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Phenological growth stages according to the BBCH scale *Elaeagnus multiflora* Thunb.

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Phenology is a key trait of plants of all species, as it determines their season and duration of growth and reproduction, as well as their ability to capture variable resources. Understanding the phenology of *Elaeagnus multiflora* Thunb. a rare but promising fruit and medicinal plant of Ukraine, namely the codification of the stages of seasonal development, according to the international BBCH scale, is important for the evaluation of breeding material and the development of new varieties, improving the technological qualities of fruits. In the climatic conditions of Ukraine (M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, Kyiv), the studied genotypes of *E. multiflora* go through a full cycle of development. Plants begin the growing season with the development of leaves and shoots. According to the international BBCH scale, they clearly distinguish eight of the ten main stages of seasonal development, in particular: the development of buds (Principal growth stage 0), leaves (Principal growth stage 1), shoots (Principal growth stage 3), inflorescence emergence (Principal growth stage 5), flowering (Principal growth stage 6), fruit development (Principal growth stage 7), fruit ripening (Principal growth stage 8) and senescence and the onset of dormancy (Principal growth stage 9). The proposed BBCH scale for characterizing the phenological stages of *E. multiflora* plants can be used to guide the growers as to when to carry out orchard management practices such as canopy training and pruning, nutrient and water application, pest and disease control and post-harvest processing. Correct identification of phenological stages is of great importance for the characterization and management of *E. multiflora*. Thus, this study will ensure the dissemination of knowledge about *E. multiflora* cultivars among growers and researchers.

Keywords: *Elaeagnus multiflora*, BBCH scale, developmental stages, phenology, phenophase

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Introduction

Observations of the seasonal development of plants, both wild and introduced, have been carried out since ancient times using various techniques (Meier et al., 2009). In the middle of the last century, the need for a single, international standard for displaying the phenological stages of plant growth, regardless of taxonomic affiliation and research region, became apparent (Cautín, Agustí, 2005; Zhao et al., 2019). Based on the numerical code of Zadoks et al. (1974), the BBCH scale was developed, and then the expanded BBCH scale (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) (Berning et al., 1987a, b, c; Bleiholder et al., 1986, 1989; Lancashire et al., 1991; Hack et al., 1992). Since then, the BBCH scale has been widely used to record and describe the phases of the seasonal development of plants of various types in different climatic zones, in particular, fruit plants, namely: *Cydonia oblonga* Mill. (Martínez-Valero et al., 2001), *Olea europea* L. (Sanz-Cortés et al., 2002), *Prunus armeniaca* L. (Pérez-Pastor et al., 2004), *Actinidia deliciosa* (A.Chev.) C.F.Liang & A.R.Ferguson (Salinero et al., 2009), *Diospyros kaki* Thunb. (García-Carbonell et al., 2002; Guan et al., 2021), *Diospyros virginiana* L. (Grygorieva et al., 2010), *Mangifera indica* L. (Hernández Delgado et al., 2011), *Mespilus germanica* L. (Atay, 2013), *Persea americana* Mill. (Alcaraz et al., 2013), *Ziziphus mauritiana* Lamk. (Krishna et al., 2019), *Prunus avium* L. (Fadón et al., 2018), *Pseudocydonia sinensis* C.K. Schneid. (Grygorieva et al., 2018a), *Malus domestica* Borkh (Martínez-Valero et al., 2019), *Prunus dulcis* (Mill.) D.A.Webb (Sakar et al., 2019), *Myrtaceae* species (Guollo et al., 2020), *Cornus* L. (Klymenko and Ilyinska, 2021), *Cornus sessilis* Torr. ex Durand (Klymenko et al., 2021). In fruit growing, detailed codification of seasonal stages of plant growth is important for evaluating breeding material and breeding new varieties, improving the technological qualities of fruits; it is a good reference for growers and scientists who need uniform selection criteria, as well as the development of management methods for the expansion of commercial cultivation.

The genus *Elaeagnus* L. (Elaeagnáceae Juss., nom. cons.) includes almost 90 species distributed in Asia, southern Europe, North America, and South-Eastern Australia (Qin and Gilbert, 2007). The greatest species diversity, including 55 endemic species, is concentrated in China (Qin and Gilbert, 2007; Sun and Lin, 2010). Plants of many species (for example *E. angustifolia* L., *E. commutata* Bernh., *E. pungens* Thunb., *E. umbellata* Thunb.) have economic value and are used as a fruit, medicinal (in traditional medicine), honey-bearing or decorative (Qin and Gilbert, 2007; Lachowicz et al., 2020; Nazir et al., 2020; Bieniek et al., 2022; Yang et al., 2022) plants. *Elaeagnus multiflora* Thunb. (cherry elaeagnus, cherry silverberry, gumi) has Japanese origin and also it belongs to the number of promising fruit plants with high nutritional and medicinal potential.

The natural range of this species includes China, Japan, Korea, and the Kuril Islands; introduced into the culture in North (USA) and South America (Colombia, Brazil), as well as in 11 European countries (*Elaeagnus*, 2021). In its homeland, *Elaeagnus multiflora* has been cultivated as an ornamental, food, and medicinal plant for several centuries (Sakamura and Suga, 1987; You et al., 1994; Lee et al., 2007). Plants form thickets and sparse forests in the lowlands and the mountains from sea level to 1,800 m above sea level. r. m. They can grow on poor soils, due to symbiosis with nitrogen-fixing microorganisms living in root nodules (Qin and Gilbert, 2007; Sun and Lin, 2010), resistant to drought and frost. They bear fruit regularly and abundantly. Fruits contain carbohydrates, organic acids, amino acids, vitamins C and F, biominerals, polyphenols, flavonoids, carotenoids, chlorophylls and tocopherols, macro- and microelements, which ensures their high nutritional value (Vasyuk and Moroz, 2006; Bieniek et al., 2017, 2022). Biologically active compounds are also present in the bark, leaves, flowers, and seeds (Shin et al., 2008; Patel, 2015; Lachowicz et al., 2020).

In Ukraine, cherry elaeagnus is grown in some botanical gardens and as a fruit plant on private plots, in particular in the Lviv region (Vasyuk and Moroz, 2005; *Elaeagnus*, 2021). In M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, (NBG) cherry elaeagnus was introduced in 1980–1982. The modern collection of *E. multiflora* in the NBG includes 45 genotypes. In the climatic conditions of Kyiv, the variability of the morphometric parameters of fruits was investigated (Grygorieva et al., 2018b), their biochemical composition, and also for the first time, oil was isolated from fruits, its quantitative and qualitative composition was determined, and it was established that the seeds and pulp of fruits contain irreplaceable of acids (linoleic and linolenic) is significantly higher than in the fruits of sea buckthorn (*Hippophaë* L. sp.) (Vasyuk and Moroz, 2006). Attention is also paid to the peculiarities of vegetative growth and generative development, winter and drought resistance, the nature of fruiting, seed, and vegetative propagation of plants. A phenological study of *E. multiflora* was conducted based on the use of fairly general and not very detailed methods of Beidemann and Lapin and Sidneva, which

are widespread mostly in Eastern Europe (Vasyuk and Moroz, 2005).

These data are important, but they lack detailed information, as only the main stages of seasonal rubber development are described. Therefore, a detailed phenological characterization is needed, which covers the entire life cycle of *E. multiflora* and which meets modern international standards. Thus, the purpose of our work is to describe and codify the seasonal development of *E. multiflora*, according to the international BBCH scale.

Material and methodology

Research area

The experiment was conducted in NBG, Kyiv (latitude: 50° 27.28‘ N; longitude: 30° 31.428‘ E, altitude: 197 m above sea level), which is located in the north of the central part of Ukraine in the middle course of the Dnipro; the climate is continental with mild winters and warm summers – Dfb, according to the Köppen-Geiger classification (Peel et al., 2007).

Biological material

30-year-old genotypes of *Elaeagnus multiflora* growing in the M.M. Gryshko National Botanical Garden. Ten healthy trees were randomly selected.

Phenological studies

Phenological behavior was assessed by observation dates and photographs. Seasonal phases of development are classified based on the BBCH (Biologische Bundesantalt, Bundessortenamt und Chemische Industrie) scale (Hack et al., 1992; Meier et al., 2009). A two-digit numbering system was used, where the first digit corresponded to the primary stage of growth, and the second to the secondary one. Data were recorded at intervals of three days. Throughout the experiment (April–November 2021), basic meteorological data were monitored.

Results and discussion

Phenology is a key trait of plants of all species, as it determines their season and duration of growth and reproduction, as well as their ability to capture variable resources (Schwartz, 2003; Chuine, 2010).

Elaeagnus multiflora is a deciduous shrub or small tree with a different crown shape (from upright to spreading), rusty-red young branches, abundantly covered with peltate trichomes (scales) and with

Figure 1 *Elaeagnus multiflora* Thunb. deciduous shrub

stellate hairs along the midvein of young leaves (Figure 1).

The biological minimum of *E. multiflora*, like many other fruit plants of the temperate zone, is ≥5.0 °C (Chmielewski and Köhn, 2000; Vasyuk and Moroz, 2005). In the climatic conditions of Ukraine (NBG), the genotypes of *E. multiflora* go through a full cycle of development. Plants usually start vegetation in the first decade of April with the development of leaves and shoots when the sum of effective temperatures is 36–59 °C (Table 1, Figure 1).

Principal growth stage 0. Bud development

E. multiflora is characterized by two types of buds such as vegetative (developing on proleptic, epicormic, and sylleptic shoots) and complex, vegetative-generative, which are formed on replacement proleptic shoots.

The development of vegetative buds of gum occurs in the same way as in other woody plants of the temperate zone. At rest (phenophase 00), the buds are round, half-open, and covered with brown scales. At the beginning of swelling (phenophase 01), buds increase in size. At the end of swelling (phenophase 03), they reach their final size, after which brown bud scales

Table 1 Phenological growth stages of *Elaeagnus multiflora* Thunb. according to the BBCH scale

BBCH Code	Description
	Principal growth stage 0: Bud development
00	bud dormancy
01	beginning of bud swelling
03	end of bud swelling
07	budding begins: the first silvery-green tips of the leaves are just visible
09	silvery-green leaf tips about 3 mm above bud scale
	Principal growth stage 1: Leaf development
10	first leaves separating: silvery-green leaf tips about 10 mm above the bud scales
11	the first young leaves unfolded, the rest of the leaves have not completely unfolded yet
15	more leaves unfolded, but not yet at full size; petioles visible
19	the first leaves have reached the characteristic size for the species
	Principal growth stage 3: Shoot development
31	beginning of shoot growth: axes of developing shoots visible
32	20% of the expected typical shoot length (annual shoot) achieved
34	50% of the expected typical shoot length (annual shoot) achieved
39	90% of the expected typical shoot length (annual shoot) achieved
	Principal growth stage 5: Inflorescence emergence
51	reproductive buds swelling in leaf axils: buds closed, greenish-brown scales visible
54	bud burst: scales separated, the silver-white tops of the buds are visible
55	sepals visible, but still united, flower pedicel elongating (green bud)
56	flowers still closed; sepals slightly begin to separate
59	first flowers form a hollow ball
	Principal growth stage 6: Flowering
60	first flowers open (sporadically)
61	beginning of flowering: about 10% of flowers open
65	full flowering: 50% of flowers open, the calyxes of the first flowers dry
67	flowering finishing; the calyxes of many flowers have dried up
69	the end of flowering: the calyxes of all flowers have dried up; fruit set is visible
	Principal growth stage 7: Fruit development
71	fruit set, beginning of ovary growth
72	fruit at 20% of final size
75	fruit at 50% of final size
79	fruit at 90% of final size, green
	Principal growth stage 8: Fruit and seed maturation
81	the beginning of fruit ripening: the color of the fruit changes from green to yellow
85	the color of the fruits progresses: it acquires a red color characteristic of the species
87	increasing color intensity; 80% of the fruits have reached technical ripeness; the flesh is crisp and sweet with typical taste and correct firmness
89	fruits colour fully developed. fruit ripe for consumption, the flesh is crisp and sweet with typical taste and correct firmness

Continuation of Table 1

BBCH Code	Description
	Principal growth stage 9: Senescence and beginning of the rest period
91	shoot growth is complete, the terminal bud is developed, but the leaves are still green
92	change in leaf color, green color started to disappear
93	the beginning of falling leaves
95	leaves at 50% fallen
97	dropping of all leaves
99	the beginning of winter dormancy

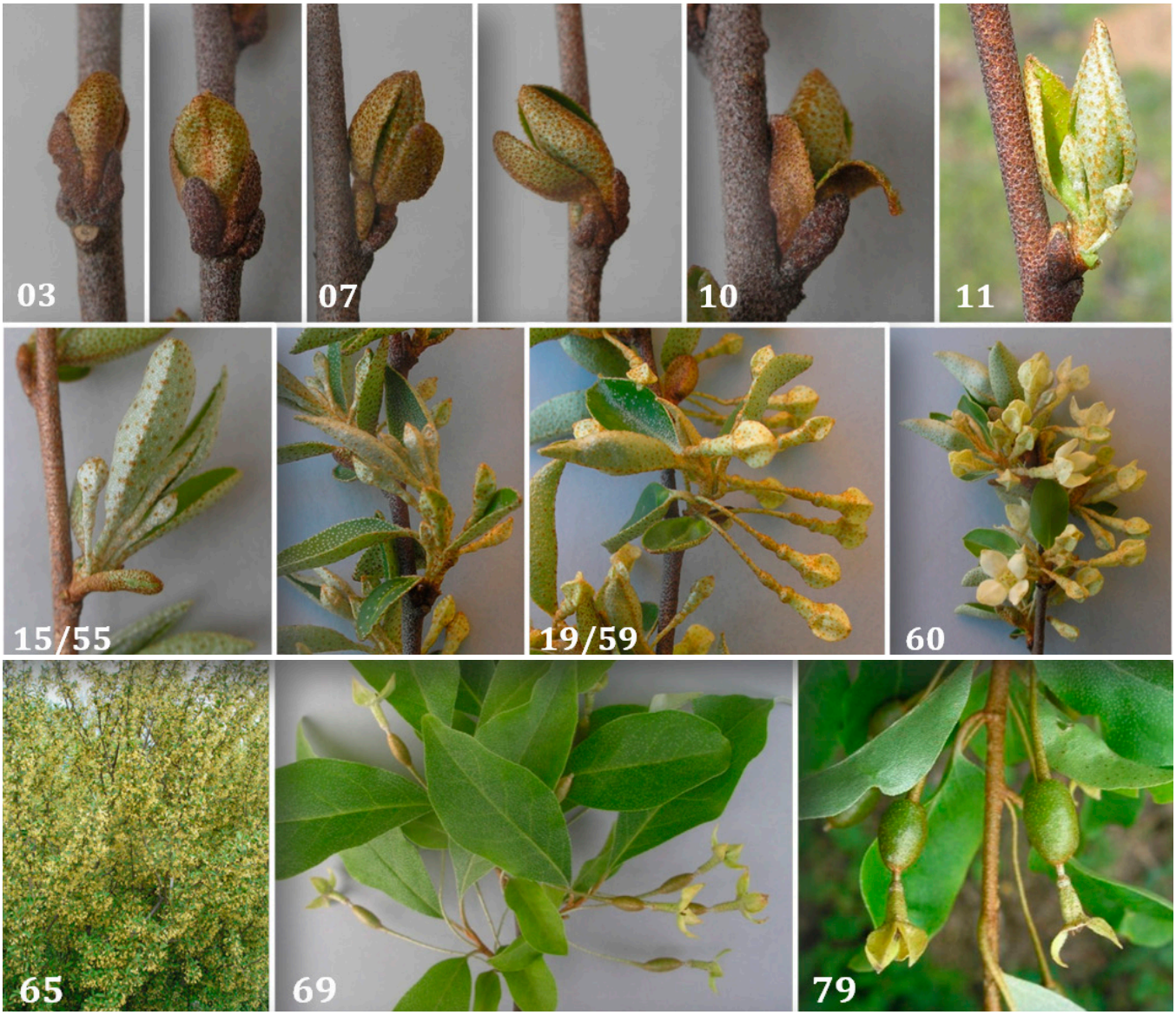

Figure 2a Phenological stages of the *Elaeagnus multiflora* Thunb. plants

Figure 2b Phenological stages of the *Elaeagnus multiflora* Thunb. plants

separately. Next, the silver-green tips of the leaves become noticeable (phenophase 07), as well as the green adaxial surface of the bud scales, thanks to which they can perform the function of photosynthesis, which intensifies the further development of the leaves. At the end of phenophase 09, all bud scales are opened, and the tips of the leaves reach about 3 mm in length.

Principal growth stage 1: Leaf development

The formation of leaves of vegetative shoots lasts from mid-April to August, and the development and growth of leaves of replacement shoots continue for about two months from mid-April to the end of May.

The first to begin development (phenophases 10, 11) are the three prophyllum. They differ from real leaves in a much smaller size, as in many other types of fruit plants. The full development of the first typical leaves (phenophase 19) completes "Principal growth stage 1".

Typical leaves of *E. multiflora* are elliptic or ovate to obovate-oblong, 3–10 × (1–)1.2–5 cm, abaxially with densely overlapping white and scattered pale brown scales, scales shallowly umbonate, adaxially stellate-pilose while young, lateral veins 5–7 per side of the midrib, base obtuse to cuneate, apex obtuse to acute or bluntly acuminate. Petiole 4–6 mm, brown scaly (Figure 3).

Principal Growth Stage 3: Shoot development (sprout from the terminal bud)

The study of shoot growth dynamics is an important indicator, as it allows us to assess the compliance of new environmental conditions with the needs of introduced plants and the course of adaptation processes.

Elaeagnus multiflora is characterized by proleptic vegetative and vegetative-generative shoots that develop from overwintering buds. In addition, epicormic vegetative shoots formed from dormant buds are also characteristic, and on which sylleptic enrichment shoots develop from buds with no period

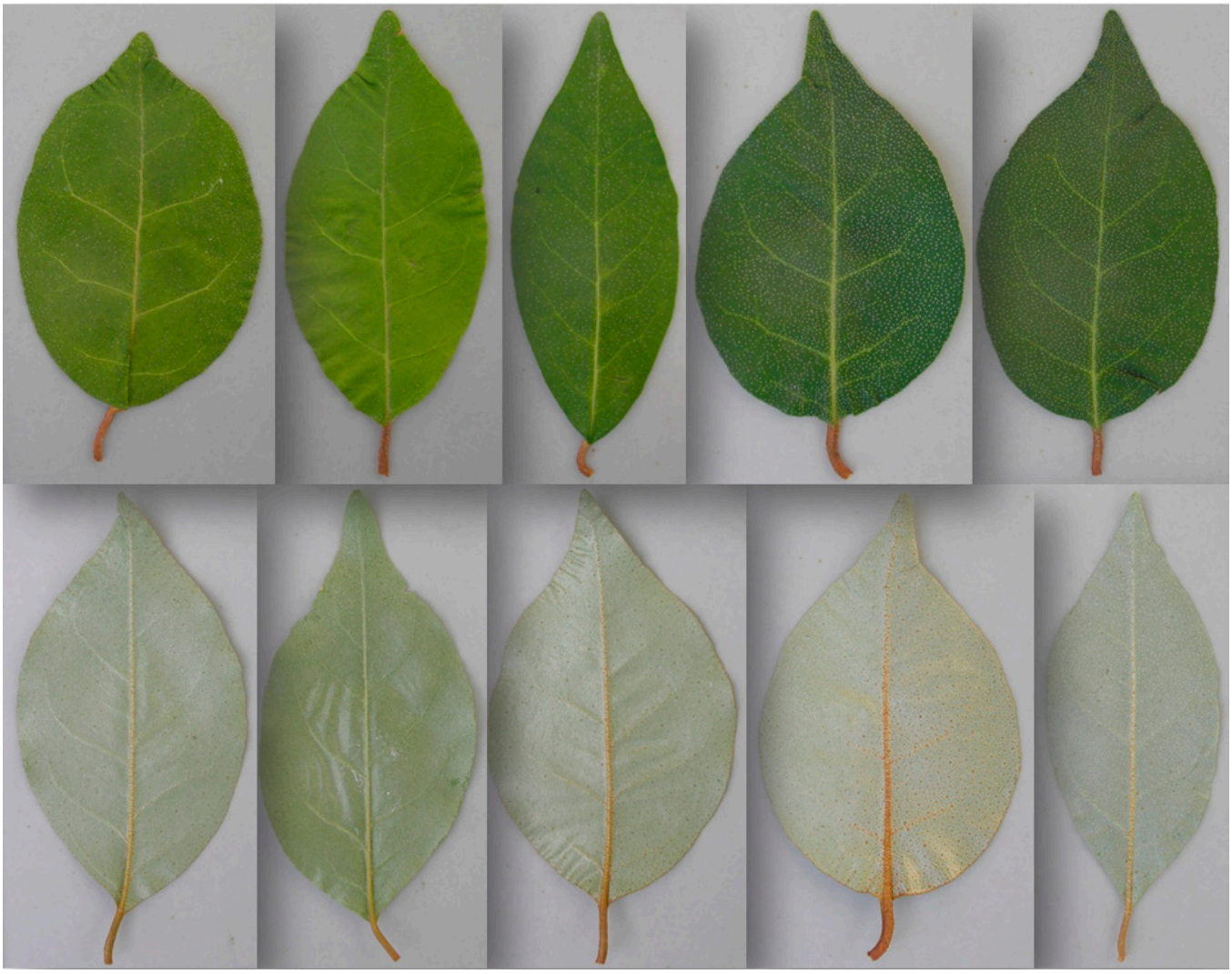

Figure 3 Leaves of *Elaeagnus multiflora* Thunb.

of growth dormancy, as in other fruit plants (Negrón et al., 2014).

Stems of one-year shoots are light brown or reddish-brown, covered with scales (Figure 1), perennial, gray-brown, very branched, and sometimes have thorns. Replacement shoots are the first start growing usually in the second half of April; they are short, 14.0–18.0 cm long. The development of vegetative shoots becomes noticeable 10–12 days later and lasts until the end of August. At the end of the season (phenophase 39), they are thickened, strengthened, gray-green, densely covered with white spots, 128.2–160.3 cm long. Sylleptic shoots of enrichment are formed in mid-July on epicormic vegetative shoots (in the middle part of their length) and are characterized by slow growth. They are short (about 4.0–6.0 cm long), and their tip is often transformed into a thorn. We studied the development of vegetative shoots.

Principal Growth Stage 5: Inflorescence emergence

Many species of the *Elaeagnus* genus are characterized by racemes or umbel-like inflorescences (Sun and Lin, 2010). In *E. multiflora*, flower buds develop one or two at a time (occasionally) in the axils of the lower leaves of the replacement shoots. They are covered with peltate trichomes, like the whole plant. On one shoot, flower buds are formed gradually, synchronously with its growth and development. Therefore, flowers and buds of various degrees of development are observed at the same time. The period of development of flower buds lasts from the end of April to the middle of May, approximately 2–3 weeks.

Principal growth stage 6: Flowering

The flowers of *E. multiflora* are regular, bisexual, fragrant, 4-membered, without petals, as in other

Figure 4 Variation in the shape and color of perianths of *Elaeagnus multiflora* Thunb.

species of the Elaeagnaceae family (Graham, 1964; Bartish and Swenson, 2004), have white, creamy-white, or light yellow perianth (Figure 4). The flower tube is tubular or funnel-cylindrical, four-lobed, 4.0–5.5 mm in diameter and 5–10 mm long, narrowed above the ovary, breaks off as the fruit develops; blades are broadly ovate, sharply pointed at the top. Stamens 4, alternate with calyx lobes; the threads are very short. The ovary is upper with one seed primordium. Style is oblong; stigma is dry. Pedicel 4–8 mm long; during fruiting, it lengthens to 1.5–5.0 cm, thin.

From two to seven to eight flowers are usually formed on one shoot, depending on the genotype and weather conditions (Figure 5). Flowering begins in late April or early May and lasts from 15 to 20 days. The duration

Figure 5 Flowering shoots of *Elaeagnus multiflora* Thunb. plants

of flowering depends both on the genotype and on weather conditions, in particular on temperature and amount of precipitation. The sum of effective temperatures at the beginning of flowering during 1999–2003 was 204.5–225.5 °C (Vasyuk and Moroz, 2005).

Principal growth stage 7: Fruit development

Fruits are propagules for the reproduction and dispersal of angiosperms and at the same time are products of many agricultural crops. Fleshy fruits play a particularly important role in a person's daily diet. The edible, juicy part of such fruits has very diverse origin. Almost all parts of the entire structure of the inflorescence can form fruit pulp in certain species (Coombe, 1976).

The fruits of *E. multiflora* are false drupes (sphalerocarps), as in other species of the Elaeagnaceae family. They are covered with a juicy hypanthium base (calyx tubes). Only the abaxial layers of hypanthium cells form the pulp, while the adaxial ones are transformed into mechanical tissue such as an eight-ribbed "stone" (Qin and Gilbert, 2007; Ye et al., 2012). The fruit (pericarp) is thin, membranous, and consists of several layers of cells, that is why cherry elaeagnus fruits are classified as false fruits namely stone-like or berry-like (Graham, 1964; Bartish and Swenson, 2004).

E. multiflora fruits develop and grow quite quickly. About three to five weeks pass from the beginning of the growth of the ovaries (phenophase 71) to reaching the final size of the first fruits (phenophase 79). Ovaries and immature fruits that have finished growing are hard, green, and abundantly covered with brown peltate scales. The rapid development of fruits is also characteristic of the Japanese variation of *E. multiflora* var. *gigantea* Araki, in which the period from flowering to fruit ripening is 6–7 weeks (Ye et al., 2012).

Principal growth stage 8: Maturity of fruit

Ripe *E. multiflora* fruits are elongated, ovoid, or ellipsoidal false drupes with a length of 7.60 to 19.54 mm and a diameter of 4.39 to 10.32 mm (Grygorieva et al., 2018b). The beginning of fruit ripening starts with a change in their color (stage 81). They initially become yellow, and then gradually acquire a red color, characteristic of the studied *E. multiflora* genotypes. Ripe fruits are red with silver or brown scales, have thin long peduncles (up to 5 cm long) (Figure 6, 7), remain on the plant for a long time, and do not fall off. On the same shoot, as well as on the same tree, the fruits ripen at different times, which corresponds to the gradual development of generative buds. The fruit ripening period begins in the first or second decade of June at the sum of effective temperatures of 616.6–790.2 °C (Vasyuk and Moroz, 2005) and lasts about two to three weeks. For use, the fruits are collected at the stage of technical ripeness (phenophase 87).

E. multiflora has abundant and regular fruiting (Figure 8). Ripe fruits are sweet with a slight astringency. The skin of the fruit is thin and fragile, which determines the specificity of the use of the species as a food crop. *E. multiflora* begins to bear fruit in the 4^{th}–5^{th} year. The most productive fruiting occurs at the age of 8 and lasts at least 12–15 years.

Figure 6 Fruits and seeds of *Elaeagnus multiflora* Thunb.

Figure 7 Fruits and peduncle of *Elaeagnus multiflora* Thunb.

Figure 8 Fruiting of *Elaeagnus multiflora* Thunb. plants

Principal Growth Stage 9: Senescence and beginning of the rest period

The stage of senescence and the onset of dormancy in the studied *E. multiflora* genotypes is extended in time, as in other fruit plants of a temperate climate (Martínez-Valero et al., 2001, 2019; Grygorieva et al., 2010, 2018; Atay, 2013; Klymenko and Ilyinska, 2021; Klymenko et al., 2021). First, the oldest leaves change color and fall (phenophases 92, 93), and later others die. Under optimal weather conditions, November (phenophases 93, 95) in the studied *E. multiflora* genotypes is quite long and not intense. Rapidly, within several days, the fall of leaves can be caused by a significant night frost. All leaves (phenophases 97) shed infrequently. Usually, one or more leaves can remain on the tops of annual shoots for a very long time.

Conclusions

In the climatic conditions of Ukraine (NBG, Kyiv), the studied genotypes of *E. multiflora* go through a full cycle of development. Plants begin their growing season with the development of leaves and shoots. According to the international BBCH scale, they clearly distinguish seven of the ten main stages of seasonal development, in particular: the development of buds (Principal growth stage 0), leaves (Principal growth stage 1), shoots (Principal growth stage 3), flowering (Principal growth stage 6), fruit development (Principal growth stage 7), fruit ripening (Principal growth stage 8) and senescence and onset of dormancy (Principal growth stage 9). Understanding the phenology of *E. multiflora* as a rare, but promising medicinal plant of Ukraine, namely the codification of seasonal stages of growth, according to the international BBCH scale, is important for the evaluation of breeding material and breeding varieties, improving the technological qualities of fruits. The proposed BBCH scale for characterizing the stages of seasonal development of *E. multiflora* plants is important for further research on the adaptive capabilities of the species under different climatic conditions, for the practical use of the complex of gumi genetic resources, as well as for its introduction and use in agricultural production, pharmacology, decorative and landscape gardening.

Conflicts of interest

The authors declare no conflict of interest.

Ethical statement

This article doesn't contain any studies that would require an ethical statement.

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