



## Assessment of Ecological and Biological Characteristics of *Gleditsia triacanthos* L. for Agroforestry of Degraded Areas

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### ABSTRACT

*Gleditsia triacanthos* as a drought-tolerant multipurpose species, is introduced into artificial plantings in the southern regions of Russia. The aim is to study the ecological and biological features of *Gleditsia triacanthos* in the conditions of the northern border of their cultivation (Volgograd Oblast, Russia). The studies were conducted from February to October annually (from 2019 to 2023) in the field (assessment of winter hardiness and drought resistance, measurement of taxonomical and reproductive indices, recording the passage of phenological phases, selection of plant samples – leaves, fruits) and laboratory conditions (freezing of shoots, study of water deficit parameters, water-holding capacity, assessment of fruit quality). It was established that the northern limit of cultivation of *Gleditsia triacanthos* is V zone of winter hardiness. Taxation parameters of *G. triacanthos* and the influence of soil-climatic conditions on them were revealed. Ranking of species by winter hardiness was carried out. *G. triacanthos* in the first variant (at –37 °C) had insignificant damage to buds (from 6 to 18%), annual shoots (from 4 to 20%) and perennial shoots (from 2 to 10%). The variability of fruiting indicators under the conditions of stress factor exposure was revealed. The average and low level of variability was revealed for most morphological traits of fruits and seeds. Weather conditions during fruit ripening have a noticeable effect on seed weight. Reproductive parameters of *G. triacanthos* var *inermis* are more influenced by environmental conditions, which reflects correlations between morphometric parameters of fruits and seeds.

**Keywords:** *Gleditsia triacanthos* L.; Introduction; Bioecological features; Artificial plantations.

### Article History

Article # 24-591

Received: 23-Apr-24

Revised: 29-Apr-24

Accepted: 17-May-24

### INTRODUCTION

The area of lands subject to forest protection is 156 million ha (about 75% of all agricultural lands). Two macro-regions of anthropogenic desertification spread have been identified: the European part of the arid belt – 700 thousand km<sup>2</sup> and the Asian part – 1500 thousand km<sup>2</sup>, that is within the boundaries of geographical regions 108 million ha of agricultural lands (Kulik et al., 2023). During the first experiments (early and mid-twentieth century) to establish forest belt systems, many tree species died or were short-lived due to systematic exposure to habitat stressors (Latkina and Latkin, 2018). Forest belts protect against wind erosion, form microclimate in the adjacent areas. The higher the forest cover, the more favorable the microclimate for living things (Boklag, 2020). In the dry-steppe zone of

Volgograd region, soils with low humus content (1.7-2.5%), medium loamy, light loamy granulometric composition prevail. Due to the active wind regime, soils are subject to wind erosion, which does not contribute to favorable conditions for farming (Tkachuk and Viter, 2022). To reduce these negative factors, it is necessary to carry out forest reclamation with expansion of forest plantations that will be tolerant to abiotic factors (Tkachuk and Pankova, 2021; Lavrov et al., 2021; Tunyakin et al., 2022).

Analysis of taxonomic composition of natural woody vegetation of the region has shown that it is represented by *Quercus robur* L., *Tilia cordata* Mill., *Ulmus laevis* Pall., *Fraxinus excelsior* L., *Acer platanoides* L., *A. tataricum* L., *Prunus spinosa* L., *Amygdalus nana* L., *Prunus fruticosa* Pall., *Caragana frutex* (L.) K. Koch., *Cytisus ruthenicus* Fisch., *Spiraea vanhouttei* (Briot.) Zab., *Rosa canina* L., *R. cinnamomea* L.,

**Cite this Article as:** Khuzhakhmetova A and Melnik K, 2024. Assessment of ecological and biological characteristics of *Gleditsia triacanthos* L. for agroforestry of degraded areas. International Journal of Agriculture and Biosciences 13(2): 112-117. <https://doi.org/10.47278/journal.ijab/2025.017>



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*Pyrus communis* L., *Malus sylvestris* (L.) Mill.). some of the listed species are widely used in agroforestry and are recommended as the main assortment for afforestation and reforestation.

Due to the trend of increasing average monthly temperature values and decreasing precipitation during the growing season, the relevance of ecological and biological research on the selection of adapted woody plants is increasing. According to the estimates of the Institute of Global Climate and Ecology for the Volga region in the winter period (1976-2021) the coefficient of linear trend for the average temperature was from +0.3 to +0.8°C/10 years, in the summer period for the amount of precipitation from -5 to -10% of the norm / 10 years.

In this regard, *Gleditsia triacanthos* L., which naturally grows in North America (Zakharenko et al., 2017), is of potential interest for green building and reforestation. In the natural range, longevity can reach more than 100 years. *Gleditsia triacanthos* has large prickles (up to 30 cm) on branches that are well suited for creating hedges. Chinese and Korean folk medicine widely uses plants of the genus *Gleditsia* L. in pharmacological direction (Chaudhary et al., 2022). Under in vitro conditions, the presence of antispasmodic and cytotoxic activities, anti-inflammatory, antimutagenic and antimicrobial activities in the plant material of *Gleditsia* L. have been confirmed (Jinqian et al., 2018; Azimova et al., 2019; Jahan and Rahman, 2022). The fruits of *Gleditsia triacanthos* contain nutrients that are promising and interesting sources of feed for small ruminants (Medjekal et al., 2018; Shestopalova et al., 2021; Malarkodi et al., 2022; Sinchana and Raj, 2023). The tree with openwork crown is known for its drought tolerance (Muratgeldiev, 1975; Semenytina et al., 2022; Khuzhakhmetova and Melnik, 2023) and is tolerant to slight soil salinity.

The aim is to study the ecological and biological features of *Gleditsia triacanthos* in the conditions of the northern border of their cultivation (Volgograd Oblast, Russia). Information on ecological and biological features of *Gleditsia triacanthos* is of scientific and practical importance for the development of effective technologies for the restoration of degraded lands.

## MATERIALS & METHODS

### Objects, Location of the Study

The objects of research are trees *Gleditsia triacanthos* L., *Gleditsia triacanthos* var *inermis* Pursh, growing in collections and forest strips of the Federal Scientific Center of Agroecology of the Russian Academy of Sciences (34:34:000000:122; 34:36:000014:177; Fig. 1). The remoteness of the two sites with the studied plants are located at a distance of about 190 km from each other.

Soils of the plantation location (Kamyshin, 34:36:000014:177; 50.129115° 45.392846°) are chestnut soils of medium- and light loamy granulometric composition, variation along the profile (0-1,40 m) of humus content 0,74-1,71%, with very low content of



**Fig. 1:** Objects of research on cluster dendrological collections of FSC Agroecology RAS (a – *Gleditsia triacanthos* L. *inermis*; b – forest strips with participation of *Gleditsia triacanthos*).

Nutrient elements (total nitrogen – 0,019-0,023%, total phosphorus – 0,03 - 0,09%). Potassium supply is high (total potassium – 0.58-0.91%), pH – 8.5-8.7. Soils of the site (Volgograd, №34:34:000000:122; 48.633705°, 44.421633°) are light chestnut soils, medium-wet, medium-loamy, characterized by lower humus content (0.54-0.94%) in the A horizon.

The content of nitrogen, phosphorus, potassium is typical for these soils, pH – 6.8-7.4. The content of exchangeable calcium decreases and magnesium – increases up to transition from chestnut to light-chestnut soils.

### Data Collection

The studies were conducted from February to October annually (from 2019 to 2023) in the field (assessment of winter hardiness and drought resistance, measurement of taxonomical and reproductive indices, recording the passage of phenological phases, selection of plant samples – leaves, fruits) and laboratory conditions (freezing of shoots, study of water deficit parameters, water-holding capacity, assessment of fruit quality). The sample volume was 100 trees. Under field conditions, winter hardiness was assessed in May for a number of years (2010-2023) according to the scale (Table 1).

**Table 1:** Winter hardiness scale

Score	Diagnosis of winter hardiness by plant condition	Frost index in laboratory conditions
I	No or minor damage (up to 10% of annual shoot length)	1.0
II	Damaged at 50% of the length of annual shoots	0.8
III	Damaged at 100% of the length of annual shoots	0.6
IV	Shoots (2-3 years old) and skeletal branches freeze off	0.4
V	Above-ground part (above snow level) is killed	0.2
VI	Above-ground and underground parts of plants freeze off	0

In each sample, shoots were harvested in February, when the trees were in deep rest. In the experiment on shoots freezing in the climatic chamber KHT-0.22, two temperature regimes were selected:  $-37^{\circ}\text{C}$ ,  $-41^{\circ}\text{C}$ ; the duration of temperature exposure on experimental samples was 12 hours. After freezing the shoots at different temperature regimes, staining was carried out with a solution of methylene blue (100mg/L) in 2.5%  $\text{KH}_2\text{PO}_4$ . Further, according to the character of shoot tissues staining, lesions (stained with blue color) were counted using a microscope (Micromed 3 variant 3-20M).

Damaged and non-viable cells stained blue faster than live cells. The freezing index was calculated using the formula:  $Y = 100 \times \lambda \times d\Delta / h \times d$ , where  $d\Delta$  – diameter at the base of the frozen part of the branch, m;  $\lambda$  – length of the frozen part of the branch, m;  $h$  – plant height, m;  $d$  – trunk diameter (below the first branch), m (Semenyutina and Melnik, 2021).

Indicators of water deficit and water-holding capacity of leaves were evaluated by weight method (Polevoy et al., 2001) from June to August during the period of stress factor exposure (absence of precipitation for 7-15 days against the background of high air temperatures from  $30.5$  to  $41.2^{\circ}\text{C}$ ). Pigment lability was measured with a DUALEX SCIENTIFIC chlorophyllometer. Characteristics of weather conditions in the years of study were supplemented with data from the electronic resources "Weather and Climate" (Pogodaiklimat.ru) and Hydromet (Meteoinfo.ru).

Generative development included consideration of fruiting intensity: where 1 point – no fruits on the tree, 2 – single fruits on separate branches in the upper and middle parts of the crown, 3 – insignificant number of fruits, mainly in the upper and middle parts of the crown, 4 – average number of fruits growing evenly or in groups on a significant number of branches in the upper and middle parts of the crown, 5 – fruits are present on almost the whole crown. To study morphometric characters of fruits and seeds of *Gleditsia triacanthos* and *G. triacanthos* var *inermis* and their variability, we randomly selected fruits (20 fruits in 10-fold repetition). The reliability of the obtained results was confirmed by long-term experimental studies with the use of certified equipment and statistical processing of meteorological, laboratory, field observations.

## RESULTS AND DISCUSSION

A collection of representatives of the genus *Gleditsia* has been formed in the collections of the Federal Research Center of Agroecology of the Russian Academy of Sciences, the introduction testing of which has been carried out since

1913. The natural range of *Gleditsia triacanthos* includes vast areas of North America, which belong to the Va-IXa zones of winter hardiness with average values of the minimum temperature range from  $-28.9^{\circ}\text{C}$  to  $-3.9^{\circ}\text{C}$ . Promotion of the North American species to more northern areas of the country is limited by low winter temperatures (below  $-35^{\circ}\text{C}$ , winter hardiness zones III-I).

The Volgograd region, as a place of localization of collections with introduced species, is referred to zone V (from  $-28.9^{\circ}\text{C}$  to  $-34.4^{\circ}\text{C}$ ). The analysis of average values of the main meteorological indicators revealed an increase in average monthly temperature values and a decrease in precipitation during the growing season. According to long-term data, the coldest period in the city is the second decade of January with an average temperature of  $-5.1$  to  $-5.6^{\circ}\text{C}$ . The warmest period comes in mid-July, with an average daily maximum temperature of about  $+26^{\circ}\text{C}$ . The average maximum was  $+28^{\circ}\text{C}$  in the city of Volgograd in the second decade of July. Volgograd Oblast is characterized by unstable weather, with frosts occasionally alternating with thaws. Precipitation is mainly snow or sleet. January 2022 was snowier (100 mm) over the period of observation (2019-2023). Winters were warm (2019, 2020, 2023; exceedances of the norm of average monthly temperatures by  $3.1-6.1^{\circ}\text{C}$ ) or relatively warm (2021; 2022; exceedances of the norm by  $2.2-5.8^{\circ}\text{C}$ ) and favorable for tree overwintering, absolute minimum temperatures did not fall below  $-30^{\circ}\text{C}$  (Fig. 2).

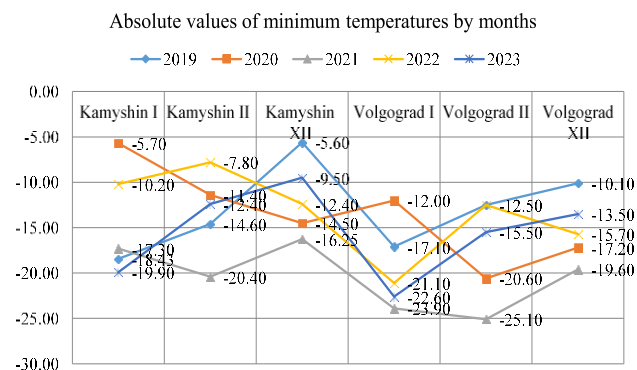
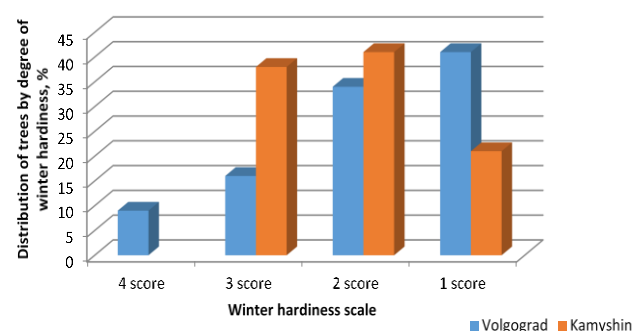
According to the data absolute minimum air temperature recorded during the cold periods of 1966-2018 in Volgograd  $-35^{\circ}\text{C}$ , in Kamyshin  $-37^{\circ}\text{C}$ . The temperature of the coldest days with a probability of 92% and 98% for Volgograd is  $-26^{\circ}\text{C}$  and  $-28^{\circ}\text{C}$ , for Kamyshin  $-28^{\circ}\text{C}$  and  $-30^{\circ}\text{C}$ , respectively. To identify critical values of minimum temperatures, experiments on their effect on shoots of *Gleditsia triacanthos*, *G. triacanthos* var *inermis* were additionally conducted (Table 2).

As a result of the experiment, the minimum temperature value ( $-41^{\circ}\text{C}$ ), which has a limiting effect on the species' advancement to northern areas, was confirmed. At the same time, it should be noted that unfavorable conditions for collections of *Gleditsia* species occurred in the winter of 1968/69. Temperatures dropped to  $-21.9^{\circ}\text{C}$  in December,  $-31.6^{\circ}\text{C}$  in January, and  $-29.8^{\circ}\text{C}$  in February. *Gleditsia* plants died during this period due to the low temperatures. The conditions were particularly difficult for root systems. The soil temperature in the 50 cm layer was kept at  $13.1^{\circ}\text{C}$  due to prolonged snowlessness, which caused the death of plants (Khizhnyak, 1982).

The average degree of frost resistance of *Gleditsia triacanthos* and *G. triacanthos* var *inermis* was established. If earlier air temperature within  $-20-22^{\circ}\text{C}$  was considered

**Table 2:** Evaluation of frost resistance of *Gleditsia triacanthos*

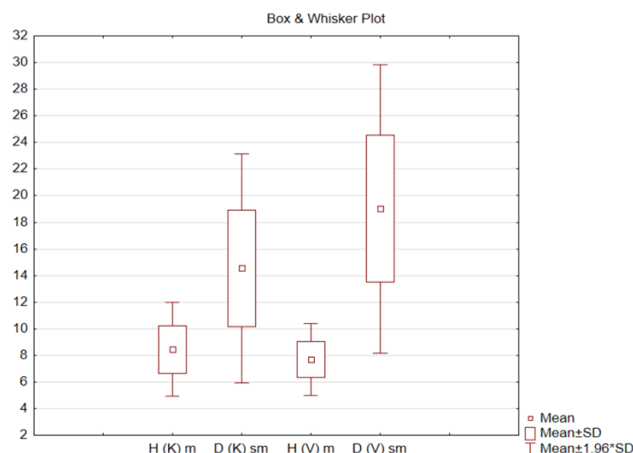
Year	Species	Average index freezing index at temperature		Average value of damage in points (%)					
				Buds		Annual shoot		Perennial shoot	
		-37°C	-41°C	-37°C	-41°C	-37°C	-41°C	-37°C	-41°C
2019	<i>G. triacanthos</i>	0.94±0.04	0.89±0.03	1(7)	2(18.5)	1(4)	2(20)	1(2)	1(20)
	<i>G. triacanthos</i> var <i>inermis</i>	0.97±0.03	0.89±0.04	1(8)	2(22.5)	1(4)	2(20)	1(2)	1(15)
2024	<i>G. triacanthos</i>	0.87±0.01	0.83±0.02	2(18)	2(22.5)	2(20)	2(25)	1(4)	1(14)
	<i>G. triacanthos</i> var <i>inermis</i>	0.95±0.04	0.87±0.03	1(6)	2(2)	1(8)	2(32.5)	1(10)	2(20)

**Fig. 2:** Absolute values of minimum temperatures in January (I), February (II), December (XII).**Fig. 3:** Distribution of *Gleditsia triacanthos* by degree of winter hardiness.

critical for *Gleditsia*, then, as long-term monitoring of plant condition showed, they crossed this threshold in the second generation in the process of gradual acclimatization and selection. *G. triacanthos* and *G. triacanthos* var *inermis* in the first variant (at -37°C) had minor damage to buds (6 to 18%), annual shoots (4 to 20%) and perennial shoots (2 to 10%). Under field conditions, where the species is affected by a complex of factors and their different combination, different degrees of winter hardiness were revealed (Fig. 3).

Comparative characterization of ecological plasticity in relation to low negative temperatures showed that in Kamyshin conditions 79% of trees had damage to young shoots. In Volgograd, single specimens of trees with 4 points were recorded, the percentage of trees with an excellent degree of winter hardiness was higher than in Kamyshin.

The age of the forest belt in Volgograd was 45 years and in Kamyshin 20 years. The preservation of these forest belts is very high: 91.3% in Volgograd and 93% in Kamyshin. The average height of trees in the two forest belts ranged from 7.6 to 8.7m, and the average diameter varied from 14.2 to 19.0cm (Fig. 4).

**Fig. 4:** Variation of height (H, m) and diameter (D, cm) of *Gleditsia triacanthos* in the forest belt (K – Kamyshin, V – Volgograd).

Taxation indices were established for groups of trees with different degrees of winter hardiness. Specimens of *Gleditsia triacanthos* with a high degree of winter hardiness (1-2) had a maximum height of 10 to 12 m in the forest belt (Kamyshin), a group of trees with a 3 degree of winter hardiness had a height ranging from 5.5 to 5.8 m, which exceeds the values of trees with the same degree of winter hardiness (4.8-5.2 m) growing in light-chestnut soils of Volgograd. Deterioration of forest conditions affects growth vigor, differentiation and drought tolerance of the stand in a negative direction. In the years of research *Gleditsia triacanthos* has different duration of shoot growth period, as this indicator depends on air temperature and soil moisture. The summer period was characterized by hot sunny weather, maximum precipitation (122 mm) was recorded in July 2020. The following year (2021) was recognized as the wettest year during the study period, as the sum of annual precipitation amounted to 484 mm. In Kamyshin, the highest total precipitation also occurred in 2021 - 582mm. against the background of high temperatures and prolonged absence of precipitation, leaf water content indices from June to August varied insignificantly: 67.4% (VI) to 62.0% (VIII), indicating drought tolerance. Water deficit indices ranged from 7.1 to 14.3%. The water loss in June after 4 hours of experiment is 29.2 to 34.5%, and in August this figure is 37.6 to 41.4%. By the end of the growing season, the water retention capacity of isolated leaves decreases. The highest average value of chlorophyll in *G. triacanthos* species is 20.4 mg/cm<sup>2</sup>, which grows on the territory of the Nizhnevolzhskaya tree plant breeding station, which confirms the best conditions of chestnut soils for the taxon.



**Table 3:** Fruiting intensity of *G. triacanthos*

Cadastral number of the plot	Fruit weight from 1 tree, kg (fruiting score)				Average score
	2020	2021	2022	2023	
34:34:000000:122	12.05 (4)	25.86 (5)	13.9 (4)	14.58 (5)	4.5
34:36:000014:177	0.65 (2)	1.67 (2)	0.76 (2)	1.32 (2)	2.0

**Table 4:** Comparative assessment of morphometric indices

Indicators	Mean	Min	Max	Std.Dev.	Mean	Min	Max	Std.Dev.
2022								
			<i>Gleditsia triacanthos</i>				<i>G. triacanthos</i> var <i>inermis</i>	
Fruit length (cm)	28.19	24.0	34.6	2.89	25.38	21.0	28.2	1.77
Fruit width (cm)	2.35	1.8	3.2	0.40	2.52	2.20	2.80	0.14
Fruit weight (g)	9.27	6.51	18.05	3.65	6.59	4.07	7.81	0.81
Number of seeds (pcs.)	12.95	4.0	25.0	5.89	14.90	10.00	20.0	2.55
Seed length (cm)	0.86	0.70	1.10	0.11	0.96	0.80	1.00	0.06
Seed width (cm)	0.61	0.50	0.80	0.07	0.60	0.50	0.70	0.04
Seed weight of 1 fruit (g)	1.85	0.70	3.55	0.88	1.86	1.11	2.48	0.37
Seed yield (%)	19.83	9.70	35.95	6.14	28.59	15.57	38.39	5.92
2023			<i>Gleditsia triacanthos</i>				<i>G. triacanthos</i> var <i>inermis</i>	
Fruit length (cm)	30.25	22.0	37.5	4.29	20.97	13.00	27.00	3.62
Fruit width (cm)	2.77	2.0	3.2	0.33	2.02	1.50	2.50	0.29
Fruit weight (g)	12.16	5.0	18.6	3.57	5.99	2.83	8.42	1.71
Number of seeds (pcs.)	17.75	12.0	25.0	3.37	14.2	9.00	20.00	3.19
Seed length (cm)	0.90	0.6	1.0	0.12	0.83	0.60	1.00	0.15
Seed width (cm)	0.52	0.5	0.6	0.04	0.49	0.30	0.80	0.10
Seed weight of 1 fruit (g)	2.94	1.32	4.2	0.72	1.41	0.73	2.36	0.44
Seed yield (%)	25.04	15.70	36.8	5.28	24.26	14.33	35.89	5.86

During a prolonged dry period, there is a decrease in photosynthesis, chlorophyll synthesis, pigment ratio. The most drought-tolerant species slow down the processes of water exchange at the lack of air and soil moisture, creating more favorable conditions for the plant.

Comparative evaluation of fruiting parameters showed satisfactory flowering and fruiting of *G. triacanthos* in Kamyshin (2 points). A drop of single fruits was recorded in the upper and middle part of the crown, mainly on the southern side within 30 days. Under Volgograd conditions, *G. triacanthos* in the strip had fruiting intensity from 4 to 5 points, which is associated with high fruit set (97.3%) and stable annual fruit formation from 12 kg (in dry years) to 25.86 kg/tree in wet year 2021 (Table 3).

A reliable excess of values of morphological indices of *Gleditsia triacanthos* fruits in 2023 compared to 2022 was revealed (Table 4), which was also reflected in the total fruit productivity of the species.

Since the second ten-day period of May 2022, no precipitation was observed, in June a precipitation deficit of 5.0 mm was noted. In the period of flowering and fruit setting, water availability of plants was better in 2023 (total precipitation 29.0 mm). In the drought year 2022, pronounced positive correlations were established in *G. triacanthos* between the following traits: fruit length and width (0.725), fruit length and weight (0.779), fruit length and number of seeds (0.736), seed weight and fruit length (0.877). Seed weight correlated with number of seeds (0.874), fruit width (0.706) and fruit weight (0.849). The relationship between other morphometric characters of fruits and seed weight is lower and, as a rule, unreliable.

*G. triacanthos* var *inermis* showed lower morphometric indices of fruits and seeds in 2023. In 2022, *G. triacanthos* var *inermis* showed a close correlation only between length and fruit weight (0.865), seed weight and fruit weight (0.731), seed weight and number of seeds in the fruit (0.871). In 2023, the correlation between the above indices was medium (0.567 to 0.684) and moderate (0.434).

The revealed dependencies of morphological traits are largely determined by differences in the duration of phenophases (from flowering to fruit ripening) and weather conditions during these periods.

## Conclusion

Under the conditions of the northern border of cultivation, winter-hardy specimens of *G. triacanthos* (frost index 0.83-0.89) with good taxation indices (up to 10-12 m) were isolated and the significance of the taxon for agroforestry was confirmed.

In difficult forest conditions of Volgograd region the species retains the ability to regenerate by regrowth. Stability of fruiting (4-5 points) in artificial plantations of Volgograd was established. The average and low level of variability was revealed for most morphological characters of fruits and seeds. Coefficients of variation were as follows: fruit weight – 10.2-29.0%, fruit width and length – 11.0-14.1%, seed length – 7.4-13.0%. At moisture deficit during the period of fruit and seed setting and formation, the variability of morphological parameters increases. Weather conditions during fruit ripening have a noticeable effect on seed weight. Reproductive parameters of *G. triacanthos* var *inermis* are more influenced by environmental conditions, which reflects the correlations between morphometric parameters of fruits and seeds. According to the Cheddock's scale, *G. triacanthos* has a high correlation (in different years it ranged from 0.706 to 0.877) between fruit length and width; fruit length and weight; fruit length and the number of seeds in it; seed weight and their number in one fruit; seed weight and fruit length, width and weight.

Thus, as a result of the ecological and biological studies, it has been established that *Gleditsia triacanthos* is a prospect for wide use in agro- and urban landscapes of the dry-steppe zone. This species is a valuable taxon for landscaping of settlements and creation of forest strips in the Volgograd region.

### Author's Contributions

Conceptualization, A.Kh. and K.M.; Data curation, Methodology, Investigation, Supervision, Validation, A.Kh.; Funding acquisition, Visualization, K.M.; Writing – original draft, A.Kh. and K.M.; Writing – review & editing, A.Kh. and K.M. All authors have read and approved the final version for publication.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Acknowledgements

This study was funded within the framework of State Assignment №FNFE-2020-0004 (registration number 121041200195-4), Federal Scientific Centre of Agroecology, Complex Melioration and Protective Afforestation of the Russian Academy of Sciences.

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