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PRESOWING TREATMENTS TO BREAKING SEED DORMANCY OF *PTEROCARYA STENOPTERA* C. DC. AS AN INDICATOR OF POTENTIAL INVASIVENESS

Abstract: The Small-winged wingnut is a 12 to 20 metres tall, deciduous and fast growing tree with large substantial branches that spread as wide as the tree is tall. The 15 to 30 *cm* long winged fruit catkins, which turn brown in the autumn, are suspended below the branches. The species is not described by domestic authors in Serbia. The seed was introduced from Sofia (from the Arboretum of the Forestry University of Sofia, Bulgaria - Лесотехнически университет, София), and subjected to classical stratification and naked stratification during the period of 1 and 2 months. Germination capacity varied among the treatments. The largest number of germinated seeds was observed in the shorter stratifications, (1 month) the classical (56.5%) and the naked (51.0%) with no significant difference. The values of real germination of all treatments were between 69-88%. High value of RG, compared to GC, indicated that more than 40% of the seeds were empty. However, the results of the control as well as other parameters of seed germination clearly point to the fact that practically no deep embryo dormancy was observed, but the seed is recalcitrant and microbiotic. The results indicate a non-invasive character of the species in terms of reproductive potential.

Key words: *Pterocarya stenoptera*, seed dormancy, stratification

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ПРЕДСЕТВЕНИ ТРЕТМАНИ ЗА ОТКЛАЊАЊЕ ДОРМАНТНОСТИ СЕМЕНА *PTEROCARYA STENOPTERA* C. DC. КАО ИНДИКАТОРА ПОТЕНЦИЈАЛНЕ ИНВАЗИВНОСТИ

Извод: Ускокрила или кинеска птерокарија, је листопадна брзорастућа дрвенаста врста до 20 *m* висине, са гранама које хоризонтално расту стварајући крошњу чија ширина је приближна висини. Плодови су висеће скупине крилатих орашица, дужине 15-30 *cm* које током сазревања у јесен попримају браон боју. Врста није описана од стране домаћих аутора. Семе ускокрилате птерокарије је сакупљено са матичног стабла које се налази у Арборетуму Шумарског универзитета у Софији (Лесотехнически университет, София). Семе је стратификовано у перлиту и без супстрата, тзв. гола стратификација током периода од једног и два месеца. Вредности техничке клијавости указују на разлике између третмана. Највећи проценат клијавости дали су краћи третмани, једномесечне стратификације у перлиту (56.5%) и голе једномесечне стратификације (51%), без међусобно статистички значајне разлике. Апсолутна клијавост свих третмана се креће од 69.8% до 88%. Овако високе вредности апсолутне клијавости у поређењу са техничком клијавошћу указују да је преко 40% семена у узорку штуро. Резултати контролних третмана осталих параметара клијања указују да код врсте није присутан дубок облик ембрионалне дормантности, али да је семе рекалцитрантно и микробиотско. Резултати указују на неинвазиван карактер ове врсте.

Кључне речи: *Pterocarya stenoptera*, дормантност, стратификација

1. INTRODUCTION

The introduction of plants into a new environment is related to a potential risk of its invasiveness. The introduced plant species adjusts to the new environment in varying degrees by overcoming a few barriers, ranging from the extent that overcoming geographical barriers to become non-indigenous to the extent that overcoming the spread barrier becomes invasive (Richardson *et al.*, 2000). Invasive alien species cause significant global changes and influence losses in the economy, biodiversity and functioning of affected ecosystems. Habitat destruction and invasive species are now recognized as the two most important factors that threaten biodiversity at the global level. The Curitiba Declaration on Cities and Biodiversity is the basis for studies of invasive species in cities as the primary centres of their introduction and distribution. The greatest number of invasive species is recorded as a category of ornamental plants. According to research in the U.S.A, where they have had a long-term monitoring (150 years), 85% of the invasive plants are ornamental (Reichard *et al.*, 2001). EEA (European Environment Agency) considered invasive species as one of the main threats to biodiversity (set up homogocene - homogeneous ecosystem with few dominant species) (Scalera, 2008).

In order to prevent possible harm to biodiversity by invasive species, it is important to examine the mechanisms of invasion and ecological and biological characteristics of invasive species. Knowledge of these mechanisms and characteristics of invasive

species allow finding the right ways to control them. It is quite understandable that the study of biological properties taken before the introduction has a special value. There are regulations that provide guidance on the introduction of neophytes in many countries. For example, in Slovenia the Regulations on the nature of risk assessment and license (The Official Gazette of the Republic of Slovenia, No. 43/02), which contains regulations considering invasive species, defines the conditions and methods for assessing the risk to the environment before the introduction of alien plants and animal species to the environment.

Biological properties that are related to reproduction are of primary consideration. Abundance and frequency of fruiting, the beginning of the generative stage of ontogeny, sexual expression of the species, self-pollination, types of seed dispersal, dormancy and seed longevity, are only some of the qualities crucial for the emergence of the next generation and the rate of spread of the introduced plant.

Small-winged wingnut or Chinese wingnut, Feng Yang (*Pterocarya stenoptera* C. DC.) is a species that has not been introduced in Serbia until now. It is a tree up to 30 *m* high that naturally occurs in China. The flowering is in April-May, with leaf emergence. The pendulous catkins remain on the branches during the winter.

Caucasian wingnut (*Pterocarya fraxinifolia* (Lam.) Spach), is a species that has been successfully grown in parks in Serbia (Petrović, 1952, Vukićević, 1974, Jovanović, 1985) for over 100 years as an ornamental and it belongs to the same genus. Prostran *et al*., (2010) reported that the age of five trees of *Pterocarya fraxinifolia* in the Zemun Park is 115 years. *Pterocarya fraxinifolia* do not show signs of invasiveness in Serbia. Despite regular fruiting, it very rarely gives spontaneous seedlings.

The dominant method of propagation of the genus Pterocarya is by seed, although the *Pterocarya fraxinifolia* could be propagated by layering as well. Green cuttings of *Pterocarya* × *rehderiana* C.K. Schneid. (*Pterocarya fraxinifolia* × *Pterocarya stenoptera*) root well in May and June when the young shoots are treated by IBA powder (1%), while later in July the concentration of IBA powder is reduced to 0.1%. The rooting substrate contains peat and sand (2:1); rooting takes place in greenhouse with mist system, and in 5 weeks there were 75% of rooted cuttings (Grbić, 2004).

The aim of this paper is to investigate some of the seed traits, in fact dormancy (type and intensity), of *Pterocarya stenoptera*, the species that is present in neighbouring countries and whose seed was collected in Sofia, by different presowing methods.

2. MATERIAL AND METHOD

The parent tree is located in the Arboretum of Forestry of the University of Sofia (Лесотехнически университет, София) (Fig. 1). The fruits were collected from the tree on September 9th 2007. The seeds were stored wet in a plastic bag in a refrigerator, until the stratification or the germination test.

Figure 1. The parent tree of small-winged wingnut in the Arboretum of Forestry of the University of Sofia

Слика 1. Матично стабло кинеске птерокарије у Арборетуму Шумарског факултета у Софији

The pendulous catkins on the long hairy pedicle 30 *cm* (10-45), remain on the branches during the winter. There are 36 (28-41) fruits in one group. Nuts are with two narrow and long-ribbed wings (1.2-2.5×6-7 *mm*) (Fig. 2). The wings are connected to each other at a sharp angle. Small nut 6-7 *mm* long, slightly hairy or bare is mature in mid-September (Fig. 3). The mass of 1000 cleaned seeds is 40-55*g* and the percentage of sound seed is 70. Five-month stratification is recommended (Milev *et al*., 2004).

A germination test was carried out in a growth chamber, Type 1291/TPC-1/LP-113, on filter paper (4×100 seeds per treatment) at 20°*C* (±2). Photoperiod was 16/8 (light/dark). The control was tested immediately, October 19th 2007, and the rest of the seed was stratified before the test. The stratifications were: (1) one-month naked, (2) two-month naked, (3) one-month in perlite and (4) two-month in perlite. The testing lasted for 21 days, while germination (EC) was calculated on the 7th day.

Naked stratification was performed in transparent polyethylene bags. Seeds were pre-soaked in water at room temperature for 48 hours after which they were treated with fungicide (Previcur), surface drained, and then they were put into bags that were closed and placed in a refrigerator at a temperature of 3-5°*C*. Each week, the seeds were washed and aerated. Classical stratification was carried out in boxes filled with perlite. In order to investigate the longevity and recalcitrancy of the seeds, the rest of the seed had been stored for a year and germinated after that period.

The result was presented by means of nine indicators of germination. Some of them reflect only the quantitative value of germination (germinative capacity (GC), real germination (RG), and germinative energy (GE)), others only the dynamics (mean germination period (MGP), germination intensity (GI), the coefficient of the rate of germination (CRG), and the coefficient of uniformity of germination (CUG), and still others refer to both groups (germination value by Czabator (GV (Cz)) and germination value by Djavanshir and Pourbeik (GV (MC)).

Figure 2. Pendulous catkins of nuts with two narrow and long, ribbed wings
Слика 2. Висећи скуп орашица са два уска и дуга ребраста крила

The definitions of all the indicators of germination and their causes are given as follows. The abbreviations used in the text and tables are given within the brackets. Germinative capacity (GC) can be defined as a percentage of seeds that germinates during a period of time and ends when the germination is practically complete (time of duration of the test) (Schopmeyer, 1974). Real germination (RG) is the percentage of sound seeds that germinate (Schopmeyer, 1974). Germinative energy (GE) is the percentage of seeds that germinate during a specified time interval that is determined by the peak germination rate (Schopmeyer, 1974). The mean germinative period (MGP) is equal to $\Sigma(Z \cdot N)/\Sigma N$, where Z is the number of days of germination from the point of observation (the last day is day 0) and N is the number of seeds germinated on day Z (Sarnavka, 1954). Germinative intensity (GI) is $\Sigma(Z \cdot N)$, where Z and N are the same parameters as in MGP. The germination intensity is expressed by the seed-day (Sarnavka, 1954). The coefficient of the rate of germination (CRG) is $R \cdot 100$, where R is the mean germination rate. The rate of germination can be defined as the reciprocal of the time taken for the process to be completed, starting from the time of the test beginning ($\Sigma N/\Sigma(T \cdot N)$), where T is the time in days, starting from day 0, the beginning of the test, and N is the number of seeds completing germination on day t (Bewley *et al*., 1994). The coefficient of uniformity of germination (CUG) is given by $\Sigma N/\Sigma((MTCG-T)^2 \cdot N)$. $MTCG$ is equal to $\Sigma(T \cdot N)/\Sigma N$, where T is the time in days, starting from day 0, the beginning of the test, and N is the number of seeds completing germination on day t (Bewley *et al*., 1994). Germination

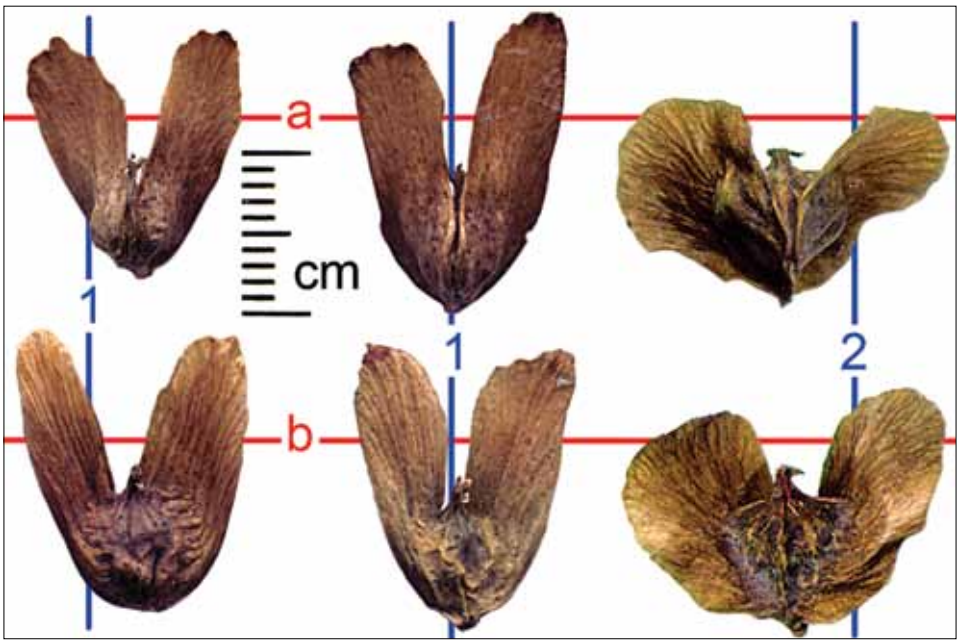

Figure 3. Dorsal (a) and ventral (b) sides of small-winged wingnut fruit (1) and Caucasian wingnut fruit (2)

Слика 3. Дорзална (а) и вентрална (б) страна орашице ускокриле кинеске (1) и кавкаске птерокарије (2)

value - Czabator's formula (GV (Cz)) is as follows *MDG·PV*, where *MDG* is mean daily germination calculated as the percentage of seeds germinated at the end of the test divided with the number of days of test duration, *PV* is peak value, or the maximum quotient derived from all of the cumulative full-seed germination percentages of any day divided by the number of days to reach these percentages. The peak value is a mathematical expression of germination (Czabator, 1962). Germination value - a formula by Djavanshir and Pourbeik (GV (MC)) is as follows: $\Sigma(SBS/N)\cdot(GP/10)$. *SBS* is daily germination speed, which is obtained by dividing the cumulative percent germination with the number of days. *GP* is the percent of germination at the end of the test. *N* is frequency of the *SBS* (Djavanshir, Pourbeik, 1976).

3. RESULTS

As the seed of small-winged wingnut belongs to the non-albuminous seeds and at the time of full maturity contains only a well-developed embryo, morphological dormancy (type B, Nikolaeva, 1977) was excluded. Exogenous dormancy types (A), caused by physical or chemical properties of the outer layer of seeds, are also not recorded, because

the seeds absorb moisture and increase weight during soaking in water. All parameters that reflect the germination, neither of qualitative nor of quantitative or combined, indicate endogenous type of dormancy - C dormancy (Nikolaeva, 1977), and the differences between the results obtained with control seeds and seeds stratified indicate that this is C_1 moderate (shallow) physiological dormancy (Table 1).

Germinative capacity indicates a significant difference between the control and the treatments. After one month stratification in perlite, the largest number of seeds germinated (56.5%). Seeds from one month naked stratification germinated to a lesser extent (51%), but there was no statistically significant difference between these and the previous treatment. Both types of two-month stratification affected the lower germination but did not show significant difference between them. Real seed germination puts control between the homogeneous group of one-month stratification (with higher values: 88 and 85%) and two-month stratification (lower: 75.6 and 69.8%). The following three parameters (Germinative energy, Mean germinative period, Germinative intensity) indicate that the germination of stratified seeds was more dynamic, particularly of the ones that were in perlite. Combined indicators of germination, which mainly indicate the quality and dynamics of germination, singled out stratified seeds as better with the exception of the coefficient of uniformity of germination that is best for control.

Table 1. Параметри клијања контролног и третмана једномесечне и двомесечне стратификације (голе и у перлиту)

Table 1. Parameters of seed germination of control, one and two months stratification (naked and in perlite)

Третмани Treatments	**КТ**	**КА**	**ЕК**	**СВТК**	**ИК**	**КРК**	**КЗК**	**ВК$_{(Cz)}$**	**ВК$_{(Dj)}$**
	GC	**RG**	**GE**	**MGP**	**GI**	**CRG**	**CUG**	**GV$_{(Cz)}$**	**GV$_{(Dj)}$**
контрола control	16.5^{c}	80.5^{ab}	0.0^{c}	5.1^{c}	41.7^{d}	6.3^{d}	7.3^{a}	0.3^{d}	0.45^{d}
1 месечна гола 1 month naked	51.0^{ab}	85.0^{a}	9.0^{c}	10.6^{b}	236.5^{c}	10.0^{c}	0.3^{b}	3.6^{cd}	5.8^{c}
1 месечна перлит 1 month perlite	56.5^{a}	88.0^{a}	48.0^{a}	15.2^{a}	425.7^{a}	17.8^{b}	0.2^{b}	9.8^{ab}	12.5^{ab}
2 месечна гола 2 months naked	46.5^{b}	75.6^{b}	40.0^{b}	15.3^{a}	356.2^{b}	17.6^{b}	0.3^{b}	6.6^{bc}	8.5^{bc}
2 месечна перлит 2 months perlite	48.5^{b}	69.8^{b}	47.5^{a}	17.2^{a}	413^{a}	26.6^{a}	0.4^{b}	13.9^{a}	15.4^{a}

Legend: *GC* - germinative capacity, *RG* - real germination, *GE* - germinative energy, *MGP* - mean germination period, *GI* - germination intensity, *CRG* - coeff. of the rate of germination, *CUG* - coeff. of uniformity of germination, and germination value by Czabator (GV (Cz)) and germination value by Djavanshir (GV (Dj))

Легенда: КТ - техничка клијавост, КА - апс. клијавост, ЕК - енергија клијања, СВТК - сред. време трајања клијања, ИК - интензитет клијања, КРК - коеф. размере клијања, КЗК - коеф. здружености клијања, ВК(Cz) - вредност клијања по Czabator-y, ВК(Dj) - вредност клијања по Djavanshir-y

Means in the columns followed by the same letter do not differ significantly at p=0.01 (Duncan's test)

Средње вредности означене истим словом не разликују се на нивоу значајности p=0,01 (Данканов тест)

Seed that had been stored for one year and germinated after that period gave negative results as the germination did not occur. This result is indicating the microbiotic character of small-winged wingnut seed and its recalcitrant nature.

4. DISCUSSION

Stratification of seeds (in perlite or naked) resulted in the highest germination percentage and germination rate after 30 days. This definite response to stratification is typical for the dormant embryo. Similar results were revealed in a study of Cicek (2008) with *Pterocaria fraxinifolia* - Caucasian wingnut. After a five-week cold naked stratification (without a medium), seed of *Pterocaria fraxinifolia* had the highest germination rate. Young and Young (1994) stated that the same species had seed dormancy and seed germinated after 3 months of cold stratification. Shao (1989) found that *Pterocarya stenoptera* had more than 70% germination when treated with a temperature varying from 20°*C* (or 15°*C*) to 30°*C* before stratification. Pretreatments and seed germination can vary greatly among seed lots (Edwards, El-Kassaby, 1996, Baskin, Baskin, 1998, Tilki, Guner, 2007, Jull, Blazich, 2000). Seeds generally have an inherent high genetic variability, which results in great heterogeneity in their behaviour and particularly in their germinability following stratification procedures. In the present study, real seed germination and distribution of homogeneous groups in all treatments indicated that more than 40% of the seeds were empty - control treatment with 80.5% was between one-month stratifications (with higher values: 88 and 85%) and two-month stratifications 75.5 and 69.8%. Avsar (2002) similarly stated that most of the *Pterocaria fraxinifolia* seeds were empty.

In the final study, the germination did not occur in the treatment with seeds that had been stored for one year. This result points out the microbiotic character of the seed and its recalcitrant nature. Recalcitrant seeds cannot survive drying below relatively high moisture content in the seed itself (often in the range 20–50% wet basis) and cannot be successfully stored for long periods. Many of the large-seeded hardwood species with nuts fruit in the temperate zone were recalcitrant e.g. *Corylus* spp., *Castanea* spp., *Fagus* spp., *Quercus* spp., *Junglans* spp. (Hartmann, 1990). According to the research of Gordon *et al*. (1982), species *Pterocarya fraxinifolia* belongs to this group. When fresh recalcitrant seeds begin to dry, viability is first slightly reduced as moisture is lost, but then it starts being reduced considerably at a certain moisture content in the seed that is determined as the „critical moisture content". If drying continues further, the viability is reduced to zero.

5. CONCLUSIONS

Small-winged wingnut has been introduced in the Balkans (Sofia, Bulgaria) where it successfully grows and fruits. Similar climatic conditions in Belgrade and Sofia point

out that the area of Belgrade (and Serbia) is suitable for the introduction of this species. The properties such as fast growth, ornamental flowers and fruits are valuable attributes for its application in landscape architecture and horticulture.

On the other hand, treatment of one month stratification is enough to break the seed dormancy of small winged wingnut. For spring sowing in nursery production, this form of seed preparation is recommended. Autumn sowing without a presowing treatment is a proper replacement for stratification. This method is generally recommended for species with intermediate physiological dormancy (Grbić, 2003). All the results indicate the non-invasive character of the small-winged wingnut seed.

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ПРЕДСЕТВЕНИ ТРЕТМАНИ ЗА ОТКЛАЊАЊЕ ДОРМАНТНОСТИ СЕМЕНА *PTEROCARYA STENOPTERA* C. DC КАО ИНДИКАТОРА ПОТЕНЦИЈАЛНЕ ИНВАЗИВНОСТИ

Резиме

За семе кинеске птерокарије (*Pterocarya stenoptera* C. DC) као могуће нове врсте на зеленим просторима Београда (Србије) испитана је потенцијална инвазивност. Инвазивне алохтоне врсте су један од индикатора биодиверзитета, односно једна од главних опасности по биодиверзитет, губитак и фрагментацију станишта. У пројектима који се баве биолошком инвазијом и у литератури која се односи на исту тему, наглашава се да највећи број инвазивних биљака припада групи хортикултурних. Њихово ширење у вези је са начином коришћења земљишта, индустријом, присуством ботаничких башти и дистрибутивних центара за гајење и трговину биљакама (гарден центри, расадници).

Ове, алохтоне врсте украсних биљака уносе се садњом у приватним вртовима и јавним градским површинама. После извесног времена оне се могу отети контролисаној култивацији због одсустава одређених мера контроле ширења. Због потенцијалних опасности које носе у новом окружењу, упознавање биолошких карактеристика као што су појава и учесталост плодоношења, потенцијал и начин ширења врсте, дормантност и дуговечност семена само су неке од особина које је неопходно познавати о врсти која се уноси на нова станишта. *Pterocarya stenoptera* C. DC. је дрвенаста врста која веома добро расте и плодоноси у Бугарској (Софија). Ово дрво достигне у Кини висину преко 30 *m,* где се од природе јавља. Врста цвета у априлу-мају паралелно са олиставањем. Скупине крилатих орашица остају да висе дуж грана током зиме.

С обзиром да врста није описана од домаћих аутора и да није познато да расте на територији Србије, у раду су истражене особине сетвеног материјала ове врсте (крилате орашице) који је сакупљене у Арборетуму Шумарског универзитета у Софији (Бугарска). Поред испитивања дуговечности семена, примењени су и предсетвени третмани за одређивање степена дормантности. Семе је стратификовано у перлиту и без супстрата (тзв. гола стратификација) током 1 и 2 месеца. Техничка клијавост варирала је у зависности од третмана. Највише исклијалог семена дала је једномесечна класична стратификација (56,5%), а затим гола у истом трајању (51,0%). Двомесечни третмани са статистичком значајношћу се издвајају као лошији, али без појаве разлике у односу на медијум који је коришћен приликом стратификације. Клијавост и код двомесечних третмана прелази 45% и са једномесечном голом стратификацијом показују статистички нижи ниво значајности разлика (појава једноструког преклапања хомогених група). Ово указује да и двомесечни третмани дају добре резултате. Резултати апсолутне клијавости и појава једноструког преклапања између хомогених група за средње вредности апсолутне клијавости указује да разлике за овај параметар нису на високом нивоу значајности (за све третмане се крећу од 69,8% до

88%). С друге стране, високе вредности овог параметра указују да је број штурих зрна у свим понављањима висока.

Према типу дормантности, може се закључити да *Pterocarya stenoptera* нема дубљи облик дормантности и да су једномесечни предтретмани хлађења довољни да се ове сметње у клијању отклоне. Испитивање вијабилности семена након годину дана које је дало негативне резултате јасно указују да је семе кинеске птерокарије рекалцитрантно и микробиотично. Сви добијени резултати указују на неинвазивни карактер семана испитиване врсте.