The reproduction of *Yucca carnerosana* (Trel.) Mc Kelvey in Crimea

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Abstract. Seed reproduction and bio-ecology of the genus *Yucca* (*Yucca* L.) species promising open ground ornamental crops for the South of Russia are of great importance for the Southern Coast of the Crimea (SCC). At homelands *Yucca Carnerosana* (*Yucca carnerosana* (Trel.) Mc Kelvey) has an obligate mutualism and is closely tied to a specific pollinator, which is an endemic species of moth *Tegeticula yuccaselia* C. V. Riley. Under the conditions of introduction due to the lack of moths *Yucca* species bloom, but do not bear fruit. In the period from 1984 to 2014, works on artificial pollination of flowers *Y. Carnerosana* were conducted using the method invented in 1989 (RU Patent 1470245). The real productivity of seeds in fruits is 70…80% of the potential one. The average class of seeds’ development ranges from 3.57 to 3.63. The embrio-spectra development of seeds is given. Germination, germination energy and seed viability, calculated according to the formulas, are directly dependent on the index of *Cave* and reflect the potential possibility of obtaining seed reproduction. Statistics of endogenous variability in seed of *Y. Carnerosana* obtained by the way of autogamy and mixed geitonogamy are given. The main species of *Yucca* pests in the parks of the SCC region are identified and the measures to combat them are described.

1. Introduction
Species of the genus *Yucca* (*Yucca* L.) are of great interest for landscaping in the South of Russia and the medical industry [1,2]. These are highly indigenous exotic woody and shrubby plants for landscaping of the southern coast of Crimea (SCC) and Black Sea coast of the Caucasus. Pollination of *Yucca* species in the homeland is carried out by the endemic species of the moth *Tegeticula yuccaselia* C. V. Riley (1872), which lays its eggs in the stigma of the pestle and applies viscous pollen to it, closing the eggs from their loss. Pollen germinates to ovules and fertilizes them. Larvae, emerging from eggs, penetrate a developing fruit, following by the germinated pollen, and until pupation, eat up the part of the seed of the growing fruit. Having passed the certain stages of development (pupation), the moles leave a seed case through a round hole on the side [3,4]. The close attachment of the plant to a specific pollinator is expressed by the concept of "obligate mutualism".
Almost all kinds of Yucca in the homeland have built their lives this way. But under the conditions of introduction, where this kind of moth is absent, Yucca bloom, but do not bear fruit. Only aloe-leaved Yucca (Y. aloifolia L., 1753) under the conditions of introduction forms fruits with viable seeds without artificial pollination. But the reason for this phenomenon (deviation from obligate mutualism) has not yet been studied. However, as it turned out as a result of artificial pollination of various degrees, some species and hybrid forms of Yucca do not set fruits with viable seeds in the usual way of artificial pollination. Y. Carnerosana (Y. carnerosana (Trel.) McKelvey, 1938) is belong to such species, and it is of considerable interest for landscaping purposes in terms of its habitable and decorative characteristics [5-8].

In Russia, this species is represented only in the Nikitsky Botanical Garden in a very limited number (7 specimens). Only in one specimen of the plant, we were able to carry out the set of fruits with viable seeds through the use of the new method of artificial pollination of difficult-to-polluted yucca species invented by us. Yucca species are introduced on all continents. Work on their introduction has also been successfully conducted in Russia. The bio-ecological study of the species of Yucca, including Y. Carnerosana as one of the most promising ornamental plants of open ground on the South of Russia, is important, has a notable scientific novelty and is of great practical importance. However, the success of the introduction work is determined not only by the sufficiently high resistance of Yucca species to environmental factors, but also by the possibility of obtaining seed reproduction [1, 2, 5, 9]. In recent years, a number of works related to species of the genus Yucca, their phylogenesis, peculiarities of their reproduction and practical use have been published [10-13]. The homeland of Y. Carnerosana – East Texas (the USA) and Northern Mexico (the states of Coahuila, Chihuahua, Zacatecas, San Luis Potosi and Nuevo Leon). Naturally grows to a height of 2200 m above sea level (figure 1).

![Distribution overview of the Paxtoniaceae Series](image)

**Figure 1.** Disjunctive natural habitat of Y. Carnerosana [14, 15].

This species forms a trunk from 5 m and more and over 50 cm in diameter with rosettes of symmetrically arranged leaves from 50 to 100 cm long, 5-7 cm wide and thick from 1 to 2 cm. Flowering begins after 15-25 years, when the trunk reaches a height of 1.5 to 2.5 m from the soil level (figure 2).
Figure 2. The group of Y. Carnerosana at the main entrance to the Arboretum of the Nikitsky Botanical Gardens in different years of observations.

Figure 3. Flowering Y. Carnerosana in the Arboretum of the Nikitsky Botanical Gardens in different years of observations.

After flowering, the trunk branches and forms lateral branches with new rosettes of leaves. The terminal inflorescence is a double dibotry, reaches a height of 100-150 cm and a diameter of 80 cm with 20-30 side branches of the second order with a length from 15 to 60 cm (figure 3).

Figure 4. Flowers Y. Carnerosana: a – general view of flower; b – pistil and stamen.

The flowers are tulip-shaped, white hanging from 6.5 to 9.5 cm long and from 4.5 to 7.5 cm in diameter (figure 4). The fruits are juicy, greenish-brown, barrel-shaped, smooth, up to 10 cm long and 4 cm wide. The consistency of the fruit is similar to sweet candy, with a small bitterness of taste (figure 5). It is edible. Seeds are black, flat, matte-shiny, oval-triangular.
The aim of our work is to analyse the possibility of obtaining \textit{Y. carnerosana} fruits with viable seeds using the artificial pollination and to estimate the quality of obtained seeds used for the reproduction and selection of the species.

2. Experimental part

In the Arboretum of the Nikitsky Botanical Gardens, the species is represented by 7 specimens of 87-year aged Bisexual hermaphrodites. Their sexual preference was observed by us since 1985. Then 3 specimens were referred by us to conditionally female and the remaining 6 - to conditionally male. In the nineties, two female specimens dropped out of the collection of the Arboretum for unknown reasons. Now in the collection of Arboretum only one conditionally female specimen (figure 1) and six conditionally male specimens are preserved. Up to 2018 specimen has not changed its existing from the very beginning growing status.

In the vegetation periods of 1984, 1995, 2001, 2006, and 2014 years we conducted the experiments on the study of seed reproduction of various species and garden forms of \textit{Yucca} and proved the possibility of their industrial cultivation. Since the spring of 1984, artificial pollination of flowers of all species of \textit{Yucca} presented in the Crimea has been carried out. During the period of research (over 30 years) we regularly observed flowering and fruiting \textit{Y. Carnerosana} and what insects visited its flowers. Mainly bees showed the interest in the flowers of this species. But we did not receive the evidence of pollination of flowers by bees.

For several years the artificial pollination of flowers of this species with the use of its various types by simple application of pollen on the style did not give the desired results [9]. As a result of the complex of bio-ecological studies we have developed a new method of artificial pollination of hard-to-pollinate species of \textit{Yucca}, including \textit{Y. Carnerosana}, using special know-how and obtained the USSR copyright certificate for the invention.

The study of seed quality \textit{Y. Carnerosana} is an important part of the researches for developing technologies of its seed reproduction. It should be noted that the quality of seeds of species of \textit{Yucca}, and \textit{Y. Carnerosana} in particular is still insufficiently studied [9].

The real productivity of seeds in the fruit was established by calculating the percentage of the filled seeds from the total number of seeds. The data obtained for each fruit were averaged. The length and width of the seed were measured by a caliper, the thickness – by a micrometer, and the mass was determined on VLK-500 scales. All measurement data were processed using statistical methods.

At the NBG \textit{Y. Carnerosana} bloom first (from April 29th) 15-18 days pass from the beginning of the terminal buds before its branching and the emergence of the flowers. Flowering lasts 16 days. After the invented method of artificial pollination, the flowers begin to form fruits in 3-5 days, which finish their growth and fully mature in 90 days. The number of seeds in the fruit varies from 90 to 160 pieces. We have created a polytomical key for determining the species of \textit{Yucca} L. by...
fruit, introduced to the Crimea, it was published earlier. Therefore, the description of the fruit of Y. Carnerosana is not given in this article. The results of long-term observations showed that Y. Carnerosana blooms once in 2-3 and sometimes 4-5 years. One mature Y. Carnerosana tree during flowering forms on the average from 3 to 7 stalks from the apical buds of main and lateral trunks. In the year of flowering with the invented method of artificial pollination, regardless of the type of pollination it can be possible to pollinate only half of all the flowers of inflorescences and get at least 20-30 fruits from each of the 3 inflorescences, that is from 60 to 90 fruits from one plant. This figure varies depending on the number of pollinated flowers of the inflorescence and the quality of pollination itself within significant limits, from 0 to 20 fruits from one tree. Since the number of seeds in one average fruit is 125 pieces, then the harvest of the tree will be from 2500 to 20000 pieces, and with breaks in flowering for 3 years. These are indicators of real productivity. The potential productivity in the case of formation of seeds from 100% of ovules will be for 20-30% or more % is more realistic, but in nature it is not happening. At the same time, the real proportion of seed reproduction of the 87-years aged Y. Carnerosana plant may be from 1:800 to 1:6500 per year.

The quality of seeds was determined by x-ray examination [16–18 and others] on "Svetlana" x-ray generator. For a more visual expression of the qualitative assessment of seeds, embryo-spectra were built. The quality of seeds was determined by decoding radiographs on the degree of endosperm development and the presence of the embryo in it, taking into account the intensity of fixation of the endosperm and the embryo on the spathella. All the seeds were divided into 3 categories and 5 embryo classes. In addition, the average class of seed development ($C_{ave}$) was calculated by the formula:

$$C_{ave} = \frac{1n_1 + 2n_2 + 3n_3 + 4n_4 + 5n_5}{100},$$

where $n_1, n_2, n_3, n_4, n_5$ are the numbers of seeds of the corresponding development class (endosperm class) as a percentage of the total number in a sample. The seeds with developed endosperm and embryo were classified as complete, and the ones with underdeveloped endosperm and embryo – to the category of underdeveloped, and seeds without endosperm and embryo – to the category of empty.

On classification of N. D. Smirnova [19], the seeds of Yucca aloefolia are referred to the third group, that is, to a group of seeds with a fairly well-visible embryo lying in the cavity of the endosperm. On the basis of this we have identified the following embryos-classes: I - seeds are empty (without endosperm and embryo); II - embryo and endosperm fill less than ½ of the length of the seed; III - the embryo and endosperm are filled from ½ to ¾ of the length of the seed; IV - the embryo and endosperm are filled with more than ¾ of the length of the seed, but the periphery remains unfilled space between the endosperm and the skin of the seed; V – endosperm fills the entire volume of the seed, tightly adjacent to the seed skin, and the embryo fills the entire cavity, tightly adjacent to the endosperm.

The seeds of Y. Carnerosana are flat, large in size compared to other types of Yucca, triangular-ovate. Therefore, their preparation for x-ray examination (laying on adhesive tape enclosed in a frame) and the correct orientation in the frontal plane provide a good comparability of all elements of their internal structure. Due to this, the x-ray images of seeds give correct and reliable information about their quality. The x-ray images show clearly the endosperm with shadows of different intensity, the degree of its development, various injuries and an oblong embryo, located along the entire length of the endosperm of the seed.

3. Results and discussion

The X-ray examination showed that the quality of the seeds obtained from Y. Carnerosana turned out to be quite high (figure 6).

The seeds are presented in three categories: full, underdeveloped and empty. The average class of seed development ($C_{ave}$), giving a more complete comparative assessment of the quality of seeds in the average sampling of all groups of fruit inflorescence is $C_{ave} = 3.57$. In the average sampling of seeds from large fruit inflorescence $C_{ave} = 3.63$. These differences are particularly evident in the embryonic spectra (figure 7). The quality of seeds of the second sampling is higher than that of the first one,
which indicates that large fruits form a larger number of seeds of a typical flat-oval-triangular shape and a smaller number of atypical seeds from pyramidal to round shape. This testifies that the average sampling of seed from the large fruit is slightly higher than from all the groups of fruit buds.

Figure 6. Radiographs of seeds Y. Carnerosana: a – average sampling of seeds from all the groups of fruit blossoms; b – average sampling of seeds from the large fruit buds.

Figure 7. Embryo-spectra of seed development Y. Carnerosana: a – autogamy; b – geitonogamy I, II, III.

Generally, when using the invented method of artificial pollination of hard-to-pollinate species of Yucca, the quality of seeds is higher when using the type of pollination of mixed geitonogamy than autogamy. However, the difference in the quality of seeds is small and the use of different types of artificial pollination has little effect on their quality indicators. The use of the invented method of artificial pollination indicates the possibility of obtaining high quality seeds using different types of artificial pollination. Pairwise comparison of the distributions of seeds for the endosperm classes taking into account the embryo development in them, performed on the basis of Kholmogorov’s and Smirnov’s criterion [9] at P= 0.95, significant differences between seeds of Y. Carnerosana formed by autogamy and as a result of mixed geitonogamy, and also with an average sample I and II were not revealed (table 1).
Table 1. The distribution of seeds Y. Carnerosana on embryo-classes of development (I-V) and indicators of quality of seeds depending on the type of pollination.

| Type of artificial pollination | Seeds quantity, pcs. | C_{ave} | Ger, % | V, % | GE, % |
|-------------------------------|----------------------|---------|--------|------|-------|
| Autogamy                      | I class 6 II class 2 III class 18 IV class 41 V class 8 Total 75 | 3.57    | 90.67  | 77.33| 36.78 |
| Geitonogamy                   | I, II, III 8 class 4 II class 5 IV class 42 V class 11 Total 70 | 3.63    | 85.71  | 79.29| 38.98 |

It is established that the greatest potential productivity of flowers (number of ovules) is laid in the first half of the flowering period. The ovules are formed, mainly, from the base to the first half of inflorescence shoots first (primary) and second order. The largest fruits with a large number of seeds are formed at the base of the branch of the elementary inflorescence, that is, next to the peduncle (main inflorescence) in its lower part. As we move towards the end of the branches of the second order and the top of the peduncle of the first order, fruits are smaller and with fewer seeds, as they are laid in flowers with fewer potential and formed with fewer ovules, ready for fertilization. Therefore, at the ends of the shoots of the first and second orders of inflorescence small and very small fruits are formed. Often the fruits grow ugly.

During the preparation of the fruit of Y. Carnerosana it was found that their length and form depend on the topography of the ensuing seeds in seed boxes. The diameter of the fruit is formed on the basis of the size of the set seeds, and with the full filling of the seed box is the same at any length of the fruit for a certain type of Yucca, including Y. Carnerozana. Only in the case of a partial (one-way) filling of the box with seeds, their diameter may be smaller. The length, thickness, width, radius and weight of the seeds depend on their location in the seed box. Typical triangular and flat seeds are located in the center of the box and are equal to 60 to 90% of the total number of them in the fruit. At the end of the fruit there are seeds in the form of pyramids or other, more unusual shapes, which have been formed under the cramped conditions of a narrowing box. The weight of the seed does not depend on the length, width, thickness and shape. The dependence of the fruit weight on the number of seeds set in it is directly proportional. That is why large fruits have the greatest seed productivity. On the other hand, they are the weak link when occurring often in terms of its native habitat's hurricanes in which large fruit due to a significant sailing capacity and big weight drop the first. Because of their small weight and a small sailing capacity the medium and smaller fruits are more resistant to such cataclysms. In this case nature has taken care about the optimum type of interaction of an organism with the environment through self-regulation of the amplitude of the endogenous variability of the size and weight of the fruit of Y. Carnerosana. It is known that the richness and moisture regime of the soil play an important role for this species of Yucca. The plants that are weakened and growing under extreme conditions on strongly arenose thin and dry soils usually have fewer leaves and smaller size of generative bodies.

Germination, germination energy and seed viability are directly dependent on the index of C_{ave} (table 1) and reflect the potential possibility of obtaining seed reproduction. They are calculated by the following formulas:

\[ V = 0.5n_1 + n_2 + n_3 \]

\[ Ger = 0.5n_2 + n_3 + n_4 + n_5 \]

\[ GE = \frac{n_3^2 + n_4^2 + n_5^2}{100} \]
where: \( V, \) \( Ger, \) \( GE \) – estimated the viability, germination and germination energy percentage; \( n_1, n_2, n_3, n_4, n_5 \) – the number of seeds of the corresponding development class (endosperm class) as a percentage of the total number in a sample.

The calculated values of the Spearman rank correlation coefficients \( (r_S) \) between these three parameters and \( C_{av} \) value are in the range from 0.7 to 0.9, which is significantly higher than the standard value \( r = 0.42 \) for \( n = 17 \) and \( P = 0.95 \) and confirms the close correlation between the studied parameters.

This paper presents the results of the study of endogenous variability in number, size and weight of Y. Carnerosana’s seeds obtained by using the invented method and two types of artificial pollination. All of the investigated biometric indicators of Y. Carnerosana’s seeds vary depending on conditions of their formation on the inflorescence, environmental factors, physiological state of individuals and is genetically inherent potential productivity of flowers (table 2). However, their form, surface texture and color are constant species-specific traits.

### Table 2. Endogenous variability in the size and weight of seeds of Y. Carnerosana (\textit{Yucca carnerosana} (Trel.) McKelvey) depending on the type of artificial pollination.

| Type of artificial pollination | Feature          | Statistic       |
|-------------------------------|------------------|-----------------|
|                               | \( \bar{x} \) (Sx) | \( V, \% \)    | \( X_{\text{min}} - X_{\text{max}} \) |
| Autogamy                      | Length, mm       | 8.0 (0.1)       | 9.4        | 5.0 – 9.5 |
|                               | Width, mm        | 6.9 (0.1)       | 12.1       | 5.0 – 9.0 |
|                               | Thickness, mm    | 3.3 (0.1)       | 24.4       | 2.0 – 6.0 |
|                               | Weight, mm       | 60.0 (0.2)      | 26.9       | 20.0 – 90.0 |
| Geitonogamy                   | Length, mm       | 8.9 (0.1)       | 10.9       | 6.4 – 12.3 |
| I, II, III                    | Width, mm        | 6.9 (0.1)       | 12.3       | 4.4 – 10.5 |
|                               | Thickness, mm    | 3.0 (0.1)       | 30.9       | 1.3 – 6.4 |
|                               | Weight, mm       | 110.0 (0.2)     | 34.5       | 20.0 – 220.0 |

The subsequent germination of seeds on filter paper in Petri dishes (laboratory germination) confirmed the data obtained by x-rays examinations. A comparative study of seeds of different classes within the species showed that they do not differ in size. Seeds of I and II classes were not germinated under laboratory conditions, seeds of III class sprouted by 25-45 %, and seeds of IV and V classes – by 81-100 %. The seeds of classes IV and V came up earlier and more synchronously than the seeds of class III. Further picking of sprouts in the container and grow them standard seedlings took place without losses from fungal diseases and insects. Soil germination was 17-23 % less laboratory, and its reduction was explained by the death of seeds and seedlings from fungal diseases developing in waterlogged soil.

The main species of Yucca pests in the parks of the Southern Coast have been identified. These pests are: cactus scale (\textit{Diaspis tchinocacti} Bouche) and tropical polyphagous scale (\textit{Abgralaspis cyanophylli} L.), which belong to the family Diaspididae – scales. They damage the leaves, branches and a trunk, the settlement of which extends throughout the Yucca plant from the multicipital caudex. As a result of the mass reproduction of pests on the leaves are yellow spots (destruction of chlorophyll) and the plant loses its decorative (figure 8).

To combat scales, it is advisable to conduct early spring spraying of plants at a temperature of +4 - +8 °C with mineral oil emulsion (Preparation 30) in a concentration of 2.5-3.0% during the winter stages of pests, when predators are in wintering places. During the growing season, it is recommended to use the preparations from the group of organophosphorus and hormonal compounds against females and hatching larvae (swarmers).
Figure 8. Damage to the organs of tropical Yucca with cactus scale: a – damage to the trunk; b – damage to the leaves. Original photos from 2016.

However, on sunny locations with a high level of agrotechnics Y. Carnerosana is quite resistant to fungal diseases and insects under the conditions of the Southern Coast, starting from germination and ending with the adult plants.

4. Conclusion

Seeds of Y. Carnerosana of different classes of development (embryo-classes) within a species do not vary in size. Fruit setting, real productivity of seeds in the fruit, size, shape and weight of seeds, as well as their quality do not depend on the type of pollination within the species and are specific characteristics of the species. The amplitude of the endogenous variability of the size and mass of seeds of Y. Carnerosana varies widely and depends, in the first place, from characteristic and, to a lesser extent by species. For structural features the growth correlations are of the greatest importance; they reflect the cycle of ontogenetic development and realize the optimal type of interaction of the body with the environment by self-regulating the amplitude of endogenous variability. The quality of seeds does not depend on the size of the fruit and during the artificial pollination by the invented method, the seed productivity of this species is sufficient to ensure its reproduction under the conditions of introduction not only on the Southern Coast of the Crimea, but also on the Black Sea Coast of the Caucasus. To combat scales, it is advisable to conduct early spring spraying of plants at a temperature +4…+8°C mineral oil emulsion (Preparation 30) in a concentration of 2.5–3.0% during the winter stages of pests when, predators are in wintering places. During the growing season, it is recommended to use the preparations from the group of organophosphorus and hormonal compounds against females and hatching larvae (swarmers).

The mass introduction of Y. Carnerosana in the green areas of the southern coast of Crimea (SCC) will greatly enhance their decorative value and increase the attractiveness of domestic resorts. The scientific novelty is that for the first time the quality of the seeds of this Yucca species formed as a result of the use of the invented method of artificial pollination was studied and fruits with viable seeds were obtained, from which plants were grown, which took their rightful place in the landscaping of the SCC. The practical value of the results of our research is in the enrichment of exotic trees and shrubs of all green plantations of the SCC, what will significantly increase their decorative and aesthetic value.
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