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Germination biology of *Parthenocissus quinquefolia* (L.) Planch. (Vitaceae)

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Abstract: We tested germination of seeds of *Partheno-cissus quinquefolia* (L.) Planch. (Vitaceae) in laboratory in response to different ecological factors: light intensity, temperature, humidity, acidity and salinity. Our results show that the most critical ecological factor is humidity. The highest rank of germination was 73 % in condition of sufficient moisture, 37 % in condition of excessive moisture and only 3 % in condition with insufficient moisture. Among variants with different reaction of medium the most successful germination was at pH 7 (73 %) and pH 9 (44 %). Another significant factor for seedlings germination of *P. quinquefolia* is lighting, since at full-dark, dark and semi-dark conditions the level of germination was 0 %, 2 % and 24 % respectively. The percentage of *P. quinquefolia* germination decreases with increasing salinity level. The most optimal germination regime was at +25 °C. Further study of *P. quinquefolia* biology will allow to clarify the difference in naturalization level between this species and closely related *P. vitacea* as well as to estimate the possibility of transformation of the introduced species into invasive.

Keywords: germination, ecological factors, *Parthenocissus quinquefolia.*

Introduction

The genus *Parthenocissus* Planch. (*Vitaceae*) consists of 12 species, which are native to the Himalayas, Eastern Asia and North America (Nie et al. 2010). Some of these species are invasive in different regions of the Earth (Müller & Okuda

1998; Protopopova et al. 2006; Dehnen-Schmutz et al. 2007; Végh et al. 2015). In Ukraine 3 species are recorded (Mosyakin & Fedoronchuk 1999), but only for *Parthenocissus vitacea* (Knerr) Hitchc. (= *P. inserta* (A. Kern.) Fritsch) are known confirmed occurrences of escaping this species from the places of cultivation (Mosyakin & Fedoronchuk 1999; Vasilchenko 1996). For a long time, Ukrainian botanists did not distinguish between *Parthenocissus vitacea* and *P. quinquefolia* (L.) Planch. due to close morphological similarity of these species. As a result, in several floristic works (Petrovych & Burda 2012; Churilov & Yakubenko 2014; Galkin & Dojko 2015; Kotsun & Kuzmishyna 2016) *P. quinquefolia* was listed as invasive species by mistake. *P. quinquefolia* is native to North America (Canada, the USA and Mexico) and Central America. It has been widely introduced as an ornamental creeper and can be found naturalized in Europe (Oprea & Sîrbu 2006; Petrović et al. 2013; Maslo 2014; Vladimirov & Grigorievskaya 2015), tropical and temperate Asia, southern Africa, and Australia (GRIIS 2017; USDA-NRCS 2017). Preliminary results of our field study in all biogeographical zones of Ukraine did not confirm cases of naturalization of *P. quinquefolia* s.str. in semi-natural and natural habitats (Kalista & Kovalenko 2018). Nevertheless, a question about the possibility of invasion of this species onto the territory of Ukraine is still open due to uncompleted data about its distribution and described cases of invasion in natural habitats in nearby Russian Federation (Vladimirov & Grigorievskaya 2015). *P. vitacea* are morphologically and genetically similar to *P. quinquefolia* with overlapping distribution (Nie et al. 2010). That is why extended study of biological, phytosociological and ecological features of *P. quinquefolia* is necessary.

Seed germination is one of the first steps for colonizing new habitats for some alien species. The most important environmental factors controlling seed germination and seedling establishment are temperature, light, acidity, salt regime and soil humidity. Invasion success of alien plant species heavily depends on its germination mechanisms, wherein broad or narrow tolerance to key ecological factors can predict a possibility to occupy a certain habitat. Seed germination studies are important for understanding the biological and ecological traits of plant species (Borghetti & Ferreira 2004; Vieira et al. 2010). Ecology of germination can explain biogeographic distribution of many plant species and predict possible environments susceptible to invasion (Cordell et al. 2002; Borghetti 2005; Vieira et al. 2010). This information is useful for developing effective management programs on control of alien plant species expansion.

The aim of this study was to investigate biology of germination of *P. quinquefolia*. Our investigation focused on biological traits of germination and seedlings development. In order to understand what factors can limit or facilitate colonizing of natural and semi-natural habitats by *P. quinquefolia*, influence of light, temperature, moisture, acidity and salinity were examined.

Material and methods

Seed collection

P. quinquefolia has a long seed maturing period in Ukraine, with ripe infructescence available from September to November. Seed material of *P. quinquefolia* was collected on 5 December 2017 in Kyiv, 2 Academic Glushkov Avenue, on the wall of Educational and Scientific Centre "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv (GPS-coordinates: 50°23'00''N 30°28'37.4''E). Herbarium specimens are stored in herbarium collection of National Museum of Natural History of National Academy of Sciences of Ukraine.

After fruit collection, seeds were extracted and air-dried for 12 hours. Seeds had long 31 day-stratification in freezer at the temperature of -18°C (Ergasheva & Nimadzhanova 2011). The fruits of *P. quinquefolia* are rounded (1–1.2 cm in diam.), bluish-black juicy 14 seeded berries. Seeds are obovoid, base with short, acute rostrum and rounded apex (Chen & Jun 2007) (Fig. 1A). The seeds have two sides. The ventral side is closer to the centre of berry, and dorsal – to the periphery of fruit. The ventral side has longitudinal notches with seed suture between them, which passes to the dorsal side and ends with a deepening – chalaza. The seed cover is dark-brown (Ergasheva & Nimadzanova 2011).

Experiment arrangement

We designed a set of experiments for testing the tolerance and response of *P. quinquefolia* seeds to important environmental factors such as light, temperature, humidity, acidity and salt regime. After stratification, all seeds were planted on filter paper in Petri dishes under laboratory conditions. In every variant we used a set of 100 seeds. Calculation of seedlings was made on 31st day since the beginning of the experiment. Twice a week, seed germination was scored, with simultaneous replacement of filter papers and water solutions. After 31 days, the non-germinated seeds were removed to filter papers moistened with deionized water to check if the seeds were still viable.

All experiments were conducted in the randomized manner. Each Petri dish was positioned randomly inside the growth chamber and rearranged daily. For each treatment, at least 4 replicates were performed. Results of germination at temperature of 25˚C in constant light condition with moistening by deionized water was included as a control in analysis.

Effects of regime of moisture on seed germination. Seeds were put on filter paper discs moistened by deionized water in Petri dishes. Seeds were grown at 25˚C in light for germination. We had 3 regimes of moisture: insufficient moisture – 1 ml of water per day, sufficient moisture – 5 ml per day, excessive moisture – 10 ml per day. After 24 hours the paper discs were changed.

Effects of light intensity on seed germination. The seeds were germinated on filter paper discs moistened by sufficient amount of deionized water in Petri dishes at 25˚C with 12 hours photoperiod provided by white fluorescent lamps. We established 4 regimes of light: with 20 lx, 100 lx, 500 lx and 2500 lx according to environmental conditions of this species habitats. We used parchment paper for wrapping Petri dishes with seeds.

Effects of temperature on seed germination. Seeds were grown on filter paper discs moistened by sufficient amount deionized water in Petri dishes in light. We tested 3 temperature regimes: +10°C, +15°C, and +25°C. We checked viability of non-germinated seeds at 25˚C.

Effects of different pH level on seed germination. The seeds were exposed to HCl or NaOH aqueous solutions of pH = 3 (10^{-3} mol.L^{-1} HCl solution), pH = 5 (10^{-5} mol.L^{-1} HCl solution), pH = 9 (10^{-5} mol.L^{-1} NaOH solution), and pH = 11 (10^{-3} mol.L^{-1} NaOH solution) accordingly in comparison with the seeds treated with deionized water as the control (pH = 7). Seeds were grown at 25˚C in light with simultaneous replacement of filter papers aqueous solutions twice a week.

Effects of salinity level on seed germination. The effect of salinity was scored in NaCl aqueous solutions of three concentrations (concentration of natrium chloride 1g/l, 2g/l, 4g/l), in comparison with the control (deionized water).

Data analysis. Data are presented as means and standard errors and subjected to one-way ANOVA and Fisher's and Tukey's criterions ($p < 0.05$) using Statistica 10.0 (StatSoft) (Tab. 1).

Results

Biology of germination

P. quinquefolia has above-ground germination, some of seedlings can bring the seed cover to the surface of soil on the apexes of cotyledons. At the first stage of germination the embryo root crash the seed cover (Fig. 1B). The main root reaches a size of 0.7–2.2 cm, and about 0.1 cm in diam. It contains 3–4 lateral roots. The hypocotyl is white or anthocyanin, 1.2–1.8 cm in length and about 0.1 cm in diam. Cotyledons have short petioles (0.2–0.8 cm). The leaf blade is heart-shaped or ovoid (0.7–1.1 cm in length, 0.6–0.9 cm in width), light green with 3 expressive veins, glabrous (Fig. 1C)

Effects of moisture regime on seed germination

Obtained data show that germination of *P. quinquefolia* is low in condition of insufficient and excessive moisture. Only in with sufficient moisture (Fig. 2, Tab. 1) the level of germination was 72 %. In this study, the lack of water had stronger effect on limitation of germination than high moisture content.

Effects of different pH level on seed germination

The seeds of *P. quinquefolia* germinated over the pH range of 3–11, but the percentage of germination was higher in the pH range of 7–9 comparing to other levels (Fig. 3, Tab. 1). More acidic medium is a significant factor that decreased percentage of germination to 12–22 %. In contrast, under slightly alkali conditions the germination of *P. quinquefolia* was 43 %.

Fig. 1. ***Parthenocissus quinquefolia*****: A – seeds, B – primary stage of germination, C – seedlings**

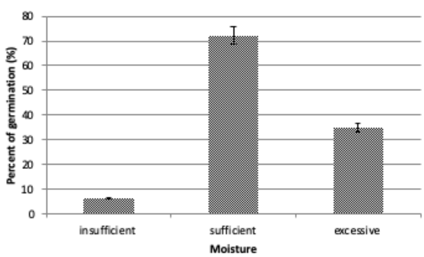

Fig 2. Seed germination of *Parthenocissus quinquefolia* under the various regime of moisture

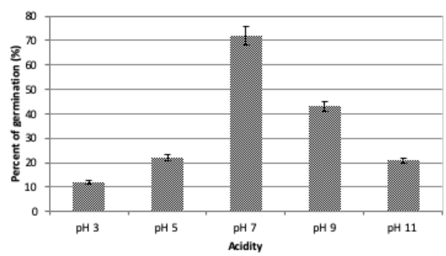

Fig 3. Seed germination of *Parthenocissus quinquefolia* under the various levels of acidity

Tab. 1. One-way analysis of variance for germination of *Parthenocissus quinquefolia* seeds.

Factor	df	F	P
Moisture	2	5265,47	<0,001
pH	4	2108,57	<0,001
Temperature	2	6737,57	<0,001
Salinity	3	1677,44	<0,001
Light intensity	3	3581,103	<0,001

Note: df = degrees of freedom, F = a value on the F distribution, p = value is, for a given statistical model, the probability that, when the null hypothesis is true.

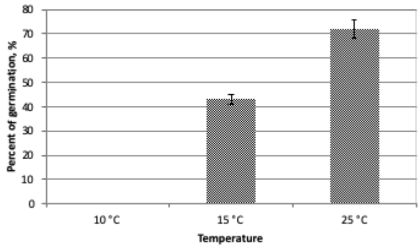

Fig. 4. Seed germination of *Parthenocissus quinquefolia* under the various temperature

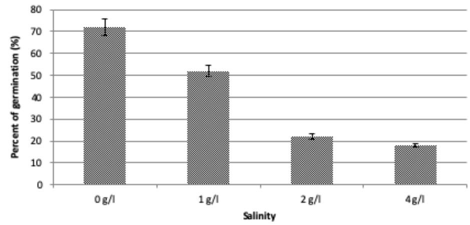

Fig 5. Seed germination of *Parthenocissus quinquefolia* under the various levels of salinity

Effects of temperature on seed germination

According to temperature the germination of *Parthenocissus quinquefolia* was unsuccessful in variant with 10°C (Fig. 4, Tab. 1). 43 seedlings we observed in condition of 15°C. According to our data, optimal condition for germination was 25°C.

Effects of salinity level on seed germination

The NaCl solution with different concentrations significantly reduced the cumulative germination (Fig. 5, Tab. 1). Only 18% of seeds germinated under the treatment with solutions of NaCl (4 g/l). The medium percent of germination decreased with increasing salinity level. However, we observed that seedlings can develop even in medium with high salinity.

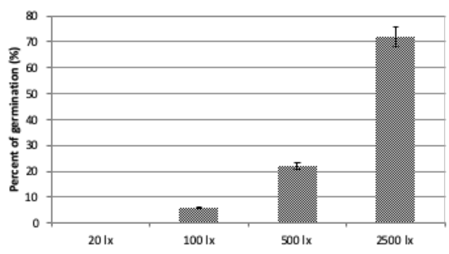

Fig 6. Seed germination of *Parthenocissus quinquefolia* under the various intensity of light

Effects of light intensity on seed germination

Successful germination of *P. quinquefolia* required a high level of illuminance (Fig. 6, Tab. 1). In almost full darkness no seedlings were observed, even illuminance on level 100 lx and 500 lx was insufficient for production of large number of new individuals. Only in variant 2500 lx we observed high number of seedlings.

Discussion

Moisture regime of is one of the most important factors for every plant species. Wide tolerance for this parameter can be a key feature for invasive success of alien species, as seen with *Bidens frondosa* L., which can invade riparian habitats, as well as dry grasslands on sandy soils (Kovalenko 2018). Many other alien species have good adaptation to bare ground habitat characterized by high temperatures and water stress (Yuan & Wen 2010). Water deficit limits germination of alien species less, than native species (Gioria et al. 2016). It was shown in Banksia woodlands in south-western Australia by Pérez-Fernández et al. (2000) as well as by Young et al. (2015) for perennial grasslands in north-central California. These results were obtained for *Ambrosia artemisiifolia* L. (Sang. et al. 2011), *Malva pusilla* Sm. (Blackshaw 1990), and *Solanum viarum* Dunal (Akanda et al. 1996), which can germinate under high water stress. This explains why these species can grow on habitats with high and low humidity soils.

According to our findings, *Parthenocissus quinquefolia* has high level of germination only in condition of sufficient moisture (Fig. 2). Ecological features of classes *Artemisietea vulgaris, Chenopodietea* and *Agropyretea repentis* (Chokha 2006; Fitsailo & Osypenko 2007) are inappropriate for germination *Parthenocissus quinquefolia* due to its xeric or mesoxeric regime of moisture as well as excessive moisture conditions in communities of class *Galio-Urticetea.*

The wide tolerance to acid regime of soils is an important factor for invasive plant species. Only for some alien species with expansive strategy, such as *Ageratina adenophora* (Spreng.) King et H. Rob. (Lu et al. 2006), strict range of pH tolerance (germination is possible only in buffer solutions of pH 5 to 7) was shown. Other studied invasive species *Ambrosia artemisiifolia* (Sang et al. 2011), *Urochloa subquadripara* (Trin.) R. D. Webster. (Teuton et al. 2004), *Bidens pilosa* L. (Reddy & Singh 1992), *Asclepias syriaca* L. (Evetts & Burnside 1972), *Euphorbia heterophylla* L. (Brecke 1995), *Solanum viarum* (Akanda et al. 1996) and *Pueraria montana* var. *lobata* (Willd.) Sanjappa et Pradeep (Susko et al. 1999) successfully germinate in a wide range of pH level. Germination in a broad range of pH values is an indicator of the further spreading of invasive plant species.

According to our results, *P. quinquefolia* has higher probability for germination on neutral or weakly alkaline substrates. Such levels of pH exist in deciduous forests, parks and tree plantations as well as on trampled habitats without any vegetation, or in coenosis of *Polygono-Poetea* class. These data also predict that *P. quinquefolia* cannot occupy brown forest soils of pine and spruce forest or meadows. Overall, this species has a relatively wide range of tolerance to pH for germination.

Temperature condition is very important for early life-stages of every plant species. Many alien plant species came to Ukraine from submediterranean and subtropical climates. Narrow range of tolerance to temperature is a significant barrier for further distribution. These species form heterogeneous groups of colonophytes and ephemerophytes. Usually, invasive species have wide range of temperatures under which they germinate. Such data were obtained for *Ambrosia artemisiifolia* which corresponds with the wide distribution of the species in China (Sang et al. 2011), and is again reflected by other successful invasive weeds, such as *Cortaderia jubata* (Lem.) Stapf (8–35°C) (Drewitz & DiTomaso 2004), *Solanum viarum* Dunal (10–35°C) (Akanda et al. 1996) and *Pueraria lobata* (Willd.) Ohwi (Susko et al. 1999).

According to our results, individuals of *P. quinquefolia* in primary periods of germination has a narrow amplitude for a temperature factor, which predicts low invasive success in Ukraine. Territory of Ukraine is under diverse temperature conditions. January average temperature is from -8°C in the north-east of Ukraine and in the Carpathian highlands, up to +4°C on the south coast of Crimea. July temperature is from +17°C in the northwest and +19°C in the Carpathian highlands to +23°C in the south. In mountain areas, temperatures are lower during the year than in the lowlands. The average yearly temperature in Ukraine varies between +5 – +7°C in the north and +11 – +13°C in the south (Lipinskyi et al. 2003).

Salinity is important factor in urban environment. Sodium chloride is used in winter period against ice and snow shield on the roads. This practice is a reason of excessive emission of salt in rural community. In some cases, it causes development of halophytic vegetation. Sometimes, small amounts of salts have a positive effect on germination. These results were obtained for some halophytes (Bajji et al. 1998; Matoh et al. 1986). Several studies demonstrate a significant decrease of seed germination in condition of high salinity for invasive plant species (El-Keblawy & Al-Rawai 2005; Kołodziejek & Patykowski 2015; Javaid et al. 2018).

According to salinity regime, the germination of *P. quinquefolia* is successful in oligotrophic and mesotrophic conditions.

The seeds of invasive plants usually germinated successfully under the light (Xu et al. 2010), while the germination of non-invasive species does not show significant differences in condition of darkness and under the light. However, seeds requiring light for germination have a barrier for invasion, because they cannot emerge when buried deep in the soil (Chauhan 2016).

Positive correlation of percent of germination also was shown for *Clausena excavata* Burm. f. (Vieira et al. 2010), *Piper aduncum* L. (Rocha et al. 2005; Dousseau et al. 2011), *Crassocephalum crepioides* (Benth.) S. Moore, *Ageratum conyzioides* L. (Yuan & Wen 2018), *Rumex confertus* L. (Kołodziejek & Patykowski 2015) and numerous other invasive plant species. But in contrast, *Celastrus orbicularis* Thunb. (Greenberg et al. 2001), *C. canadensis* (L.) Cronq. (Yuan & Wen 2018), *Calotropis procera* (Ait.) R. Br. (Leal et al. 2013), *Ailanthus altissima* (Mill.) Swingle (Kota et al. 2007; Martin et al. 2010) showed a different behaviour, a relative germination percentage was achieved in the dark compared to light germination, in some times depending on incubation temperatures. Some invasive plant species have slightly photoblastic nature of the seeds. It indicates that germination is successful in condition of different seed burial depths, as shown for *Physalis angulate* L. and *P. philadelphica* Lam. var. *immaculate* Waterfall (Ozaslan et al. 2017). It is key-feature for distribution of such species in diverse habitats.

Our data indicate that *P. quinquefolia* is positively photoblastic. However, similar species *P. vitaceae* may successfully invade into communities of *Galio-*

Urticetea, but conditions of such habitats have low level of illuminance. Further studies on difference in success germination of these two species are needed.

Conclusion

The seed germination of *P. quinquefolia* requires medium moisture, low soil salinity, moderate temperatures, neutral pH and the presence of light. Optimal condition for germination is 15–25°C that predicts low invasive success of *Parthenocissus quinquefolia* in Ukraine.

Parthenocissus quinquefolia is positively photoblastic and does not germinate in the dark. It may facilitate fast germination of this species from soil seed banks in disturbed areas, but at the same time it probably can be a barrier for expansion in communities of class *Galio-Urticetea*.

Our results indicate that this species has relatively wide range of tolerance to pH for germination. *Parthenocissus quinquefolia* has higher possibility for germination on neutral or weakly alkaline substrates. Such levels of pH exist in deciduous forests, parks and tree plantation as well as on trampled habitats without any vegetation, or in coenosis of *Polygono-Poetea* class. With increasing salt level in a medium percent of germination of *Parthenocissus quinquefolia* decreases, but the seedlings can develop even at high salinity.

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