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Key Features of Seed Germination of Cuscuta Japonica Choisy and C. Campestris Yunck. (Cuscutaceae Dum.)

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Abstract. The features of hard coat of two species of dodder – *Cuscuta japonica* Choisy and *C. campestris* Yunck. (Cuscutaceae Dum.), the influence of ecological and geographical conditions on hard coat for example, the Amur region. It is shown that under laboratory conditions to overcome the dormancy of these species, requires chemical stratification in concentrated sulfuric acid (H_2SO_4 conc.). Host plants do not affect the japanese dodder seed germination, therefore, the statement about the impact of the plant – hosts on the germination is not universal, at least for the species.

Any anthropogenic effect is gradually becoming the key factor determining the dynamics of the current distribution of organisms. Invasion or extinction of even a single species may cause a drastic rearrangement in the communities [3]. Parasitic plants are classified as a separate group; however, their biology remains poorly studied. Sometimes it is unclear why certain species become cosmopolitan, while the habitats of the others are limited. The parasitic nature of dodder (*Cuscuta* L.) makes it enormously challenging to control this plant. This is particularly true for *Cuscuta japonica* Choisy and *C. campestris* Yunck. (Cuscutaceae Dum.), which has a wide range of hosts [1]. One of the key features of *Cuscuta* L. as parasites is that these species have an enormously large number of seeds (several thousand seeds per plant). The seeds mature gradually: the dormancy period may last for 4–5 years (according to some reports, up to 20 years) [2]. Hence, studying the seed dormancy and conditions to overcome it is of primary importance. One of the dormancy signs, seed hardness (total water impermeability of the seed coat or, less frequently, of the pericarpium) is observed in many *Cuscuta* species. This affects the germination dynamics and makes seeds resistant to soil solarization. The activity of species in natural ecosystems usually maintains the ecosystems as their habitats.

The Handbook on Dormant Seed Germination [7] reports the data on germination of some *Cuscuta* L. species; however, no data on *C. japonica* Choisy and *C. campestris* Yunck are available. Our preliminary experiments demonstrated that the poor germinability of *Cuscuta* L. seeds is also associated with seed hardness [4, 5]. According to J. M. Hutchison and F.M. Ashton [12], germinability of immature seeds of *Cuscuta campestris* is 80%. Maturation results in natural drying of the seeds, reduces permeability, and increases the strength of pericarpium. As a result, seeds enter secondary dormancy; their germinability drops to 2%. According to J.H. Dawson [11], over 90% of mature seeds have a hard seed coat. These seeds stay viable for a long period of time and are characterized by an extended germination period. Scarification of hard seeds with sandpaper, via making incisions, or treating with sulfuric acid breaks dormancy and enhances seed germinability.

What are the factors that play the key role and affect dormancy-breaking in *Cuscuta* L. seeds in nature? This question still remains controversial. Thus, such factors as depth of seed burial, burning of plant communities (thermal treatment), winter period, animals feeding on seeds, etc. play a significant role. The contribution of chemical signals to the host–dodder (a parasite) interplay, which has been demonstrated for various parasitic animals and plants, also remains unsolved. The response of a parasite to the chemical signal of the host has been reported for some parasitic flowering plants [13]. Since the studies conducted back in 1913, it has been believed that dodder seeds germinate faster as they respond to host plant excretions. The substances secreted by the host plant are required for dodder seedlings to ensure their attachment to the host and transition to the parasitic way of life [8]. This statement has been published in all Russian and foreign textbooks as an axiom. Thus, S. Tarr [9] substantiated that germination can be accelerated by root excretions of some plants, which are not necessarily the hosts of this parasite. Stimulation of germination of parasitic plant seeds by root excretions of plants ensures certain benefits for the parasite, since they enhance the probability of successful invasion. Nevertheless, the range of these studies is confined to certain root parasite species [10, 13].

Our research was aimed at studying the germination features of seeds of the aboriginal species *C. japonica* and the adventive species *C. campestris* in the Amur region.

Material and Methods

Mature *Cuscuta japonica* and *C. campestris* seeds collected in 2004 and 2012 – 2013 were the study object. Seeds of identical size with no visible defects were selected for the experiments. Twenty-five seeds were placed on moistened filter paper (a substrate) in Petri dishes and germinated at room temperature. Sprouting seeds with the axial organs of the sprouts longer than half-width of the seed were considered to be germinated.

Chemical scarification was performed by treating seeds with concentrated sulfuric acid (H_2SO_4 conc.) with different exposure time (10 min, 15 min, 20 min, 30 min, 40 min, and 1 h). The seeds were subsequently washed with running water. Twenty-five seeds were placed on moistened filter paper in Petri dishes; germination was carried out at room temperature. Intact seeds were used as a control. Chemical treatment was performed in compliance with the requirements specified in the Handbook on Dormant Seed Germination [7]. The viability of the treated seeds was evaluated according to the number of seeds that germinated. The seeds that remained ungerminated after 4 days were considered unviable. Four biological replicates were used for measurements.

Results

Hard seeds of agricultural crops are usually subjected to various physical or chemical treatment prior to planting. The treatment techniques include scarification, soaking in concentrated sulfuric acid, scalding with boiling water, etc. [7]. There are various reasons behind germination inhibition of dormant seeds; hence, the conditions of dormancy breaking differ as well. M.G. Nikolaeva et al. [7] reported that sometimes seeds of the same plant species are not characterized by seed hardness: it depends on the degree of seed maturity, maturation and storage conditions. In addition to the varied dormancy depth in different species, it may frequently vary within the same species and even plant. Taking the aforementioned fact into account, the treatment method should be determined experimentally.

The earlier [6] and recent experimental data demonstrate (Fig. 1) that *Cuscuta japonica* and *C. campestris* seeds are characterized by poor laboratory germinability (3 and 14%, respectively) under normal temperature conditions (22 – 25^{0}C, since the optimal temperature range for most species 10 – 30^0 C) and high percentage of hard seeds (97 and 86%, respectively).

The research into seed hardness has revealed a narrow variation range for this indicator depending on the genotype. It was found by studying 100 samples of each species in 2012 and 100 samples in 2013 that the seed hardness in doddle species fluctuated within 96 – 98 % for *C. japonica* and 84 – 88% for *C. campestris* during these years. We have also found that viability of seeds was not lost after the seeds were stored for 10 years (seeds collected in 2004), while the degree of seed hardness remains at the same level. All these facts attest to the strict genotype control over the indicatory and high degree of adaptation of these *Cuscuta* species to the parasitic way of life. There can be a significant variation in the degree of adaptation within the genus: e.g., from the presence of chlorophyll in some species to complete absence of any photosynthetic activity in other species. Furthermore, the research gives grounds for claiming that the number of hard seeds is independent of their shape, seed coat color and pigmentation, hilum color, and seed coat quality [7], unlike other plant species.

The seed hardness is typically determined by ecological and geographical conditions of species formation; in particular, the low humidity results in an abrupt increase in the share of hard seeds [7]. However, our data for *C. japonica* are identical to the data obtained by Japanese researchers [14], despite the fact that the conditions of seed formation differ drastically in terms of this factor. All these facts attest to the narrow variation range of this indicator and strict genetic control.

A reasonable method for accelerating *Cuscuta* seed germination (treating seeds with concentrated sulfuric acid, which allows one to obtain seedling mass of these species for further research, since examination of their juvenile stage is difficult because of seed hardness) was also determined in our experiment. Unlike the control (3%), the germination percentage of *C. japonica* seeds (Fig. 1) treated with $H_2SO_{4\ conc.}$ (exposure time 1 h) was as high as 70%.

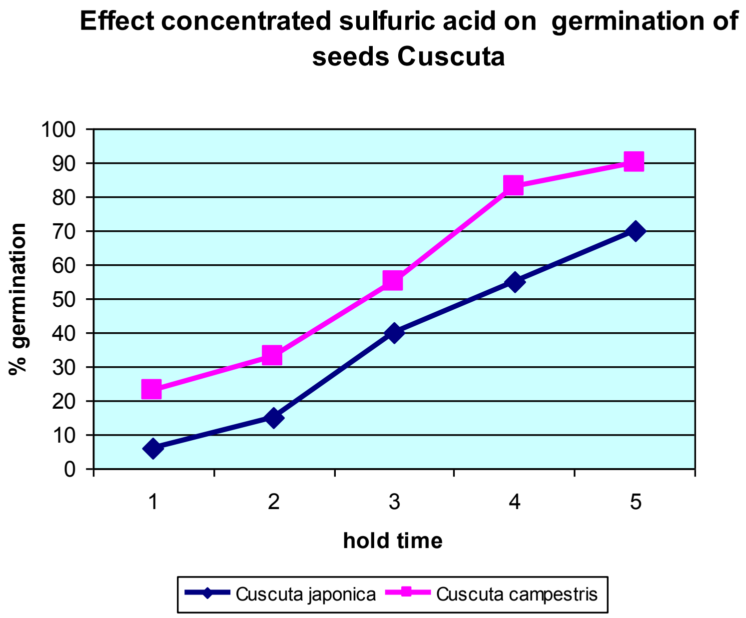

Fig. 1. Effect concentrated sulfuric acid on germination of dodder seeds: *C. japonica* Choisy and *C. campestris* Yunck.

Germination of *C. campestris* required shorter treatment time (40–45 min): the germination degree was 83%, while being 89–90% when the seeds were treated for 1 h (control, 14%) (Fig. 2). Different response to seed treatment is most likely attributable to their size (*C. japonica* – 2–3 mm, *C. campestris* 1–1.5 mm) and interspecies differences.

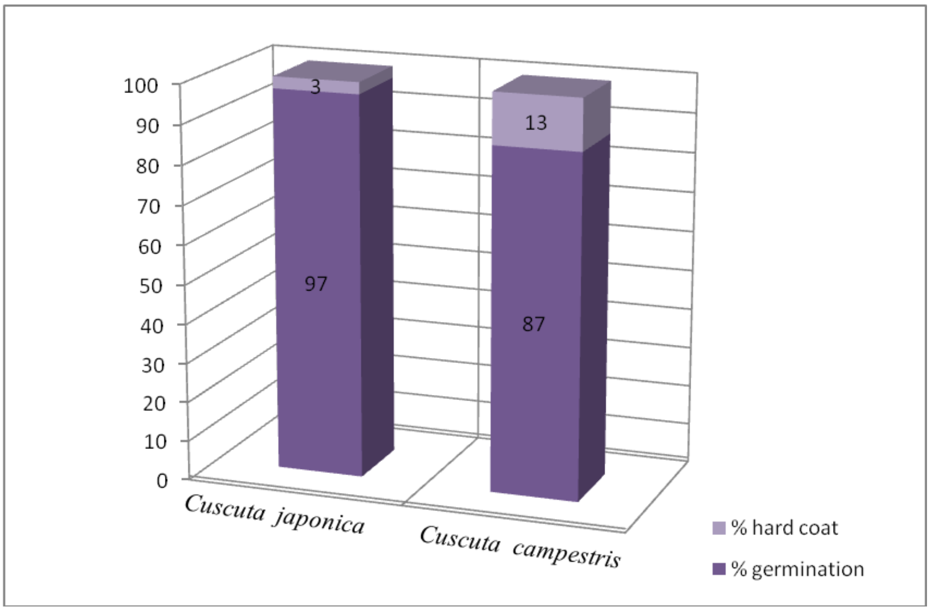

Fig. 2. Features of seed germination of *Cuscuta japonica* Choisy and *C. campestris* Yunck. (shows the average value of the sign)

Seed germination and attachment of parasitic plants to the host plant is mediated by chemical signals, the root excretions of some plants [9, 13]. This fact was experimentally confirmed for root parasitic plants [9, 13, 10]. Hence, *Cuscuta* L., the stem obligate parasite is believed to have features similar to those of other parasitic plants; *Cuscuta* L. seeds are characterized by accelerated germination caused by responding to host plant excretions. Stimulation of seed germination of the parasitic plant by root excretions of other plants ensures certain benefits by increasing the probability of successful invasion. We have experimentally demonstrated (Fig. 3) that the presence of seeds and seedlings of host plants (*Capsicum annuum* L., *Brassica oleracea* L., *Helianthus annus* L., *Cucumis sativus* L., *Raphanus sativus* L., *Avena sativa* L.) has no effect on seed germination of *C. japonica*.

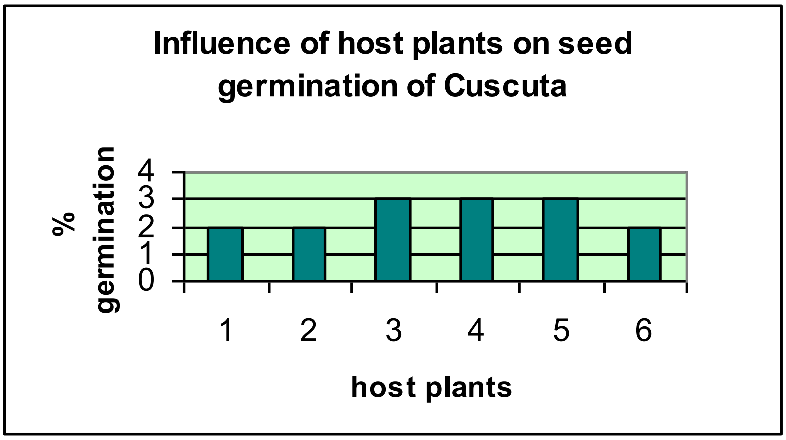

Fig. 3. Influence of host plants on seed germination of *Cuscuta japonica*.

Data labels: * 1. – *Capsicum annuum* L.; 2. – *Brassica oleracea* L.; 3. – *Helianthus annus* L.; 4 - *Cucumis sativus* L.; 5 – *Raphanus sativus* L.; 6 – *Avena sativa* L.

This can be caused by total water impermeability of the seed coat of *Cuscuta* L. Thus, the claimed effect of host plants on germinability is not valid for all plants (at least for *C. japonica*).

Conclusions

The reasonable method for accelerating *Cuscuta* seed germination (treating seeds with concentrated sulfuric acid) was determined in this study. The optimal treatment time is up to 1 h for *C. japonica* and 40–45 min for *C. campestris*. The wider variance of the indicator for *C. campestris* attests to the different strategy of the species striving to occupy its potential habitat. This may be attributable to the cosmopolitan nature of *C. campestris* as a more universal parasite that can live in different environments. Unlike it, *C. japonica* (an aboriginal species) has already developed an adaptive mechanism to live under these conditions. The statement on the effect of host plants on germination of parasites is not universal, at least for *C. japonica.*

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