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Population biology of the endemic species *Pimpinella tragium* Vill.

Abstract. For the first time for the European part of Russia, a study of the population biology of the endemic species of chalk and limestone *Pimpinella tragium* Vill. in natural habitats of the Voronezh region was carried out. The species is an obligate calcephyte, prefers open chalk substrates. Biomorphological analysis made it possible to classify *Pimpinella tragium* as a herbaceous polycarpic taproot plant. Due to the presence of a powerful taproot, the species promotes the fixation of weak chalky substrates. In the future, open groups of calciphilic vegetation with the participation of *Pimpinella* may appear on the Cretaceous substrate. Six-year monitoring of the main stages of development of *Pimpinella tragium* made it possible to identify 4 ontogenetic periods and 9 age states: latent (seeds), pregenerative (seedlings, juvenile, immature and virginal individuals), generative (young, middle-aged, old generative individuals), postgenerative (senile individuals). The paper presents the morphometric characteristics of individuals of different age groups. The ontogenetic structure of *Pimpinella tragium* cenopopulations was studied, the type of the basic spectrum was revealed. Most cenopopulations have a left-sided spectrum; the absolute maximum is more often represented by young individuals, the local maximum – by middle-aged individuals. For some populations, a centered ontogenetic spectrum was noted with maxima on middle-aged generative individuals. The formation of the ontogenetic structure of *Pimpinella tragium* cenopopulations is significantly influenced by the physical characteristics of the Cretaceous substrate, and hence the physiological characteristics of this species. The ability of the species to reproduce only by seed is of great importance, since in different years the germination and survival rate of seedlings can differ sharply.

Key words: *Pimpinella tragium* Vill., population biology, ontogenesis, coenopopulations (CP), Cretaceous substrate, endemic species, Voronezh region.

Introduction

The problem of studying and effectively preserving the biodiversity of the vegetation cover is one of the most pressing problems of our time. In this regard, the study of the unique flora of the Cretaceous outcrops, which includes a number of endemic, rare and endangered species, is of undoubted interest [1-4].

The aim of this work was to study the ontogeny and structure of populations of endemic species of chalk and limestone *Pimpinella tragium* Vill from the Apiaceae family. The studies were carried out in the Voronezh region.

Voronezh region is located in the south-west of the European part of Russia between 49° 34' and 52° 06' north latitude and 38° 09' and 42° 55' east longitude. It is located in the central part of the East European (Russian) plain, at the junction of the Central Russian and Kalach uplands and the Oka-Don Plain, the region stretching from north to south for 277.5 km, and from west to east for 352 km, its area is 52.4 thousand km^2. Voronezh region in the north and northeast borders with Lipetsk and Tambov, in the west – with Belgorod and Kursk, in the east – with Saratov and Volgograd, in the southeast – with Rostov and in the south-west – with the Luhansk region of Ukraine (Figure 1) [5]. Voronezh region is located at the border of two botanical-geographical zones – forest-steppe and steppe, which provides a rich floristic composition and heterogeneity of landscape-ecological conditions.

The territory of the Voronezh region has passed a long and difficult path of geological development, which is reflected in its modern landscape and ecological features, in turn, leaving an imprint on the vegetation cover. Along with the covers of Quaternary sediments, the Cretaceous system rocks are most widespread in the region. Cretaceous deposits are represented by Lower Cretaceous and Upper Cretaceous rocks, which in a fairly large part of the region can come to the surface along the slopes of river valleys, gullies and ravines [6]. The most frequently noted outcrops of Upper Cretaceous rocks in the northwestern, southwestern, southern and southeastern parts of the region. V. Mikhno [7], based on long-term field observations and analysis of literature data, comes to the conclusion that the time of appearance of calciphilous vegetation in different regions of the East European Plain is asynchronous, since the territory was populated with relict plant species both autochthonously and by migrations from mountainous regions of the Mediterranean, the Caucasus, Central Asia and other regions. The main prerequisite for the convergence of relics of different eras was the physicochemical properties of the Cretaceous rocks, the originality of their denudation, as well as specific microclimatic conditions. The most important environmental factors affecting the spread of chalk plants are [7,8] temperature, humidity and chemical composition of the nutrient substrate. So, D. Sakalo [8] indicates that the main typomorphic element of chalk substrates is calcium, the need for which determines the peculiarities of the ecology and physiology of calcephilous species, in particular, their ability to use for photosynthesis the carbon dioxide of calcium bicarbonates entering through the root system has been shown. There is also information in the literature [9] that representatives of the Cretaceous flora are very selective about the chemical purity of carbonate soils, preferring pure chalk substrates to contaminated chalk, where calcium is readily available for plants and is absorbed in the required amount by the calciphilic flora.

The material for the analysis was collected in Ostrogozhsky, Liskinsky, Kamensky, Podgorensky, Rossoshansky, Olhovatsky and Kantemirovsky districts (Figure 1, the author indicates the approximate location of the studied habitats). Route and stationary expeditions have been carried out annually since 2008.

Figure 1 – Voronezh region map. From: https://www.shutterstock.com/es/image-vector/detailed-map-voronezh-region-russia-175714598

The study of the unique flora of chalky outcrops is of great interest since it is composed of many endemic species of plants. In Voronezh region, the outputs of chalk and marl are confined to the riverine slopes and slopes of hills composed of chalk (North-Western, Southern, South-Western and South-Eastern areas). Chalk landscape – specific natural-territorial complexes, where the main role is played by chalk-loamy rocks. Natural specificity is determined by the erodibility of the terrain, high reflectivity, lack of the developed soil, predominance of sparse vegetation of calciphyte groups. By the flora of the cretaceous outcrops a set of species associated in its distribution with the chalk substrate are understood.

According to modern reports more than 500 species of angiosperms growing on the outputs of chalk and marl are marked in Voronezh region [10]. A significant portion of them belongs to endemic and relict species which existed in pre-glacial period. Depending on the degree of adaptation they can be divided into four groups: obligate calciphyte, optional calciphyte, insensitive to calcium content types, calciphobous species.

Cretaceous outcrops have a number of common features such as lack or weak development of soils, the mobility of the Cretaceous rocks, specific microclimate, physical and chemical properties of chalk as a substrate on which the plants grow. In this regard in the composition of the Cretaceous flora species certain life forms dominate such as subshrubs and perennial herbaceous plants with a powerful taproot penetrating to the depth of 50 cm to 2 m and more. It was repeatedly noted that such plants strengthen chalks [11].

Our interest in *Pimpinella tragium* as an object of population monitoring was caused by a number of factors: endemism, confinement to Cretaceous substrates of various genesis, the ability to dominate both in primary groups of vegetation and in stable communities. A comprehensive study of ontogenesis, structure and dynamics of cenopopulations (CP) of the species has not been previously carried out, we only studied the features of development and ontogenetic structure of CP of *Pimpinella tragium* [12].

Materials and methods

In carrying out this work, studies were carried out at various levels of the organization of plant systems: organ, organismic, population and coenotic. The methods of modern biomorphology, population biology, phytocenology and statistics were used.

Organ level of the structural organization. To study the underground organs of individuals in expeditionary conditions, the methods of dry excavation along the roots and the method of horizontal excavation were used. [13,14].

Study of an individual as a separate organism. The life form of plants was characterized according to the concept of I. Serebryakova [15] for individuals of a mature generative ontogenetic state. To isolate the ontogenetic states of *Pimpinella*, the generally accepted methods of population biology were used [11]. On the basis of morphological changes in the ontogeny of individuals, the age periods are distinguished and the ontogenetic states are characterized, which are further indicated in the text by symbols: **se** – resting seeds, **p** (plantulae) – seedlings, **j** (plantae juveniles) – juvenile, **im** (plantae immaturae) – immature, **v** (plantae virginiles) – virginal, $\mathbf{g_1}$ – young generative, $\mathbf{g_2}$ – middle-aged generative, $\mathbf{g_3}$ – old generative (plantae generativae), **s** (plantae seniles) – senile individuals.

Study of Pimpinella tragium populations. The ontogenetic structure and abundance were analyzed on accounting plots of 1 m^2, laid out in a systematic way. A plant of seed origin was used as a counting unit. When analyzing the CP structure, the recovery index I_r and the aging index I_a [11] were calculated, which are important population parameters. I_r reflects the share of undergrowth participation in the CP (or how many offspring at a given time point per one generative individual), and I_a – the share of participation of the aged fraction in the total sample.

Study of phytocenoses with the participation of Pimpinella tragium. To characterize plant communities on Cretaceous substrates, geobotanical descriptions were made on stationary plots, each with an area of 100 m^2. Subsequent analysis made it possible to compile a complete floristic list of the study areas. The coenotic significance of each species was assessed on the scale of abundance by Braun-Blanquet [16].

The morphometric data obtained in the course of work were processed statistically using the EXCEL software package.

All photographs were taken by the author personally (Figure 2, photos 1 and 2 – Kuvshin tract, Podgorensky district, May 02, 2021 and June 03, 2020, respectively; photo 3 – Marki village, Kamensky district, July 31, 2013. Figure 3, photo 1 – Volokonovka village, Kantemirovsky district, July 23, 2008; photo 2 – Ezdochnoe village, Ostrogozhsky district, July 28, 2018. Figure 6, photo 1 – May 08, 2012, photo 2 – August 11, 2012, both photos made near a chalk quarry on the western outskirts of the regional center Podgorensky). Figure 4 is made by the author on the basis of the ontogenetic herbarium of *Pimpinella tragium*.

Results and discussion

The genus *Pimpinella* comprises 150 species, distributed in Eurasia and Africa, over 16 of which are present in Europe [17]. The species is confined to the valleys of Volga and Don, Black sea coast of Caucasus. For the Voronezh region (Figure 2) it is observed on the Northern border of the area.

Figure 2 – Cretaceous landscapes of the Voronezh region

Obligate calcephyte, on chalk outcrops of the South, West and North-West region occurs everywhere: on the moving screes, dense indigenous outcrops of chalk and plumes and cones and outcrops with a mixture of humus and fine-grained deposits (Figure 3) [6,10,12].

According to the literature data, the taxon *Pimpinella tragium* has significant morphological variability [18,19].

We found that, on all of the listed types of Cretaceous substrates, *Pimpinella tragium* is part of the primary groups of vegetation. As both loose and dense chalk outcrops become overgrown, the species strengthens its cenotic positions and often acts as a dominant. The composition of subdominants depends on the type of Cretaceous substrate: *Scrophularia cretacea* Fisch.ex Spreng., *Hyssopus cretaceus* Dubjan. (mobile chalk talus), *Linaria cretacea* Fisch. ex Spreng., *Matthiola fragrans* Bunge (dense chalk), *Teucrium polium* L., *Thymus calcareus* Klok. et Shost., *Onosma simplicissimum* L., *Gypsophyla altissima* L. (mixture of chalk crushed stone with fine earth), *Festuca valesiaca* Gaudin (areas with turf).

The life form of *Pimpinella tragium* was determined on the basis of the classification developed by us earlier for tap-root grasses [11]. The biomorph of this plant is a polycarpic tap-root species with semi-rosette shoots. The ontogeny of the species for European part of Russia was described for the first time (Figure 4).

Figure 3 – Cenopolyulations on *Pimpinella tragium* on various chalk substrates

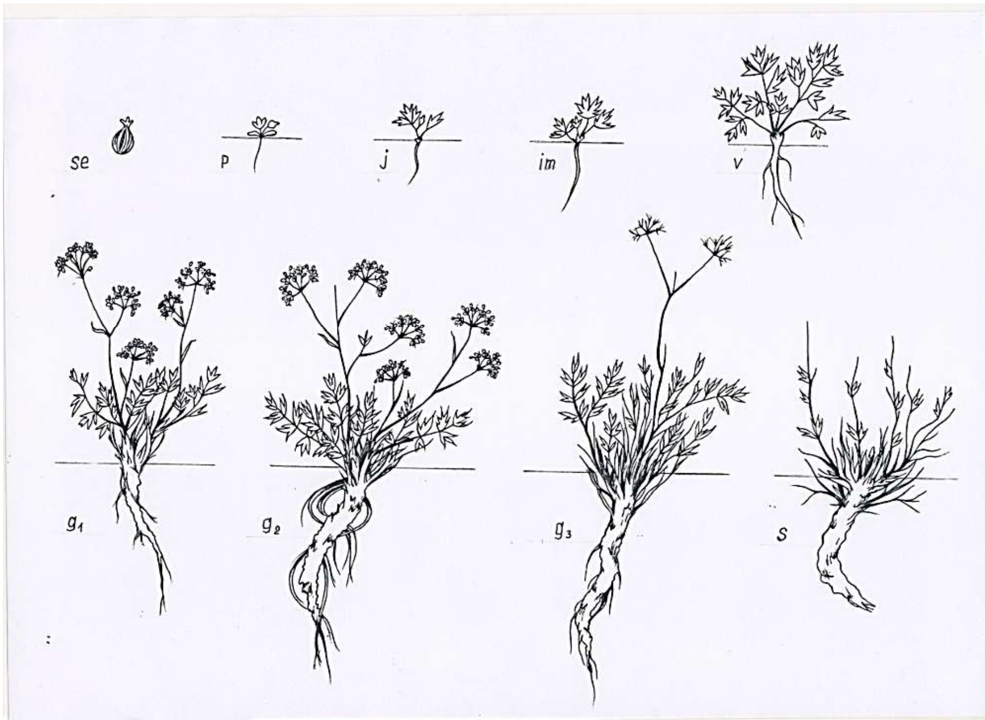

Figure 4 – Ontogenetic states of *Pimpinella tragium*. Developed by the author

The morphometric characteristics of individuals of different ontogenetic states are presented in Tables 1 and 2.

The embryonic period. Achenes (se) are dark brown, pear-shaped, with a wide base and a narrowed apex, on which an epispid disc is formed in the form of a thickened head. The surface is covered with thin threadlike ribs. Length and width – 1.5-2.5 cm.

The pregenerative period. Seedlings (p). Germination is aboveground. The hypocotyl is short. Cotyledons are oval-oblong, 10-15 mm long, 3-5 mm thick, on petioles fused at the bases. The first leaves are alternate, rounded, three-pentagonal in outline, with sharp teeth. Petioles up to 35-40 mm long, pink-violet at the base. The third leaf is weakly tripartite, with wide toothed lobes. Root length up to 30-50 mm, lateral roots are absent.

The author has found that the complete development cycle includes 4 periods and 9 age-related conditions, namely:

Table 1 – Morphometric characteristics of pregenerative and postgenerative individuals of *Pimpinella tragium*

Parameters	Ontogenetic states			
The number of vegetative rosette shoots	j	im	v	s
Total number of rosette leaves	1±0.00	1.56±0.02	2.65±0.24	3.12±0.46
Length of rosette leaves with petioles, cm	3.25±0.16	11.85±0.7	28.55±1.85	16.07±0.78
Rosette leaf width, cm	1.34±0.04	3.1±0.13	5.95±0.22	5.11±0.18
Caudex diameter, cm	0.51±0.02	0.99±0.05	1.55±0.08	1.34±0.05

Table 2 – Morphometric characteristics of generative individuals of *Pimpinella tragium*

Parameters	Ontogenetic states			
The number of axial generative shoots	g_1	g_2	g_3	g_1
Height of axial generative shoots	2.85±0.25	11.85±0.93	5.13±0.56	2.85±0.25
The number of lateral generative shoots	28.7±0.49	35.85±1.21	22.72±0.52	28.7±0.49
Number of inflorescences per individual	9.76±1.38	44.5±3.86	12.76±1.45	9.76±1.38
Number of stem leaves per individual	14.17±2.35	53.4±4.05	21.7±2.24	14.17±2.35
Caudex diameter, cm	13.18±1.76	46.22±3.12	17.33±1.98	13.18±1.76

Juvenile (j) plants have a rosette shoot with 3-5, rarely 7 leaves on petioles up to 4-6 mm long. The leaf plate is finger-dissected, the lobules are triangular. The length of the root is up to 15-20 cm, it is slightly tapering, lateral roots of the first order are formed in the lower third. Due to the contractile activity of the root, caudex appears and branches very early, and already in imature (im) individuals, the formation of 2-3 rosette shoots with 5-7 leaves and petioles from previously dead ones is possible.

The caudex diameter is 3-4 mm; transverse folds are clearly visible on it. The length of the petioles is up to 20 mm. The main root penetrates up to 20-25 cm, lateral skeletal roots up to 2 mm thick are formed at a depth of 2-5 cm.

Virginiles (v) plants have from 2 to several rosette shoots with numerous leaves, leaf plates without pubescence, oblong or ovoid in outline, with a petiole 3-8 cm long and 1-2.5 cm wide, twice pinnately dissected, primary lobes are ovoid in outline, 3-8 mm long and 1-4 mm wide. The caudex is branched, up to 20 mm in diameter, the height of individual heads is 10-15 mm, they are densely covered with stalks of dead leaves. As virginiles individuals develop, the root gradually deepens to 70 cm –1 m, along its entire length a few thin lateral roots of 1-3 orders of magnitude extend from it.

The generative period. Young generative (g_1) plants vary greatly in power, therefore they can have from 2-3 to 5 or more semi-rosette orthotropic shoots 15-40 cm high. Basal leaves are petiolar, completely coinciding in morphology with virginal-type leaves. Stem leaves are few in number, smaller. Umbrellas 2-4 cm in diameter, with 10-15 naked rays, almost equal in length, umbrellas 5-8 mm in diameter. Petals are white, about 1 mm long, shortly pubescent outside. One shoot has 1-3 umbrellas. The heads of the caudex are up to 25 mm in height, the main root is 1 m or more long, in the basal part it is densely speckled with transverse folds. The color of the root is very light, grayish-white. Young generative plants usually stay in the g1 state for 3-4 years. Middle-aged generative (g_2) plants, under optimal conditions of development, reach maximum power and can contain up to 15-20 semi-rosette orthotropic generative shoots, some shoots are only rosette. The number of umbrellas on one shoot increases on average to 5-7, the number of rays in each increase, which significantly increases the seed productivity of an individual. Caudex with a diameter of up to 20 cm, the height of the heads reaches 6 cm and more. Simultaneously with the transition of individuals to the g_2 state, a gradual partial destruction of caudex begins, associated with the transition of individual chapters to the secondary vegetation, and then death. However, in general, in middle-aged plants, the processes of neoplasm significantly exceed the dying off. This condition lasts for 5-8 years or more. On the contrary, in old generative (g_3) plants, the processes of withering become more and more important. Tissues die off not only in caudex, but also deeper, in the main root, in the formed cavities a chalk substrate is stuffed, mixed, depending on the type, with soil particles, fine gravel, etc. The confinement of the *Pimpinella tragium* to the slopes with a steepness of 10-50^0 and the mobility of the upper layer of the chalk substrate contribute to the mechanical detachment of weakly fixed caudex branches, its diameter decreases to 10-15 cm. An increasing number of shoots are no longer able to form flower-bearing stems, the number of fully ripening fruits is reduced. The bark of the root in some areas dies off and gradually sloughs off.

The postgenerative period. Senile (s) individuals are only capable of postgenerative vegetation. There are no more than 5 shoots, each rosette consists of 3-7 petiole leaves, which are morphologically similar to the leaves of imature individuals. The destruction of caudex, as well as the dying off of the main root, is accelerated. The caudex diameter does not exceed 5-8 cm. The total life span of senile individuals lasts no more than 3 years.

Simultaneously with the study of ontogeny, the ontogenetic structure CP was studied. Five communities with the participation of *Pimpinella tragium* were selected for observations: CP 1 and 3 were located on the southern and southwestern slopes and contained vast areas of hard chalk. CP 2, 4, and 5 were part of natural meadow phytocenoses on chalk rubble with an admixture of fine earth, chernozem-calcareous soil, or loose chalk outcrops. In CPs 2, 4, and 5, left-sided ontogenetic spectra were noted (Fig. 5) with maxima on imature or juvenile individuals, a local maximum on g_1 (CP 5) – or (CP 2, 4) g_2-individuals. These CP of the *Pimpinella* are part of sodded chalk communities with a relatively high projective cover (65-75%) and a slight steepness of the relief (5-15^0). Long-term observations allow us to talk about the optimal timing for the passage of each period of ontogenesis. The average density is 45.8 individuals per 1 m^2, I_r ranges from 1.17-1.58, I_a – 8.11-9.65. The left-sided nature of the spectra is primarily due to the seed type of self-maintenance of the species, while the dynamism of the pregenerative fraction is explained both by the short development of individuals of certain age groups and by the unevenness of seed reproduction and survival of seedlings.

Fluctuations in the number of age groups of the generative fraction depend on the duration of the young, middle-aged or old generative state in ontogenesis. At the same time, the presence and duration of development of individuals of all age groups are significantly influenced by the survival rate of seedlings during the years of research, the meteorological conditions of specific years, the compliance of ecological requirements with the conditions of the ecotope, anthropogenic factors, etc. In CP 1 and 3, centered age spectra (Figure 5) with a maximum at g_2 – individuals. We believe that this type of structure is formed primarily in connection with the physical properties of the chalk outcrops on which the *Pimpinella tragium* grows [20]. This is a hard, chalky substrate, so it is very difficult for the seed to gain a foothold and germinate. In addition, the top layer of chalk, due to seasonal changes in temperature and humidity, is gradually loosened and washed out by melting snow and precipitation.

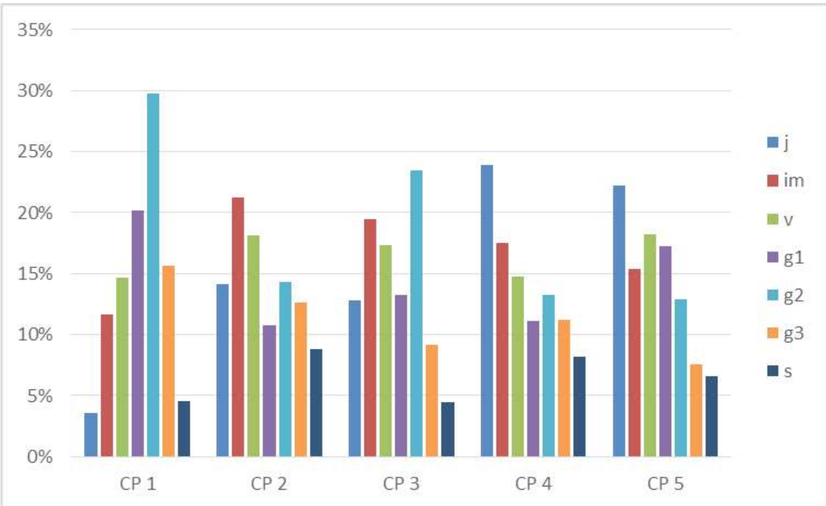

Figure 5 – Ontogenetic structure of *Pimpinella tragium* cenopopulations

The steepness of the slopes in some areas is 45-50^0 degrees. The projective cover of plants on chalk substrates does not exceed 55%, and in some loci – 5-20%. The gradual displacement of the upper layer of the chalk substrate leads to permanent death of individuals of all age groups. Individuals of the pregenerative fraction, small in size, are either washed out, or the chalky slurry curls the points of growth of the root rosette; in individuals of the generative fraction, both blocking of shoot growth points with chalk slurry and quite strong – up to 10 cm – exposure of caudex and root is observed, which significantly reduces the life of the plant. Postgenerative individuals (I_a – 4.63-4.76) die not only due to a decrease in the viability of the organism, but also because necrotic processes in caudex allow, with any mechanical effect (melt water, precipitation, wind, etc.), to come off as individual rosette shoots and heads of caudex. Among the selected CP of the *Pimpinella*, similar processes were most clearly manifested in CP 1 (density – 9.92 pcs/per 1 m^2, I_r – 0.45). Figure 6 shows the structure of the chalk substrate and the appearance of the plants after heavy rain.

Figure 6 – The state of juvenile (left) and middle-aged generative individuals (right) after the movement of water flows

Thus, the formation of the ontogenetic structure of the cenopopulations of *Pimpinella tragium*, along with the features of ontogenesis, is significantly imprinted by the physical properties of the substrate, and hence the physiological features of this species.

Conclusion

The need for a comprehensive study of the biology of rare and endangered plant species of Cretaceous outcrops is indisputable. An important aspect is the analysis of the features of ontogeny and the structure of populations of these species since these questions will help to gain an idea of the current state of phytocenoses growing on chalk and limestone substrates. This work presents the population and ontogenetic characteristics of one of the cenosis-forming species of the Cretaceous outcrops – *Pimpinella tragium*. In the Voronezh region, the species grows on the border of its range and is noted both in the forest-steppe and in the steppe parts of the region. *Pimpinella tragium* is a pioneer of overgrowing not only loose, but also dense chalk outcrops, as it tolerates compaction of the chalk substrate well. We have identified several types of phytocenoses with the dominance of this species, the subdominants are mainly tap-root perennial grasses and dwarf semishrubs, obligate calcephytes. It has been established that the species goes through all stages of ontogenesis and reproduces well enough by seed. However, the state of *Pimpinella tragium* cenopopulations is unstable and largely depends on the state of the Cretaceous substrate and the recreational load on it. In this regard, the industrial development of chalk, which has been actively developing in the Voronezh region in recent years, causes great concern. We believe that it is necessary to reduce the area of chalk quarries and prepare a program for the protection of plant communities of chalk outcrops at the regional level. Considering the ecological-cenotic and population characteristics of *Pimpinella tragium*, the species can be proposed as a model for the reclamation of significantly disturbed communities on

Cretaceous substrates. With regret, we have to state that the population biology of calcephytes for the entire territory of European Russia has been studied extremely poorly. This is all the more surprising, since the area of chalk substrates and plant communities on them is steadily decreasing under the influence of the increasing anthropogenic load. We believe that only joint efforts to study and protect calciphytic vegetation will allow preserving the unique Cretaceous landscapes and their floristic diversity.

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