral root), included in the official pharmacopoeia of the Russian Federation (IX-XIV editions, since 1961) [3], and also Republic of Belarus [4]. It is non-toxic and has no contraindications for use [5]. This species is currently absent from the pharmacopoeia of other countries in Europe, Asia and America [6, 7].

The main pharmacotherapeutic effects of *R. carthamoides* are adaptogenic, anabolic, anti-stress, anticoagulant, antioxidant, hemorheological, hypoglycemic, nootropic, and ergogenic [5]. In recent years, a series of scientific reviews have been published on the emerging opportunities and prospects for the practical use of ecdysterone-containing substances in official pharmacology and medicine for the treatment of cardiovascular, neurodegenerative and metabolic diseases [5, 8, 9]; for protection and recovery from complications of coronavirus infection (COVID-19) [10-12]; for the prevention and adjuvant therapy of malignant neoplasms, in particular breast cancer [13-15]; for preventing degenerative changes in the body associated with long-term stress and human aging [6, 16]; for overcoming heavy physical and mental stress in a healthy person, including high-performance sports where ecdysterone is not a prohibited substance [17-21]. It can also ne used as a phytobiotic and anabolic substance in feed additives for the health of farm animals and a significant increase in their average daily gain and productivity [22-25]. In this regard, it is interesting to note the recent discovery of a relatively high content of ecdysterone and its metabolites coming from wild plant seeds in the bloodstream of 20 species of birds of the family *Passeridae* (*Aves*: *Passeriformes*), which contributes to protection from harmful environmental factors and a high rate of metabolic processes of growth and development [26].

The global market for ecdysterone-containing substances in 2021 was estimated at $100 billion and, according to an analytical report and forecast by the global research and consulting company Quince Market Insights (India), the need will increase significantly in the next 5 years [27]. The market for ecdysterone drugs is largely rigged due to a sharp shortage of raw materials, since it is not controlled by regulatory authorities. Products with ecdysterone declared for sale via the Internet do not meet quality and safety standards, as well as labeling [28, 29], mainly this is an extract from *Cyanotis arachnoidea* C.B. Clarke from Asian countries, prohibited for sale due to for the content of toxic substances, in particular carcinogenic aristolochic acid [30, 31].

Highly purified isolated ecdysterone is too expensive. The cost of 10 mg of 93% purity ecdysterone from such a well-known global company as Sigma-Aldritch (USA) [32] was 40 thousand rubles as of May 15, 2022, and 52 thousand rubles as of February 8, 2023, or 4-5 billion rubles/kg, so in practice surrogates are offered. The analysis showed that instead of the 100-500 mg of ecdysterone indicated on the label, the drugs actually contain on average 700 times less, 0.09-4.2 mg per capsule (or 0.38% purity), the deviation among 9 drugs was 0.017-0.75%. This absolutely does not correspond to the declared 20E concentration of 95-99% purity [30]. In July 2021, new data was published: out of 16 studied sports supplements purchased in the USA, Canada, Great Britain, Russia, and China, only 5 contained ecdysterone, and in minute quantities (from 0.00005 to 0.15%). Ecdysterone was not detected in 11 other preparations [31]. Ecdysterone is not synthesized in the body of mammals and cannot be synthesized artificially on an commercial scale (by chemical, microbiological methods or in cell and tissue culture), therefore it is obtained exclusively from plants, the number of which is very limited [9, 33].

The value of a particular potential source of 20E is determined by its uniqueness, which consists of such indicators as the concentration of ecdysterone in biomass, availability, biological activity, intended purpose, and economic feasibility. Obviously, those species that are characterized by a high content of target substances, high productivity, the absence of toxic impurities, resistance, the ability to be introduced and long-term grown in agrocenosis are of industrial interest [33].

Plant resources for industrial production of ecdysterone are subjected to several key requirements of international experts [9]. Plants must accumulate at least 0.5% 20E, have a simple ecdysteroid profile where at least 95-97% is the target highly active component ecdysterone soluble in water and alcohol. Ideally, the raw material should be free of minor and weakly active components. Purification should not require expensive chromatographic methods. The species should not be rare or protected, have little dependence on climate and be immune to pests and diseases, that is, easily introduced and successfully cultivated in an agrocenosis. The costs of cultivation, harvesting and processing of raw materials should be minimal, and the initial processing of the crop should occur close to the place of cultivation. That is, these should be perennial plants in an agricultural population, for which the annually alienated above-ground phytomass serves as the raw material.

Ecdysterone is a low-toxic substance, does not accumulate and quickly disappears from the body after ingestion. The LD_{50} for ecdysterone is 6.4 g/kg intravenously and 9.0 g/kg orally. The half-life of 20E in the body is short and depends on the dose, route of administration, intensity of absorption into the blood, and the type of experimental animals. For example, for sheep, the half-life of ecdysterone is 0.2 hours when administered intravenously, 0.4 h when administered orally, and 2.0 h when administered intramuscularly. Excretion from the body occurs through the liver and bile into the intestines and urine. In rats with a high metabolic rate, when administered intravenously, the half-life was 8 minutes. In humans, the peak content of ecdysterone in blood plasma at single dosages of 350-1400 mg occurs after 2-4 hours after which its amount begins to sharply decrease, and after 1 day only traces of 20E remain [9, 34].

In 2020, the safety of pharmaceutically purified ecdysterone (≥ 97%20-hydroxyecdysone) obtained from Leuzea safflower was studied in rodents and domestic dogs. High dosages were used (up to 1000 mg/kg daily) for 180 days for rats and 270 days for dogs. The drug, when administered orally, demonstrated a good safety profile with no observed side effects. In vitro and in vivo genotoxicity studies were negative at doses of 1.0-1.5 g/kg in rats and dogs for 28 days. The Safety Pharmacology battery of tests (animal behaviour, CNS, respiratory function, hERG test and cardiac telemetry) revealed no abnormalities [10]. Then in 2021, after completing clinical trials, the drug was registered in the United States as a pharmaceutical under the commercial name BIO101 from the American-French company Biophytis (identifier NCT03452488) to enhance muscle growth and inhibit proteolysis (against accelerated protein breakdown, muscle weakness, sarcopenia elderly people, respiratory failure of the pectoral muscles) [9, 35].

However, when using crude extracts from plants that accumulate ecdysterone, it must be taken into account that only some of them have proven safety, for example, *Rhaponticum carthamoides* (Willd.) Iljin and *Serratula coronata* L. Most other species are toxic to varying degrees due to accumulation in their organs of other chemicals. According to the special literature, highly poisonous plants that synthesize ecdysterone include species of hellebore (*Helleborus purpurascens* Waldst. & Kit., *H. caucasicus* A.Braun, *H. niger* L.), crow's eye (*Paris quadrifolia* L., *P. polyphylla* Sm., *P. incomplete* M.Bieb.), members of the genus *Vitex* (*V. canescens* Kurz, *V. scabra* Wall. ex Schauer, *V. cymose* Bert. ex Spreng.), yew (*Taxus baccata* Thunb, *T. cuspidate* Siebold & Zucc), blue cocculus *Diploclisia glaucescens* (Blume) Diels, Daurian moonseed *Menispermum dauricum* DC., morning glory *Ipomoea petaloidea* Choisy and purple bindweed *I. hederacea* Jacq. Less toxic pecies are *Pteridium aquilinum* (L.) Kuhn, *Polypodium vulgare* L., *P. lepidopteris* (Langsd. & Fisch.) Mart., species of Silene L., Brazilian ginseng *Pfaffia paniculata* (Mart.) Kuntze, *P. glomerata* (Spreng.) Pedersen, *P. iresinoides* Spreng., *Cyanotis arachnoidea* C.B. Clarke, *C. vaga* (Lour.) Schult. & Schult. f. Relatively toxic are the species of strawflower (*Achyranthes bidentat*a Blume, *A. aspera* L.), representatives of forest mushrooms *Paxillus atrotomentosa* (Batsch), *Tapinella panuoides* (Batsch) E.-J. Gilbert and Chinese polypore *Polyporus umbellatus* (Pers.) Fr. [36, 37].

Plants that are currently considered in Europe as the best sources of ecdysterone and deserve attention for the large-scale production of substances in sufficient quantities and at a reasonable price are species from the genera *Achyranthes* (strawflower from the family *Amaranthaceae*), cyanotis from the family *Commelinaceae*, *Pfaffia* (the family *Amaranthaceae*), *Leuzea/Stemmacatha/Rhaponticum* (the family *Asteracea*), *Serratula* (the family *Asteraceae*) [9]. According to data presented in a number of analytical reviews [6, 8, 10], among these groups of plants, the above-ground and underground parts of Leuzea safflower (*R. carthamoides*) are primarily suitable for obtaining ecdysterone-containing formulations of pharmaceutical quality.

Other representatives of the world flora with adaptogenic properties and relatively high ecdysterone content, e.g., *Cyanotis arachnoide*a C.B. Clarke, *Cyanotis vaga* (Lour.) Schult. & Schult. f., *Achyranthes aspera* L., *Cyathula capitata* Moq., *Pfaffia paniculata* (Mart.) Kuntze, *Pfaffia glomerata* (Spreng.) Pedersen, arenot officially approved and cannot be sold as food or feed additives due to the content of prohibited substances, toxicity and genotoxicity [36]. When comparing the effectiveness of biologically active compounds from commercially available adaptogen plants *Panax ginseng* C.A.Mey, *Lepidium meyen*i Walp., *Rhaponticum carthamoides* (Willd.) Iljin, and *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim., the *R. carthamoides* had the greatest potential for practical use [6].

Several other members of the genus *Rhaponticum*, historically originating from the subalpine zone, for example, *Rhaponticum uniflorum* DC. found in Siberia, China and Mongolia, *Rhaponticum scariosum* (Lam.) in the Alps in Europe also synthesize ecdysterone and its analogues [2, 38, 39]. However, such promising species are only being studied on the example of single specimens and do not belong to field-cultivated crops [40, 41].

Both the underground parts of Leuzea with rhizomes and the aboveground parts, namely the leaves of vegetative (rosette) shoots, are allowed to be used in pharmaceuticals and food additives for humans [42], as well as phytobiotics for animals [35]. The regulated active ingredient in both leaves and roots is ecdysterone, 0.1%, or 1000 mg/kg dry matter [3, 4]. According to the results of comparative tests of extracts from roots and leaves (extract 1:10) at the Bekhtereva Institute of Human Brain RAS (St. Petersburg), leaves of rosette shoots of Leuzea had a multiple advantage over underground organs in terms of complex activity, 66 points vs. 16 [43]. A joint work of British and Austrian scientists reported similar results [44].

Ecdysterone, after biosynthesis in roots or leaves, is redistributed and concentrated in young and developing organs and tissues (e.g., growing leaves, apical parts, buds and seeds). The leaves of vegetative shoots of *R. carthamoides*, compared to the roots, are usually much richer in ecdysterone (the content is usually from 0.25 to 0.5-0.7%) [6, 36], and, due to annual growth, this is a renewable plant raw material. Perennial roots that have completed the growing season perform primarily an anchoring function in the soil, so the low content of ecdysterone in Leuzea roots harvested in the fall is understandable. According to publications from different countries (USSR, Czech Republic, Uzbekistan, France, Austria), the real yield of ecdysterone from 30-50-65 kg of dry roots with rhizomes of Leuzea safflower was 0.013% [45], 0.0153% [46];, 0.03% [44], 0.036% [47], 0.05% [48], 0.075% [49], and 0.101% [50].

It is important to emphasize that during storage and processing of plant materials contaminated with microorganisms, in particular the underground organs of *R. carthamoides*, ecdysterone can quickly be destroyed [9, 51]. For example, ecdysterone was not detected in a powdery substance from the roots with rhizomes of *R. carthamoides* purchased from a pharmacy chain in three large Russian cities and studied in a scientific laboratory [52]. This corresponds to the information published earlier by the laboratory of analytical chemistry (Lomonosov Moscow State University) on the trace content (0.040 mg/tablet) of ecdysterone in Levzea-P tablets (rhizome powder with Leuzea roots, Parapharm LLC, Russia) [53].

In addition, if underground parts are used, the plantation ceases to exist, the medicinal raw materials prepared in this way are of low quality, they quickly lose ecdysterone during storage, and the technological process itself is economically unprofitable for the manufacturer due to high costs. Therefore, it is more promising to collect the annually growing aboveground phytomass of *R. carthamoides* with a high content of ecdysterone,

Indeed, ethnographic primary sources for the Altai-Sayan mountain region indicate that local hunters and leaders of famous tribes (Tsetsen Khan) used deer grass in the form of powder from the leaves of Leuzea, but not from the roots with rhizomes [54]. Practical information about the peculiarities of the use of *R. carthamoides* by ethnic groups from little-known and unstudied regions of Central Asia, primarily from Northwestern Mongolia, was recorded by the Russian traveler and ethnographer G.N. Potanin in 1876-1870 during expeditions of the Imperial Russian Geographical Society.

The statement that marals (mountain deer) dig out the roots of *R. carthamoides* from under the snow and feed on them during the rutting period is not true. During special research, scientists from the Siberian Branch RAAS found that deer eat cold-resistant rosette leaves that remain green under the snow that falls early in the mountains [55]. All other representatives of the animal world in high mountain pastures (horses, cows, sheep, and deer) also eat Leuzea leaves, but not the roots, and sometimes tear off the inflorescences.

Unfortunately, generally accepted technologies and the study of *R. carthamoides* in Russia and abroad do not cover such aspect as the quality of the resulting medicinal raw materials. In previously published monographs, the authors did not study the biosynthesis and accumulation of ecdysterone and its analogues (phytoecdysteroids) in plant products, much less in agricultural populations [55-57]. Another problem is that, despite almost a century-long history of cultivation (the first crops of *R. carthamoides* in the USSR date back to 1926), it is not possible to ensure long-term economic exploitation of the species. In subalpine meadows, the ontogenesis of *R. carthamoides* lasts from 50-60 to 75-120 or more years [55, 58] while in cultivation it is reduced to 5-6 years, and the duration of economic use usually does not exceed 3-4 years [33].

In this work, for the first time, an alternative technology for obtaining an ecdysterone-containing substance from the leaves of rosette shoots of Leuzea safflower *R. carthamoides* has been implemented. The proposed technology is simple and agriculturally scalable in the European North and satisfies the key requirements set by international experts and specialists for sources of industrial production of ecdysterone. In particular, the species can be successfully cultivated for a long time (up to 30 years or more) without reseeding in agrocenosis in cold and cool climates with high humidity, is resistant to diseases and pests, and serves as a source of annually renewable medicinal raw materials. Plant raw materials accumulate a large amount of ecdysterone (0.4-0.6%), the raw material has a simple ecdysteroid profile that is more than 97% ecdysterone, the substance meets all requirements of regulatory authorities.

Our goal was to analyze a 32-year experience in cultivating Leuzea safflower at an commercially exploited plantation located in the European northeast of Russia (Arkhangelsk Province), to identify the potential for longevity and productivity of the agropopulation of *Rhaponticum carthamoides* by age period, to study the patterns of ecdysterone accumulation in annually alienated aboveground phytomass, and to assess the quality of the resulting plant raw materials with regard to the content of standardized substances.

Materials and methods. The studies were carried out in the southeast of the Arkhangelsk Province, in the middle taiga subzone (Kotlas District; 61°20″N, 47°E) in the agropopulation of *R. carthamoides* (a single 1-hectare area) during 1989 to 2022. The agropopulation of seed origin was laid in 1989. The seeds were obtained from the Botanical Garden of the Komi Scientific Center, the Ural Branch RAS (Syktyvkar). Initially, the seeds originaited from the Altai natural population were primary collected in 1956, then in the same site (IB Komi Scientific Center) 3-4 reproductions were carried out with individual selection and reseeding.

The predecessors in crop rotation were potatoes, annual crops and grain crops. Pre-sowing tillage included plowing to a depth of 22-25 cm, disking and 2-fold cultivation with simultaneous harrowing. Before sowing, the area was rolled with smooth water-filled rollers. Sowing (a four-row mounted vegetable seeder SON-2.8A, Russia), was perdormed in mid-October after the onset of autumn frosts. The seed rate was 2.7 kg/ha with a field germination rate of 58%, 70 cm of row spacing, and 2-3 cm of sowing depth. Mineral fertilizers (NPK_{60-90}) were applied only in the first three years after sowing. Further cultivation was as in organic farming (no mineral and organic fertilizers, chemical protection products, plant growth regulators, and herbicides were used). The rosette leaves from the aerial parts were once harvested annually during the budding stage. The seeds were harvested during the fruiting stage.

To determine the complex of soil agrochemical parameters of the site, samples were collected and studied using generally accepted methods (Agrokhimtsentr Kirovsky, Kirov).

The study of age states in plant ontogenesis and its periodization in the life cycle, population density (number of plants per unit area), gross production of aboveground and underground organs, seed yield with regard to the actual density of the arocenosis was carried out as outlined in the publication on other *R. carthamoides* agropopulation studies in the same region [59]. Plant age state (virginal, or pregenerative, generative and postgenerative periods of ontogenesis) were assessed based on the dominant group of individuals. Seedlings (p), juvenile (j), immature (im) and adult vegetative plants (v) were distinguished in the virginal period, young (g1), middle-aged (g2) and old generative plants (g3) in the generative period and subsenile age-related state (ss) in the post-generative period. The calendar (absolute) age of populations was counted from the time of seedling emergence. We proceeded from the following criteria: young generative age state corresponds to formation of reproductive shoots, weak fruiting, absence of rhizome dying processes; adult generative state corresponds to relative maximum of reproductive shoots, high intensity of growth and fruiting processes, balance of processes of new formation and death; old generative state means a sharp decrease in the proportion of reproductive shoots, weakened growth, inferiority and frequency of fruiting, the predominance of dying processes on the branches of the rhizome. In the post-generative period, the subsenile age state was distinguished by the absence of generative shoots in most individuals, a sharp decrease in the quality of fruiting and a weakened ability to form renewal buds and rhizome particulation [60].

In phenological observations, shoot regrowth, budding, beginning of flowering, mass flowering, fruiting, shoot death, dormancy were recorded annually:. Growth dynamics were assessed by the height of the most developed shoots of the plant from the soil level to the top of the straightened shoot. The width of the leaf blade was measured at the widest point by straightening the leaf.

The gross productivity of populations was determined as dry aboveground and underground phytomass multiplied by the actual density of the agrocenosis at the studied age. Plant density was assessed by counting in 60-80 m^2 areas at 6-9 points along the diagonal of the field. Seed fruiting was assessed by counting all fruiting inflorescences within the studied community, based on the yield of completed seeds (%) and the weight of 1000 seeds (g).

Samples were collected at the optimal phases of plant development, the aboveground phytomass during budding stage (decades 1 and 2 of June), the rhizomes in the fall after the end of the growing season (October) or in early spring before the growing season (April). In the aboveground phytomass, morphologically heterogeneous organs were distinguished, i.e., shortened vegetative shoots (rosette) and stem generative (reproductive) shoots with inflorescence. In each sample, 275-300 vegetative shoots containing 1100-1500 rosette leaves and up to 30-35 generative shoots were examined. Commercial harvest of plant raw materials was carried out in late May-early June, during the budding perios, which is characterized by the maximum concentration of the active substance (ecdysterone) in the rosette leaves of vegetative shoots (a mixture of fractions of young and adult leaves). Plant material (organs, elements and fractions) was dried at 23-25 to 35-40 °C and 25-40% air humidity in accordance with the rules for the preparation and drying of medicinal raw materials. The residual moisture content of air-dried raw materials, determined by the accelerated drying method at 130 °C, was 10-12%. Samples from air-dried raw materials for further determination of the content of primary and secondary metabolites were formed by the quartering method. Before analysis, they were stored for 3-5 months in plastic bags at room temperature.

The ecdysterone in dry samples was quantified by reverse-phase high-performance liquid chromatography (RP-HPLC) with computer data processing wih the internal standard [3]. Analyzes were performed in the laboratory of biochemistry and plant biotechnology (for 1989-2000) and the biochemical laboratory of the Botanical Garden (2001-2021) of the Institute of Biology, the Komi Scientific Center of the Ural Branch RAS (IB Federal Research Center, Syktyvkar). We used a liquid microcolumn chromatograph Milikhrom-5 (80×2 mm column, Nucleosil C18 sorbent with a 5 μm particle size) (Medicant LLC, Russia). Eluent was a solution of acetonitrile and ethanol in water acidified with acetic acid, in the mode of gradient elution of components at 100 μl/min with UV detector (λ = 242 nm). The average values of 2 biological and 3 analytical replicates (% of air-dry matter) were recorded. The qualitative composition was determined by the proportion of the most active compound ecdysterone (20E) to the weakly active ecdysone (E), the 20E/E. This parameter should be ≥ 20:1, which corresponds to a ≥ 95% qualitative purity, or the ratio of ecdysterone to other ecdysteroids 95:5 [9, 61].

Chemical analyzes of dry samples for the content of potentially hazardous substances (heavy metals Hg, Cd, As, Zn, Ni, Cu, Cr, chlorine and organophosphorus compounds, radionuclides ^{90}Sr and ^{137}Cs, nitrates and nitrites) were carried out in accordance with accepted research methods (accredited laboratories of the Agrochemical Center Kirov, Kirov) (see website http://www.agrobiology.ru).

Safety of *R. carthamoides* and identified active substances were assessed based on information about poisonous plants and herbal medicines with undesirable side effects, including guidelines on toxic substances synthesized by plants [37, 62, 63], Technical Regulations of the Customs Union "On Food Safety" [42] and reviews by A.C. Brown [64-68] on toxic world flora found in food additives. The names of plant genera and species mentioned in the article are given according to the classification IPNI (International Plant Names Index) [69].

Data was processed by standard methods of variation and correlation statistics using the Statistica module in Microsoft Excel 2016. The general totality (based on the complete accounting) and sample parameters were used. To exclude systematic errors, samples were not taken from the edge areas of the agrocenosis, and the surface layers of the soil samples were not analyzed; data on single specimens that differ sharply from normal ones in appearance were excluded. The random sampling method was applied to the residual material and typical individuals. The mean values and standard deviations (*M*±SD), coefficients of variation (*Cv*, %), lim, min-max values were calculated based on the sample size collected from the population (N).

Variability was assessed on a scale expressed by the coefficient of variation for biological studies: ≤ 7% for very low, 7-15% for low, 15-25% for moderate, 26-35% for increased, 36-50% for high, ≥ 50% for very high values [70]. The correlations between the accumulation of ecdysterone and plant phytomass over 32 years of life of the agricultural population were analyzed. The experimental data approximation curves show the corresponding value of their reliability in the form of the determination coefficient, or approximation coefficient (R^2), which assesses the strength and direction of the relationship between the two quantitative variables being studied with a 95% confidence interval (at a $p = 0.05$ significance level).

Results. In the area where the experimental site is located, the terrain is slightly undulating, elevated, the soils are sandy loam, soddy-medium podzolic, formed on two-layer deposits. The upper horizon (0-28 cm) consists of sand particles; from a depth of 70-85 cm, the medium loamy fraction predominated.

According to a set of agrochemical indicators, the soil of the site was classified as highly cultivated mineral (soil samples were studied using generally accepted methods at the Agrochemical Center Kirovsky, Kirov). The arable layer is 3.6% humus and 3.1% organic matter. The acidity of the root layer was optimal (pH_{KCl} exchangeable 6.4-6.5; hydrolytic pH 0.7 mEq), base saturation was high (12.4 mEq, or 93.5%). In terms of nutrients, the supply of phosphorus was high (mobile P_2O_5 was 31.2 mg/100 g of soil), potassium was moderate (mobile K_2O was 9.6 mg/100 g); the Ca content was 6.4 mEq/100 g, Mg was 1.0 mEq/100 g.

The research area belongs to the middle taiga subzone and is part of the European-West Siberian taiga-forest bioclimatic region with moderately cool summers and moderately cool winters, a short frost-free period, significant cloudiness and a lack of sunlight in the ultraviolet range, and excess moisture. The zonal humidification coefficient (the ratio of precipitation to evaporation) is close to 1.5. The duration of daylight at the beginning of the regrowth of *R. carthamoides* (decade 1 of May) is 16-17 hours, during flowering (decades 2-3 of June) 20 hours. The growing season lasted 165-186 days and the frost-free period was 105 days. (with an amplitude of fluctuations over the years of 77-139 days). Average annual temperatures above 15 °C were 911 °C (54-57 days), above 10 °C were 1577°C (107-110 days), above 5 °C were 1936 °C (153 days). The average temperature of the warmest month (July) was +17.4 °C, the coldest month (January) was –14.3 °C. Stable snow cover with an average height of 52-58 cm appeared on November 11-16 and persisted until April 17-19.

The temperature at the depth of the tillering node of perennial grasses remained within the range of –11.5... –12.2 °C, in certain periods it decreased to –13.5... –14.0 °C. When the air temperature passed through +5 °C on April 29, the growing season of perennial crops began. However, returns of cold weather with frosts on the soil surface (up to –15...–7 °C) and repeated snowfall often inhibited the growth and development of plants until the beginning of the second decade of May. Frosts stopped completely from the second ten days of June and resumed from the beginning of September. The completion of the growing season was observed at the beginning of October, which coincided with the autumn transition of temperature through +5 °C. During the year, 495-538 mm of precipitation fell, including 367-387 mm during the warm period. The reserves of productive moisture in the soil layer of 0-20 cm during the warm period were 37-44 mm, in the layer of 0-50 cm 55-70 mm, which is enough for the most perennial crops. Average ten-day relative humidity in the daytime was 62-74%, the lowest rates were at midday, 54-57%. In some dry periods, humidity could drop to 25-35% or lower.

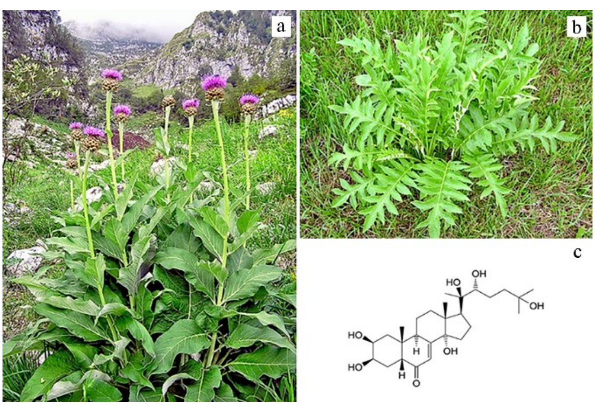


Fig. 1. Representatives of the genus *Rhaponticum* that synthesize ecdysterone: a — *R. scariosum* Lam. with generative shoots (photo by Adriano Bruna, Alps, 2009; https://www.actaplantarum.org/forum/viewtopic.php?t=13006); b — *R. carthamoides* (Willd.) Iliin with rosette leaves (photo by N.P. Timofeev; Arkhangelsk region, Kotlas district, May 2022), c — chemical structure of ecdysterone [71].

Parameters of growth and development of shoots in ontogenesis. Leuzea safflower (Fig. 1) is a perennial winter-hardy and cold-resistant plant; adult individuals form a bush 90-150 cm high (sometimes 50-250 cm). The species was introduced from the high-mountain zone of the subalpine belt (up to 3000 m above sea level) and since the early 1960s it has been introduced into production in the European North [33].

In terms of life form, *R. carthamoides* is a large herbaceous semi-rosette polycarpic plant with two types of shoots that die annually, the vegetative rosette shoots and generative stem shoots with inflorescences of varying degrees of development. It has two types of reproduction - seed and vegetative (clones). Ontogenesis of *R. carthamoides* in subalpine meadows lasts 50-75 years, where the average relative age of individuals is 25-35 years. The generative period lasts from 6-9 to 30-48 years. Senile individuals are most often absent in natural cenoses [55, 72].

Fig. 2. Plants of R*haponticum carthamoides* (Willd.) Iliin from an agro-population cultivated in the conditions of the European North-East: a — seeds, b — plants of the 1st year of life, c — plants of the generative period in the flowering phase (photo by N.P. Timofeeva; Arkhangelsk Province, Kotlas District, July 2001).

The life activity of the species in the conditions of an agropopulation (Fig. 2) based on the results of 32 years of observations could be divided into two stages: the formation of a coenopopulation, from the 1st to the 5th year of life (Table 1); further sustainable production of above-ground mass with high biosynthesis of ecdysterone (Table 2).

1. Average above-ground parameters of *Rhaponticum carthamoides* (Willd.) Iliin in a European North-East agropopulation during the first 5 years of ontogenesis (Arkhangelsk Province, Kotlas District)

Parameter	Year and plant age								N
	1990			1991		1992	1993	1994	
	p	j	im	im	v	v	g1	g1-g2	
Shoot mumber:									
total	1.0	1.0	1.0	1.2	4.2	5.7	17.2	35.6	15-20
vegetative	1.0	1.0	1.0	2.8	3.8	5.1	16.0	31.1	15-20
generative					0.4	0.6	1.2	4.2	15-20
fruit-bearing						0.01	0.16	0.84	186-4233
Height of generative shoot, cm						3-42	90.3	114.0	15-20
Height of vegetative shoot, cm	1.5	17.4	21.1	63.7		58.3	75.0	89.8	15-20
Width of rosette leaves, cm	0.6	3.0	5.0	12.0	14.0	14.5	17.5	22.5	15-20
Weight of the above-ground part:									
total, g	0.01	0.27	0.42	6.2	10.2	16.4	56.8	210.7	12-15
proportion of rosette leaves, %	100	100	100	100	100	93.3	84.0	85.4	12-15
20E content in rosette leaves, %	0.04	0.06	0.11	0.19	0.22	0.25	0.27	0.28	3-4

N o t e. 20E — ecdysterone. Age of plants in ontogenesis is p for seedling, j for juvenile, im for immature, v for virginal, g1, g2 for young and mature generative

In the first 5 years, intensive development of individuals occurred against the background of multiple annual increases in phytomass (see Table 1): seedlings in the 1st year passed through immature and juvenile age states, in the 2nd year they reached the virginal state, which continued into the 3rd year. The first year of life, and in the 4th year the transition to the generative age began, which was fixed in the 5th calendar year. Subsequently (years 6-32), the intensive growth stopped. Individuals entered adult generative (6-8 years), old generative (9-12 years) and subsenile ages (13-32 years), which differed in the ability to produce seeds, the structure and integrity of rhizomes.

2. Parameters of aboveground organs of *Rhaponticum carthamoides* (Willd.) Iliin in a European North-East agropopulation during sustainable production of above-ground phytomass with high biosynthesis of ecdysterone from the 5th to the 32nd year of life (Arkhangelsk Province, Kotlas District, 1994-1921)

Parameter	*M*±SD	*Cv*, %	max	min	*N*
Shoot mumber:					
total	31.00±12.40	40.1	60.4	17.9	15-20
vegetative	28.30±10.70	37.9	54.6	14.8	15-20
generative	2.71±2.13	78.6	3.8	0.6	15-20
fruit-bearing	0.19±0.31	167.9	1.1	0.001	20188-52
Height of generative shoot, cm	125.0±10.1	8.1	143.1	107.9	15-20
Height of vegetative shoot, cm	87.3±11.3	13.0	119.1	65.2	15-20
Width of rosette leaves, cm	24.5±3.8	15.6	33.0	19.4	15-20
Weight of the above-ground part:					
total, g	223.4±74.4	33.3	354.0	95.1	6-9
proportion of rosette leaves, %	84.4±5.3	6.3	93.9	73.2	6-9
20E content in rosette leaves, %	0.41±0.10	24.4	0.64	0.28	3-4

The senile period in the population as a whole did not occur: the plants continued to grow normally and vegetate into the 31st-33rd years of life. During an anatomical study of the underground parts at the beginning of the 33rd year of life (April 29, 2022), it was found that the processes of death and new formation of individuals are in relative dynamic equilibrium (1:1). The total number of shoots (31.5, *Cv* = 28.0%) and perennial renewal buds (52.7, *Cv* = 42.5%) was comparable to the total number of dead shoots (83.7, *Cv* = 47.7%).

Plants during the growth of aboveground organs and wintering rhizomes were not affected by diseases and pests. There were short periods in the life cycle (seedling phase) when leaves did not have the potential for resistance to leaf-eating phytophages. Birds of the family *Passeridae* were pests of seeds during ripening, *Oxythyrea funesta*, *Potosia cuprea* ssp. *metallica* (*Cetoniinae*), and *Trichius fasciatus* (*Scarabaeidae*: *Trichiinae*) were pests of inflorescences (single lesions in some years) during ovule formation [61].

Rosette leaves of vegetative shoots, the main source for the medicinal raw materials of *R. carthamoides* were large petiolate, in adult plants, more or less deeply pinnately dissected into 15-22 lobes (from no dissections to 27 lobes), light in color, yellow or dark green, formed a rosette with an average diameter of 55-90 cm (from 37 to 112 cm). When young, the surface of the leaves was cobwebby and pubescent, giving them a silvery tint. The size of adult leaves reached 60-90 cm, sometimes up to 120 cm in length and 25-33 cm in width of the leaf blade. The appearance of new leaves, their maturation and death were not confined to certain phases of development; they functioned throughout the entire growing season, changing each other over time, from the moment the snow cover melted until the onset of stable autumn frosts.

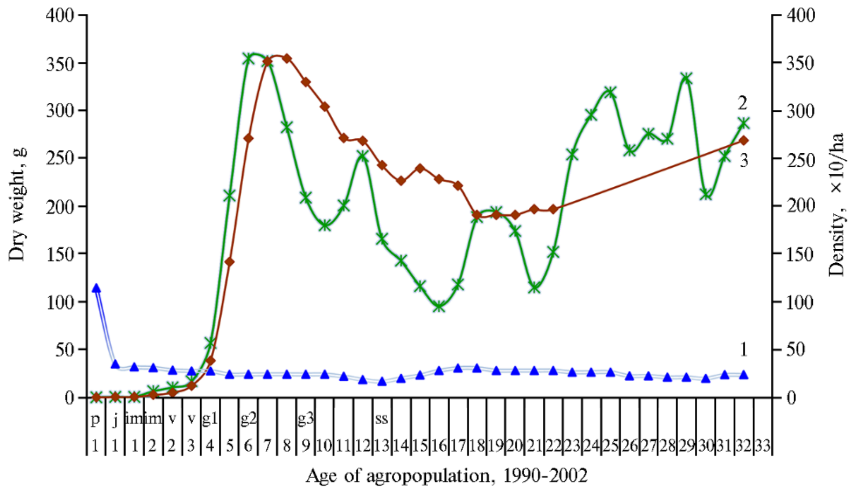
Flowering (generative) shoots had a height of 110-140 cm, sometimes up to 180 cm. The stem grew due to the intercalary growth of internodes, on which 28-55 leaves of varying structural complexity were arranged in a spiral. At the top of the hollow, unbranched stem, a single inflorescence formed was a large spherical basket with a diameter of 4-6 cm (range from 3 to 8 cm), with bisexual violet-lilac flowers. The flowering period of individuals in the agricultural population usually fell on June 14-26. At the end of June, less than 1% of the shoots bloomed. The dates of the three earliest deviations for the agricultural population are 06/10/1995, 06/10/2005, 06/09/2015; one late date is 07/07/2017. The appearance of new generative shoots and their flowering were not observed in July-September. In general, the development of *R. carthamoides* to the budding phase takes 15-23 days, flowering lasts 44-56, fruiting 72-77 days. After fruiting in mid-July, reproductive shoots died off, rosette shoots continued to vegetate until the average daily temperature dropped below 0 °C in the second decade of October, gradually decreasing in number and size. In the above-ground sphere, vegetative (rosette) shoots predominated: their share in the mass of above-ground organs in the first 3 years of life was 100-93.3%, in 4-5 years — 84.0-85.4%, subsequently (5-32 th years) remained virtually unchanged, 84.4% (with a minimum *Cv* = 6.3%). The contribution of generative shoots to the phytomass value turned out to be insignificant throughout the 32-year life cycle: 6.7% in the 3rd year, 6.0% in the 4th year, 4.6% in the 5th year, 5.6% on average for the period from the 5th to the 32nd years of life.

The total number of shoots in individuals in the first 5 years consistently increased (see Table 1): from 1.0 to 4.2 for the 2nd year; 5.7 in the 3rd year; 17.2 in the 4th year; 35.6 in the 5th year of life. The maximum number was recorded during the adult generative period during years 6-9 — 60.4-52.1, the minimum occurred at the beginning of subsenile age, during years 13-15 —17.9-20.6. During the period of stable functioning of the agropopulation with sustainable production of aboveground phytomass from year 5 to year 32 (see Table 2), the average number of shoots was 31.0 (*Cv* = 40.1%), and at the beginning of the 33rd year, it was 31.5.

The number of vegetative shoots increased until the 5th year of life (from 1.0 to 31.1), and in subsequent years the average value was 28.3 pieces (*Cv* = 37.9%). Generative shoots with inflorescences began to appear from the 3rd year, but they died off before reaching the fruiting phase (with height of 3-42 cm). In the 4th year, the plant formed on average 1.2 generative shoots, of which only a small part (0.16 pieces) bloomed and set full-fledged seeds; in the 5th year there were an average of 0.84 fruiting shoots per plant. At the subsenile age, the number of generative shoots again turned out to be insignificant (see Table 2), 0.19 per plant, which was close to the parameters of natural populations [55] and indicated a predominantly vegetative type of reproduction by clones after the disintegration of the mother plant into relatively independent daughter plants.

It is noteworthy that with a stable costs for the generative shoot formation (no more than 15-16% of the phytomass throughout the entire ontogenesis with a minimum Cv = 6.3%), the number of fruiting reproductive shoots in 27 years (from the 5th to 32 years) with average values of 0.19 per plant varied very much, from 0.001 to 1.13 (*Cv* = 167.9%). This was due both to the age characteristics of individual plants in the population and to late spring frosts up to –7... –10 °C from arctic air masses penetrating into the territory, causing irreversible damage and death of inflorescences (the rosette leaves of *R. carthamoides* are cold resistant). A similar influence of climatic factors on the variation of fruiting parameters occurred in natural mountain populations [55].

Phytomass and variability of its accumulation in ontogenesis. The productivity of the population will be determined by multiplying the number of plants per unit area by the dry phytomass of individuals. The density of *R. carthamoides* seedlings in the agrocenosis in the 1st year of life was 114 thousand/ha, from the 3rd-4th to the 10th year 27-24 thousand/ha, from the 13th to 15th 16-20 thousand/ha, then fluctuated over the years from 22-30 to 20-23 thousand/ha (Fig. 3). Over the whole subsenile age (from the 13th to the 32nd years), the average density was 24.5 thousand/ha (with fluctuations from 16.4 to 30.5 thousands, *Cv* = 16.0%). In terms of the norm for winter sowing (with a 4-5-fold reserve per unit area) with qualified care, the need for seeds for sowing was about 3 kg/ha (with an average 1000-seed weight of 15 g and field germination rate of apprx. 60%).



Fir. 3. Dynamics of density (1) and the value of dry phytomass of above-ground (2) and underground (3) organs of *Rhaponticum carthamoides* (Willd.) Iliin in a European North-East agropopulation over plant ontogenesis. Age of plants in ontogenesis: p - seedling, j - juvenile, im - immature, v - virginal, g1, g2, g3 - young, mature and old generative; ss - subsenile (Arkhangelsk Province, Kotlas District, 1990-2021).

Other seeding rates recommended in monographs on the cultivation of *R. carthamoides* and amounting to 10-15 kg/ha [57] and 20-25 kg/ha of first class seeds [56] should be considered overestimated. With high seeding rates in subsequent years, self-thinning and stabilization of the population occurred. Environmental factors causing a decrease in the number of individuals in the cenosis for *R. carthamoides* may be waterlogging, causing rotting of the root system, competitive suppression by fast-growing perennial weeds in the first three years of life, early cutting of aboveground phytomass (from the 2nd year), timing of seed sowing (spring instead of winter), soil moisture deficiency (drought), aging, etc.

The biology of *R. carthamoides* showed gradual germination over 3 years, characteristic of alpine plants: in the spring of the 1st year, in the 2nd-3rd decade of May, 85-88% of the total number of plants appeared, in the 2nd year 10-12%, on the 3rd 2-3% (provided that the soil was not waterlogged and the seeds did not rot). The first 2 months were the most vulnerable period due to the underdevelopment of the primary root system, formed by the lateral branches of the main root with a diameter of 0.03-0.05 mm, which were located in the surface layer of the soil. Mass death of individuals was possible due to suboptimal soil conditions from waterlogging and drying out. The underground sphere developed intensively after the formation of the stem root (with the appearance of adventitious roots from the hypocotyl zone), which followed in time the phase of development of the rosette embryonic shoot in juvenile age. At immature age, the proportion of the root system of plants increased from 19-21 to 43% of the total phytomass, which led to increased resistance to summer drought and ensured the growth of axillary buds of renewal into numerous lateral vegetative shoots.

In the first 3 years of life, the dry aboveground phytomass of juvenile, immature and virginal plants was 0.3, respectively; 6.2 and 16.4 g and was not of interest for alienation (Table 3). From the 4th year, when the transition of the agropopulation to the generative age began, the aboveground mass increased to 56.8 g. From the 5th year of life, a massive transition of the population into the generative state and achievement of average development parameters was observed, 210.7 g of aboveground mass per plant with an average value of 223.4 g for 5-32 years (Table 4). In the 6th-8th years (mature generative age), the maximum values of the phytomass of individuals were recorded, 354, 352 and 282 g, respectively. In the 9th-12th years of life, the population was at an old generative age, the mass of aboveground organs was 208.7-179 .9 g (see Fig. 3).

3. Productivity of the *Rhaponticum carthamoides* (Willd.) Iliin European North-East agropopulation over the first 5 years of ontogenesis (Arkhangelsk Province, Kotlas District)

Parameter	Year and plant age								N
	1990			1991		1992	1993	1994	
	p	j	im	im	v	v	g1	g1-g2	
Stand density, ×10^3/ha	114.3	34.6	31,5	30,8	28,3	27,5	27,3	24,0	40-48
Plant weight, g:									
above-ground part	0.013	0.27	0.42	6.2	10.2	16.4	56.8	210.7	12-15
undergrounf part	0.003	0.07	0.30	2.3	4.7	11.9	38.2	141.3	12-15
Number of developed inflorescences per plant						0.01	0.16	0.84	186-4233
Peoductivity, kg/ha:									
above-ground part	1.5	9.3	13.2	191	289	452	1553	5046	12-15
undergrounf part	0.3	2.4	9.5	71	133	328	1044	3384	12-15
seeds						0.17	8.0	30.3	186-4233

N o t e. Age of plants in ontogenesis: p - seedling, j - juvenile, im - immature, v - virginal, g1, g2.

4. Productivity of the *Rhaponticum carthamoides* (Willd.) Iliin European North-East agropopulation during the period of above-ground phytomass sustainable formation (from the 5th to the 32nd year of life, Arkhangelsk Province, Kotlas District, 1994-1921)

Parameter	*M*±SD	*Cv*, %	max	min	*N*
Stand density, ×10^3/ha	24.1±3.5	14.7	30.5	16.4	40-48
Plant weight, g:					
above-ground part	223.4±74.4	33.3	354.0	95.1	6-9
undergrounf part	246.3±58.6	23.8	354.4	190.6	6-9
Number of developed inflorescences per plant	0.19±0.31	167.9	1.13	0.0005	20188-52
Peoductivity, kg/ha:					
above-ground part	5338.4±1760.6	33.0	8483	2634	6-9
undergrounf part	6019.3±1337.1	22.9	8548	3977	6-9
seeds	1.31±0.65	49.6	1.98	0.49	20188-52

During plant aging, at the beginning of subsenile age (13-17 years), which was accompanied by gradual destruction of the root system of the primary plant in the main root zone and its division into daughter plants, the minimum value of phytomass of aboveground organs was 95.1 g/plant. In subsequent years, an increase in the phytomass of aboveground organs was noted in the second updated cycle of ontogenesis, when daughter individuals, vegetatively arising in the form of a clone, took the place of maternal plants, that is, a rejuvenation of the population occurred. At the same time, the average value of aboveground phytomass for the period from the 5th to the 32nd year of life (223.4 g) turned out to be close to the value for the 5th year of life (210.7 g). The increased variability of the discussed indicator over the years of observation (*Cv* = 33.3%) (see Fig. 3) is caused by the strong influence of humidity (intense rains) and air temperature (35-38 °C and frosts up to –5... 10 °C) for seasonal development.

The weight of the underground parts (roots with rhizomes in dry form) increased following the aboveground parts (see Tables 3, 4): in the first 3 years it was also insignificant (0.3, 4.7 and 11.9 g/plant), in the 4th year it was 38.2 g, in the 5th year 141.3 g. The maximum value during the generative period with an increase to a maximum from 270.6 to 354.4 g and a subsequent decrease to 303.7 g was noted in the 6th-10th years of life. The average value of the mass of underground organs at subsenile age (13-32 years) was equal to 217.3 g, and in the 33rd year of life (after overwintering on April 29, 2022) 268.5 g (*Cv* = 28.6 %). The average value of the mass of roots with rhizomes during the period with relatively stable production of above-ground phytomass (from the 5th to the 32nd year) was 246.3 g (*Cv* = 23.8%) which is slightly higher than the average value for aboveground parts (223.4 g, *Cv* = 33.3%) and indicates their importance as a storage organ for the formation of above-ground phytomass in the future period.

Thus, fluctuations in the size of the underground mass over the years were generally smoother, since it is less dependent on seasonal temperatures compared to the aboveground mass and is formed due to the outflow of organic substances from aboveground organs in the process of their gradual death.

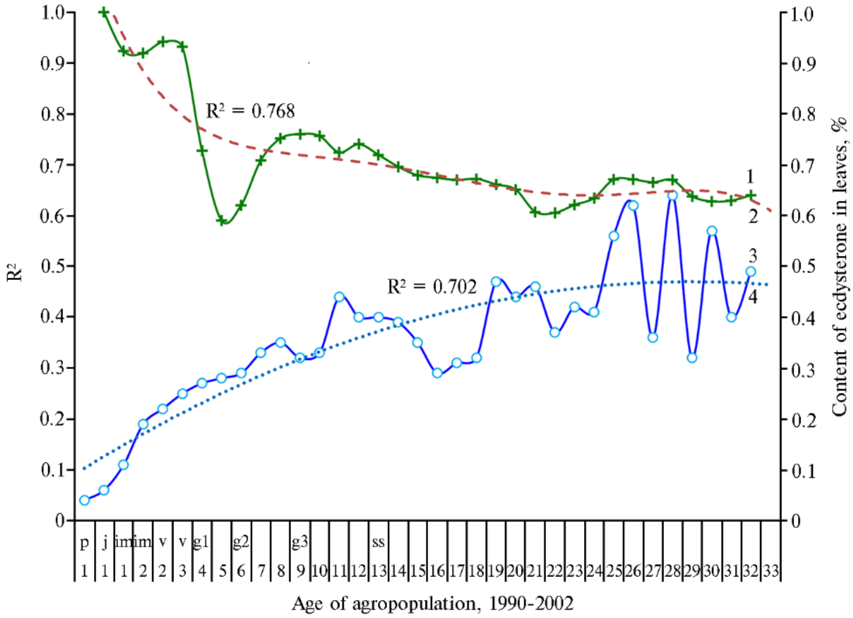


Fig. 4. Correlation between the plant aboveground mass and the content of ecdysterone (1)**, the reliability of the approximation** (2)**, the accumulation of ecdysterone 20E** (3) **and the trend of its accumulation** (4) **in the leaves of vegetative shoots of *Rhaponticum carthamoides* (Willd.) Iliin from a European North-East agropopulation during ontogeny.** Age of plants in ontogenesis: p — seedling, j — juvenile, im — immature, v — virginal, g1, g2, g3 — young, mature and old generative; ss — subsenile (Arkhangelsk Region, Kotlas District, 1990-2021).

Patterns of ecdysterone accumulation in the aboveground parts of plants. The processes of biosynthesis and accumulation of ecdysterone are dependent on growth processes in the aboveground sphere, which, in turn, are determined by the development of the root system of the mother plant and its disintegration into daughter plants. Rosette leaves of vegetative shoots of *R. carthamoides* accounted for 84-94% of the mass of aboveground organs. The ecdysterone content in them was minimal in the 1st year (0.06-0.11%), then increased consistently to 0.19-0.28% from the 2nd to the 5th year of life (see Table 1, Fig. 4). During the generative period (with fruiting flowering shoots) it was 0.29-0.33% (at years 6-10) and decreased from 0.44 to 0.40% during the transition from generative to subsenile age for years 11-13 (see Table 2).

Over the subsequent years of subsenile age (16-32 years), with minimal reproduction (on average 1 fruiting inflorescence per 5 plants), the ecdysterone content in vegetative shoots remained high - 0.41% (variation of 20E over the years from 0.28 to 0.64% when the number of fruiting inflorescences fluctuates from 0.001 to 1.13 pieces). During this period, seed reproduction sharply decreased and synthesized ecdysterone, previously redistributed with the water flow of assimilates from the leaves to the ovules of the inflorescences and seeds, remained in the rosette leaves. Previously, using the example of 8 populations of *R. carthamoides* and *Serratula coronata* under the age of 15 years, we showed that the dynamics of ecdysteroid content in their vegetative organs is inversely and strongly dependent on seed reproduction [59]. The model of mutual relationship obtained in this work ($R^2 = 0.768$) between the total value of the aboveground mass of R. carthamoides and the content of ecdysterone in rosette shoots with a coefficient of determination of about 80% (which corresponds to $r = 90\%$) can be recognized as explaining the dependence of the biosynthesis and accumulation of ecdysterone on development vegetative shoots.

We have established a set of correlative parameters of plants in ontogenesis, combined with the highest (0.56-0.64%) accumulation of ecdysterone in the vegetative shoots of *R. carthamoides*. The length of rosette leaves is 97-119 cm (maximum), the proportion of rosette leaves in the structure of phytomass is 91-94% (maximum), the number of fruiting inflorescences is 0.016-0.021 pcs/plant (minimum), the total value of above-ground phytomass (together with generative shoots) is 270-320 g (above the average value of 223 g by 20-40%).

Agropopulation productivity. Gross production per unit area of the population (taking into account density) serves as an integral indicator characterizing the ecological optimum and reflects the attitude of the organism to the entire set of factors of the external and internal environment. Under natural conditions, the yield of the aboveground mass of wild thickets of *R. carthamoides* at the Gorno-Altai Agricultural Experimental Station is 2200-4000 kg/ha. The maximum bioproductivity of individual fragments of pure thickets can reach 6500-7000 kg/ha. The productivity of underground organs of *R. carthamoides* in the Altai-Sayan mountain region ranges from 80-1500 kg/ha. The largest areas of subalpine meadows are occupied by cenoses where the average weight of rhizomes is apprx. 57-75 kg/ha, and 12-20% of resource areas have a productivity of 570-640 kg/ha [55]. In relatively small areas in the Kazakhstan Altai, the production of dry roots of *R. carthamoides* is estimated at 1.0-1.1 t/ha [73].

In culture, the rhizomes of Leuzea safflower for use as pharmacopoeial medicinal raw materials begin to be removed from the 3rd year of life. Considering that the most intensive growth of root mass occurs at the end of the growing season, harvesting is carried out in September-October. The average yield of dry roots (excluding actual density) in the Moscow region was 2000-2500 kg/ha]56]. In Finland, the expected yield of roots (in terms of square meters per hectare) after 3 years of cultivation was about 2000 kg/ha, of aboveground parts 1000-2500 kg/ha [57]. In the conditions of the Perm region, the yield of above-ground mass without the use of fertilizers on average over 6 years amounted to 2520 kg/ha [74]. In Siberia, in the fields of the experimental farm of the Central Siberian Botanical Garden, the yield of the aboveground mass of *R. carthamoides* at the age of 4-5 years reached 3600 kg/ha [56]. In older crops, a decrease in yield was noted; for example, in the Leningrad region, in crops 7-8 years old, it did not exceed 700-800 kg/ha. In the Moscow region, on medium loamy soil, at the experimental station of the Russian State Agrarian University — Timiryazev Moscow Agricultural Academy productivity in the 6th year was 3740 kg/ha and by the 9th year it decreased 3.6-fold [59].

In the studied conditions of the European North, the gross production of the agropopulation (see Table 3) was scanty in the first 2 years and in the 3rd year of cultivation and amounted to only 8% and 5%, respectively, with regards to the average values for the 5th-32nd years of life (or 452 kg/ha of aboveground and 328 kg/ha of underground phytomass). In the 4th year, the productivity of the agropopulation approximately corresponded to the literature data, 1553 kg/ha for the aboveground part and 1044 kg/ha for the underground part. In the 5th year, productivity continued to increase, amounting to 5046 and 3384 kg/ha, respectively, and reached its peak in mature generative age, about 8500 kg/ha in the 6th-7th years for aboveground and in the 7th-8th years for underground organs. At old generative and subsenile ages, phytomass decreased and varied in accordance with the patterns described above (see Fig. 4). In general, from the 5th to the 32nd years of life (over 27 years of economic exploitation), the average estimated productivity of the aboveground part of the agricultural population was about 5338 kg/ha (Cv = 33.0%), of the underground part 6019 kg/ha (Cv = 22.9%) (see Table 4).

There was no seed yield per unit area in the first 3 years. On the 4th and 5th lives it was 8 and 30 kg/ha, respectively, and the highest seed yield occurred in the 6th and 7th years, 108 and 78 kg/ha, decreasing in the 8-10th years to 50, 26 and 5 kg/ha, respectively. After the transition to the subsenile age, when vegetative reproduction dominated, the average annual seed yield for the period from the 13th to the 32nd year was 1.3 kg/ha (from 0.49 to 1.98 kg/ha). The coefficient of seed reproduction of an individual (the ratio of the number of full-fledged seeds per unit area to density) during this period was 3.3.

For comparison, in subalpine meadows of Altai, the average seed yield was 8-30 kg/ha, and in rare thickets it could reach 150-200 kg/ha [55]. In the conditions of the European North, the seed yield from experimental plots was as follows: in Finland up to 200-290 kg/ha [57], in Komi Republic up to 295-410 kg/ha. In the Perm region [75], in the 9-10th year of life, seed yield was estimated at 386-542 kg/ha (38.6-54.2 g/m^2). Moreover, abundant fruiting in such cases was accompanied by an extremely low ecdysterone content in the leaf organs, from 0.05-0.07 to 0.14% [43] and from 0.04 to 0.09% [44].

This confirms our thesis that one of the key factors in the regulation of ecdysterone concentration in leaf organs is the process of competition between seed and vegetative reproduction. Abundant production of seeds leads to the outflow of ecdysterone from vegetative shoots and the early completion of the plant life cycle due to the massive death of generative shoots after fruiting together with renewal buds, which serve as the basis for the formation in future years of new rosette leaves that synthesize ecdysterone.

An approximate estimate of the gross synthesis and accumulation of ecdysterone at the optimal exploitable age (from the 5th to the 32nd years) was about 21.9 kg/ha in the aboveground sphere with an amplitude of 20E content of 2.8-6.4 g/kg (14.9-34.2 kg/ha) annually, or about 600 kg of ecdysterone over 27 years of operation. In the underground sphere of plants, with an approximate 20E content of 0.3-0.5 g/kg (apprx. 2.4 kg/ha, from 1.8 to 3.0 kg/ha) of ecdysterone accumulated, but these plant parts can be alienated only once .

Thus, 90% of the annually synthesized ecdysterone accumulates in the aerial parts of plants from the *R. carthamoides* agropopulation. If we proceed from the potential of the life cycle of an industrially exploited population, taking into account the possibility of repeated removal of phytomass, then ecdysterone is concentrated in aboveground organs by more than 99%.

Qualitative parameters of medicinal raw materials from leaf organs. An important indicator of the quality of *R. carthamoides* medicinal raw materials for the manufacture of drugs is the ratio of highly active ecdysterone (activity in biotests 7.5×10^{-9} M) to weakly active ecdysone (activity 1.1×10^{-6} M), since less active ecdysteroids can completely or partially block the physiological effect more active compounds contained in plant extracts [8, 48]. It is desirable that the purity of ecdysterone be at least 95% (≥ 20:1), better than 97% (≥ 30:1), and ideally, an almost complete absence of minor and weakly active components (≥ 1000:1) [9]. Otherwise, the feedstock must be subjected to a complex multi-stage chromatographic procedure for purification from inactive impurities [10].

For a dry crude alcoholic extract isolated from 65 kg of dry roots of *R. carthamoides* with rhizomes, the 20E/E ratio was about 60:1 with 0.37% ecdyster- and 0.006%ecdysone [46]. According to data from the Czech Republic [50], the 20E/E ratio for dry roots of *R. carthamoides* was more than 1000:1. For the aerial parts of *R. carthamoides*, this quality indicator is not constant and depends on the intensity of the formation and development of generative shoots. Previously, using 12 different-aged agropopulations of *R. carthamoides* and *S. coronata* in the first 16 years of cultivation, we showed that in *R. carthamoides*, before entering the generative period, the composition of phytoecdysteroids was represented only by highly active ecdysterone. Ecdysone in aboveground reproductive organs was synthesized synchronously with their development: at the beginning of the growing season it was not detected in generative shoots, during flowering its share reached 9.1%, during seed filling and fruiting 17.8-18.7%. During the period of abundant fruiting, the proportion of ecdysone also increased in vegetative shoots, although to a lesser extent, from 1.5 to 4.7% in the budding stage and to 13.3% in the flowering stage [61].

The qualitative ratio of ecdysterone to ecdysone (20E/E) in vegetative shoots of *R. carthamoides* over 32 years of cultivation under agropopulation conditions changed as follows: In immature and virginal plants, it was higher than 1000:1 (1-3 years), in young generative plants plants in the budding stage, it was was apprx. 980:1. In adult generative plants, during the transition to the flowering phase, 20E/E decreased to 20-6:1, and by the beginning of fruiting to 3-4:1. In the subsenile period (13-32 years), when seed reproduction was suppressed, ecdysone was synthesized in trace amounts ($\leq 0.001\%$), the ratio 20E/E ranged over the years from 560-900:1 to 60-80:1 which met the requirement for a relative purity quality of 20E 97%.

Comparison with other industrial sources of ecdysterone showed that *R. carthamoides* has an advantage over them both in the quantitative content and in the quality of the synthesized ecdysterone. In particular, A. Hunyadi et al. [30] examined the European market of nutritional supplements made from *Cyanotis arachnoidea* C.B. Clarke extract and showed that the ratio of ecdysterone to other (minor) ES was deteriorated, apprx. 0.9:1 with a quantitative content of 0.2-2.4%/0.09-2.49%. A closely related species, *Cyanotis longifolia* Benth., grown in a pot culture in France, had a similar qualitative ecdysterone ratio: in aboveground phytomass 1.8:1 (0.095%/0.052%), in roots 0.63:1 (0.385 %/0.607%) [76]. In general, other species of the family *Commelinaceae* from natural habitats are characterized by a relatively low content of ecdysterone in dry phytomass: *Cyanotis hirsuta* Baker — 0.140%, *Cyanotis kewensis* C.B. Clarke —0.0245%, *Cyanotis longifolia* Benth. — 0.008%, *Cyanotis somaliensis* C.B. Clarke — 0.111%, *Cyanotis speciosa* (L.f.) Hassk. — 0.093% [76]. Additionally, commercial Cyanotis extracts in 2021 have been found to contain contaminants of petroleum-derived semi-synthetic ecdysteroids that interact with ecdysterone receptors as competitive agonists and antagonists, which can lead to a variety of negative pharmacological and toxicological consequences when consumed [29].

Among other indicators of the quality of medicinal raw materials, information about the accumulation of toxic compounds of natural or anthropogenic origin in it, including heavy metals, radionuclides, chlorine and organophosphorus compounds, is important. Early color reaction studies in the early 1970s suggesting the presence of alkaloids in *R. carthamoides* were subsequently not confirmed [77]. There is also no information in modern literature about the accumulation of triterpene saponins by this type, other potent, narcotic or toxic substances that are potentially hazardous to human health or that may pose a danger to animals when used as feed and feed additives (bufadienolides, cardiac glycosides, aristolochic acid, photosensitizing , cumulative or vitamin-breaking substances, etc.) [42, 63, 65-67].

The toxicity of the aerial parts of R. *carthamoides* used as feed additives has been studied previously. In long-term experiments with the inclusion of crushed *R. carthamoides* leaves in diets, no adverse effects were found. Their harmlessness has been proven in doses up to 0.3-0.5 kg of dry matter of the aboveground mass. Rats and birds could feed on the seeds of this species, which contained up to 1.5% ecdysterone [36].

5. Chemical composition of ecdysterone-containing substance from leaves of *Rhaponticum carthamoides* grown in an commercially exploited plantation (Arkhangelsk Region, Kotlas District, 2016-2020) (78)

Parameter	Norm	Actual values
Active ingredients,%:		
ecdysterone (20-hydroxyecdysone, 20E)	≥ 0.1	0.56-0.61
share of ecdysterone from PES	≥ 95.0	95.3-99.6
extractives	≥ 12.0	50.2
raw protein	≥ 16-19	19-27
raw fiber	≤ 23-26	16-19
Heavy metals, mg/kg:		
Hg	0.05	0.009-0.016
Cd	0.3	0.020-0.115
As	0.5	0.05
Ni	3.0	0.59-1.30
Pb	5.0	0.18-0.30
Cu	30.0	7.9
Zn	50.0	28.4
Organochlorine and phosphorus compounds, mg/kg:		
DDT and its metabolites	0.05	< 0.007
hexachlorocyclohexane and its isomers	0.05-0.20	< 0.001
metaphos	0.00-0.50	Not detected
karbofos	2.0-5.0	Not detected
Nitrogen compounds, mg/kg:		
NO^{2-}	10.0	0.3-2.0
NO^{3-}	2000	700-1200
Radionuclides, Bq/kg:		
^{90}Sr	100.0	5.7
^{137}Cs	600.0	4.8

Note. PES — phytoecdysteroids, ДДТ — the sum of organochlorine pesticides (dichlorodiphenyltrichloroethane, dichlorodiphenyldichloroethane, dichlorodiphenyldichloroethylene, etc.), hexachlorocyclohexane - the sum of other insecticides (lindane, hexachlorocyclohexane, heptachlor, keltan, aldrin) (data presented in accordance with testing protocols, see http://www,agrobiology).

In the conditions of industrial cultivation, during the sanitary and toxicological assessment of product safety, the priority is the compliance of the content of heavy metals with regulatory requirements. The relevance of the control of heavy metals in high-mountain plants is associated with their genetic predisposition to the accumulation of mercury, cadmium, nickel, lead and copper. When studying the pharmacopoeial characteristics of medicinal raw materials from the leaf organs of *R. carthamoides* [78], we found (Table 5) that the aerial parts of *R. carthamoides* grown and harvested in the studied agropopulation did not accumulate elements of the first and second hazard classes (Hg, Cd, As, Zn; Ni, Cu, Cr) above the background level and corresponded to the maximum permissible concentration for green mass of perennial grasses. There were no chlorine and organophosphorus compounds prohibited by sanitary standards in the phytomass. The content of radionuclides ^{90}Sr and ^{137}Cs was below the MPC (68.8 and 6.2 Bq/kg compared to the permitted 100 and 600 Bq/kg). The amount of nitrites was within the normal range (0.3-2.0 mg/kg) (see Table 5).

The first results of mass application of additives based on leaf material of *R. carthamoides* grown in the considered agropopulation were obtained in the pig-breeding complex of JSC Kotlas Pulp and Paper Mill (Arkhangelsk Province, Russia) with an average monthly population of 1.6 thousand animals, the duration of the experiment was 12 months [79]. Pregnant sows, weaned piglets and fattening livestock aged 2-4 months were daily fed granulated grass meal from the aerial part of R. *carthamoides* of the 4th year of life at a rate of 20 g/t live weight. The animals' diet was based on food waste from catering establishments with poor phytosanitary composition, which was accompanied by dyspepsia. As a result, the herd became healthier and the mortality of newborn piglets decreased by 2.1-2.7 times, and the anabolic effect was expressed in an increase in the herd's output in live weight by 40.6%.

Further studies were carried out in the breeding pig breeding farm of JSC Zarechye (Kirov Province) under strictly controlled conditions. According to the data obtained, when the substance from *R. carthamoides* was introduced into the diet of weaned piglets, their live weight exceeded that in the control by 15-22%, the intensity of the average daily weight gaine was higher by 24.0-32.8%, the incidence of animal diseases decreased by 1.6-2.5 times, safety was 100% [22]. Comparable results were obtained in experiments using chemically purified ecdysterone (20-hydroxyecdysone 96% purity) isolated from *R. carthamoide*. The anabolic effect in this case was 12-16% while reducing feed consumption by 11-17% per 1 kg of live weight gain [80].

Summarizing the results obtained, it should be noted that the biosynthesis and accumulation of ecdysterone were directly related to vegetative reproduction (with the intensity of growth of rosette shoots over the years and their power), and inversely proportional to the intensity of seed fruiting. The relationship between the total amount of above-ground mass and the content of ecdysterone in rosette shoots over 32 years of cultivation was characterized by the coefficient of determination $R^2 = 0.768$ (or about 80%) and reflected the dependence of the biosynthesis and accumulation of ecdysterone on the development of vegetative shoots. An approximate estimate of the gross synthesis and accumulation of ecdysterone at the optimal exploitation age (from the 5th to the 32nd years) was about 21.9 kg/ha annually in the aboveground sphere, or about 600 kg of ecdysterone over 27 years of exploitation. The underground sphere contained approximately 2.4 kg/ha of ecdysterone. For the factory production of medicines, food additives and phytobiotics, it is preferable to use rosette leaves of vegetative shoots containing high concentrations of ecdysterone (0.4-0.6% with a standard of 0.1%). The qualitative characteristics of medicinal raw materials prepared in the optimal phase of development (beginning of budding) were high and met the requirements for the manufacture of pharmaceuticals with a relative purity of ecdysterone of 97%. The underground parts (roots with rhizomes) did not accumulate significant concentrations of ecdysterone (on average 0.03-0.05%) and could quickly lose active substances due to infection by soil microflora. Seed productivity increased over the years (8-30 kg/ha) and reached a peak in the 6th and 7th years (108 and 78 kg/ha), decreasing in the old generative age (8-10th years) to 50, respectively. 26 and 5 kg/ha. In the subsenile period (from the 13th to the 32nd year of life and beyond), the type of reproduction changed from seed to vegetative and seed production had extremely low values (1.3 kg/ha with a seed reproduction coefficient of 3.3). The average value of the mass of roots with rhizomes from the 5th to the 32nd year of life was 246.3 g/plant, which is slightly higher than the average value of the phytomass of aerial parts, 223.4 g/plant.

From substances based on leaf material of *R. carthamoides*, ecdysterone was well extracted into aqueous and alcoholic solutions and was well preserved in them without preservatives (93-98% within 1 day). The total yield of extractives was 50.2% (with a norm of 12.0%). In biotests, the extract had a stimulating effect at a strong dilution (10^{-9}-10^{-11} M based on ecdysterone) and an inhibitory effect at a lower (100-fold) dilution (10^{-4}-10^{-5} M) [78].

In the future, it is necessary to study the influence of the multiplicity and frequency of cuttings (the amount of alienated aboveground mass) on the formation and ratio of vegetative and generative shoots of Leuzea safflower, as well as the possibility of influencing the process of ecdysterone synthesis through fertilizers, phytohormones and elicitors.

So, as a result of 32 years of research on the agropopulation of *Rhaponticum carthamoides* cultivated in the Arkhangelsk Prorvince from 1989 to 2022 using the technology of annual one-time alienation of aboveground phytomass, it was found that the natural conditions of the European North-East with cool climate, a leaching type of water regime, long daylight hours and a short growing season, are favorable for the industrial cultivation of Leuzea safflower. The duration of ontogenesis of the agropopulation was close to the parameters of natural populations in subalpine meadows and was over 30 years without a transition to the senile age state at the 33rd year of life. Starting from the 3-4th year of life, the density of plants in the agrocenosis reached optimal values of 28-23 thousand/ha. In the conditions of an agropopulation, in the first 4 years there was intensive growth and development of plants; in the 5th year (after the transition to the generative period), seed reproduction of individuals began. The average annual estimated productivity of the aboveground part of the agropopulation during the period of stable production of aboveground phytomass with a high level of ecdysterone biosynthesis (from the 5th to the 32nd years of life) was about 5300 kg/ha, of underground part apprx. 6100 kg/ha. The accumulation of the largest amount of ecdysterone in the vegetative shoots of *R. carthamoides* (0.56-0.64%) was accompanied by the maximum length of rosette leaves (97-119 cm), the maximum proportion of rosette leaves in the structure of phytomass (91-94%), the minimum number of fruiting inflorescences (0.016-0.021 per plant), The total above-ground phytomass together with generative shoots was 270-320 g (above the average value of 223 g by 20-40%). Ecdysterone from leaf organs was well extracted into aqueous and alcoholic solutions without loss of active substances (when the aqueous extract was stored for 1 day, the preservation of ecdysterone was 93-98%). The total yield of extractive substances from the leaves was 50.2%, with the standard being 12.0%. The resulting medicinal raw materials met all regulatory requirements of regulatory authorities regarding the content of radionuclides, heavy metals, herbicide residues, insecticides and other chemical plant protection products. The use of a substance from leaf material of *R. carthamoides* in commercial animal husbandry was accompanied by improvement of the herd health and a decrease in the mortality of young pigs by 2 or more times, an increase in the intensity of average daily growth by 24-33%, and a decrease in feed consumption by 11-17%.

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