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**Germination ability of the invasive knotweed seeds under
different experimental conditions**

Diploma Thesis 2024

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DIPLOMA THESIS ASSIGNMENT

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Nature Conservation

Thesis title

Germination ability of the invasive knotweed seeds under different experimental conditions

Objectives of thesis

1. To investigate the germination patterns of knotweed species (*Fallopia* spp.) under varying environmental conditions, including different substrate types, temperature ranges, and shades of light.
2. To compare germination rates of knotweed seeds in controlled laboratory conditions, semi-natural greenhouse settings, and field-based scenarios.
3. To identify the key factors influencing seed germination in knotweed species and assess how these factors interact in different experimental environments.

The thesis aims to compare germination rate in invasive knotweed taxa under different experimental conditions. Light, substrate and temperature treatments will be applied, and the influence of the environment in the greenhouse and garden will be evaluated. The germination rate under ideal conditions in climabox will be used as a control treatment.

Methodology

The germination experiment under different light, substrate, temperature and environmental conditions will be conducted. The data will be statistically analysed and the results presented in the form of tables and figures.

The proposed extent of the thesis

45 pages, 1 table, 1 figure

Keywords

germination rate, invasive plants, knotweed, environmental conditions

Recommended information sources

- Beerling, D.J., Bailey, J.P. and Conolly, A.P. (1994). *Fallopia Japonica* (Houtt.) Ronse Decraene. *J. Ecol.* 82(4), 959-979.
- Božena Šerá (2015). Effects of Soil Substrate Contaminated by Knotweed Leaves on Seed Development. *Environmental Studies.* 21(3), 713-717.
- Lavoie, C. The impact of invasive knotweed species (*Reynoutria* spp.) on the environment: review and research perspectives. *Biol Invasions* 19, 2319–2337 (2017).
- Vaseková, B. & Majorošová, Martina & Belčáková, Ingrid & Slobodník, Branko. (2022). Distribution and management of *Fallopia japonica* in riparian biotopes in Slovakia and Austria. *Biosystems Diversity.* 30. 442-452. 10.15421/012244.

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In Prague, 17.03.2024

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Abstract

This thesis examines the patterns of knotweed germination, with a particular emphasis on *Fallopia japonica*, a highly invasive plant that has substantial effects on the environment and the economy. Under carefully regulated experimental settings, the study aims to clarify the effects of environmental variables on seed germination rates, such as temperature ranges, substrate kinds, and light intensity. The germination responses of knotweed seeds to different environmental conditions were studied through a series of both indoor and outdoor experiments, using statistical analyses to find important patterns and trends.

The results show that *F. japonica* seed germination is highly dependent on substrate type and light intensity, with greater rates noted in nutrient-rich soils and partially lit (semi-shaded) environments. In the range studied, temperature by itself did not, however, show a significant effect on germination rates.

These findings offer important new understandings of the ecological strategies used by knotweed species to colonise a variety of habitats and help guide the development of efficient management plans for invasive knotweed population control. These findings have consequences for ecological restoration initiatives as well as for managing invasive species in general. This work adds to the conservation and restoration of native plant populations in invaded settings by filling important information gaps in the ecology of *F. japonica* germination.

Keywords: environmental conditions germination rate, invasive plants, Knotweed.

Abstrakt

Tato práce zkoumá se zabývá klíčením křídlatek, konkrétně taxonem *Fallopia japonica*, jedná se o vysoce invazní rostlinu, která má zásadní vliv na životní prostředí a hospodářství. V regulovaných experimentálních podmínkách se studie zaměřuje na objasnění vlivu proměnných prostředí na rychlost klíčení semen, jako jsou teplotní rozsahy, druhy substrátů a intenzita světla. Reakce klíčivosti semen křídlatky na různé podmínky prostředí byly studovány prostřednictvím řady venkovních i laboratorních experimentů s využitím statistických analýz k nalezení důležitých vzorců a trendů.

Výsledky ukazují, že klíčivost semen *F. japonica* je silně závislá na druhu substrátu a intenzitě světla, přičemž větší rychlost byla zaznamenána v půdách bohatých na živiny a v částečně osvětleném (polostinném) prostředí. Ve zkoumaném rozsahu však teplota sama o sobě nevykazovala významný vliv na míru klíčivosti.

Tato zjištění nabízejí důležité nové poznatky o ekologických strategiích, které druhy křídlatky využívají ke kolonizaci různých stanovišť, a pomáhají při vytváření účinných plánů řízení pro kontrolu populací křídlatek. Toto zjištění má důsledky pro iniciativy ekologické obnovy i pro řízení invazních druhů obecně. Tato práce přispívá k ochraně a obnově populací původních rostlin v invadovaných prostředích tím, že vyplňuje důležité mezery v informacích o ekologii klíčení *F. japonica*.

Klíčová slova: environmentalní podmínky, klíčivost, invazní rostliny, křídlatky,

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1. INTRODUCTION

1.1 Background of Study

Fallopia is a heterogenous (Desjardins *et al.* 2023) genus of herbaceous perennial plants that includes an assemblage that is well-known for its exceptional hardiness and tendency for invasion. *Fallopia japonica*, or Japanese knotweed, is one of the more well-known taxa in this genus and has gained international recognition for its quick growth and ecological devastation. Taxonomically, Japanese knotweed is placed within subtribe *Reynoutriinae* (*Polygonaceae*), which also contains the austral genus *Muehlenbeckia* (incl. *Homalocladium*) and north temperate *Fallopia* (Desjardins *et al.* 2023). Originating in East Asia and having native habitats in China, Korea, and Japan, *Fallopia* taxa have become formidable invaders in ecosystems far beyond their natural limits. These plants' effectively disseminated seeds and rhizomes have allowed knotweed species to spread to many geographical areas, making them one of the most infamous invasive plant species (Vasekova *et al.*, 2022).

The invasion of knotweed has significant and complex effects on the environment and the economy. Certain types of knotweeds are well-known for their ability to outcompete native plants in addition to their quick development. Within the impacted ecosystems, this invasion results in a decline in biodiversity. The stability of these important ecological characteristics is threatened by the density of knotweed thickets, which changes the properties of the soil and may cause erosion along riverbanks (Anderson, 2012).

The unanswered question of knotweed's widespread success emerges during the germination stage of seeds. The process of seed germination, which is essential to the growth of a new plant from a dormant seed, highlights the significance of this occurrence in the persistence of knotweed species and their ability to spread. Knotweed plants use their seeds for both reproduction and dispersal, which allows them to expand into new areas and even infiltrate virgin ecosystems (Lavoie, 2017).

The study aims to investigate the variations of knotweed seed germination in a range of environmental variables, such as temperature ranges, substrate kinds, and light intensities. This study aims to clarify the intricate interactions between variables affecting germination by means

of comparative evaluations in field-based situations, semi-natural greenhouse conditions, and controlled laboratory settings. This study aims to offer thorough insights into the germination patterns of these invasive plants by identifying the major variables influencing the seed germination in knotweed taxa and evaluating how these factors interact in various experimental conditions.

1.2 Research Problem and Its Significance

The germination of knotweed seeds under various environmental conditions is the central research subject of this work. The study specifically aims to investigate the effects of temperature, substrate type, and light intensity on knotweed species' (*Fallopia spp.*) germination patterns. This issue originates from the understanding that knotweed, one of the most infamous invasive plant species in the world, has an amazing ability to adapt and survive in a variety of environments, changing natural habitats and causing ecological and financial hardships in the process.

This research problem is particularly important in many ways:

1.2.1 Ecological Significance:

- **Management of Invasive Species:** Knotweed invasions cause major obstacles for conservation efforts in addition to upsetting natural ecosystems. Developing successful invasive species management plans requires a thorough understanding of the germination characteristics of knotweed seeds (Anderson, 2012). We can create focused containment and control strategies by determining the critical elements influencing germination, which will help to maintain natural biodiversity.
- **Ecosystem Resilience:** Because of knotweed's rapid expansion, ecosystems may become less resilient to changes in their surroundings in terms of structure and function (Aguilera *et al.*, 2010). Researching seed germination offers information about the ecological dynamics and interactions that underlie knotweed's invasiveness, information that can be used to guide conservation and restoration initiatives.

1.2.2 Agricultural and Economic Significance:

- **Agricultural Impacts:** Varieties of knotweed can spread onto farmlands, reducing crop production, and requiring more pesticide use. In regions where knotweed invasions are likely, initiatives to protect agricultural sustainability and productivity must consider the germination of seeds (Delbart *et al.*, 2012).
- **Infrastructure Costs:** Knotweed generates significant financial expenses due to its penetration into and destruction to infrastructure, including building foundations, asphalt, and concrete. Studying the germination of seeds can help manage and prevent this kind of damage, which lowers maintenance and repair costs (Cuthbert *et al.*, 2021).

1.2.3 Scientific Significance:

- **Contribution to Plant Ecology:** By deepening our knowledge of invasive plant species' seed germination patterns, this research advances the subject of plant ecology. Investigating the germination dynamics of different invasive and native plant species in various ecological situations can benefit from this knowledge.
- **Applied Research and Conservation:** By connecting the dots between basic science and practical solutions to ecological and land management issues, the knowledge gathered from this study can be used for sustainable agriculture, restoration, and conservation. In areas invaded by invasive species such as *Fallopia*, managing generative reproduction becomes crucial for controlling population spread and mitigating ecological impacts.

This research challenge highlights the importance of conducting a thorough investigation into the germination of knotweed seeds in a range of environmental settings, providing important information on the variables affecting germination patterns. By tackling this issue, the research hopes to close information gaps and advance ecological understanding, sustainable land use, and conservation in general as well as the efficient management of invasive knotweed species.

2. RESEARCH OBJECTIVES AND HYPOTHESES

2.1 Research Objectives

1. To investigate the germination patterns of knotweed species (*Fallopia spp.*) under varying environmental conditions, including different substrate types, temperature ranges.
2. To compare germination rates of knotweed seeds in controlled laboratory conditions, semi-natural greenhouse settings, and field-based scenarios.
3. To identify the key factors influencing seed germination in knotweed species and assess how these factors interact in different experimental environments.

2.2 Research Hypotheses

Hypothesis 1: Germination rates of knotweed seeds will vary across different substrate types, with specific substrates favouring or inhibiting germination success.

Hypothesis 2: Temperature will significantly affect the timing and success of seed germination in knotweed species, with optimal germination occurring within a specific temperature range.

Hypothesis 3: There will be significant differences in seed germination patterns when comparing controlled laboratory conditions, semi-natural greenhouse settings, and field-based experiments, with the latter displaying the most challenging germination behaviour that is dependent on the environment.

These hypotheses are formulated based on the identified gaps and inconsistencies in the existing literature and aim to provide a comprehensive understanding of knotweed seed germination under varying conditions. The research objectives and hypotheses guide the next experimental work and data analysis, with the aim of addressing these knowledge gaps and contributing to the field of invasive species management.

3. LITERATURE REVIEW

3.1 Global Distribution and Invasiveness

East Asia is the natural home of *Fallopia* taxa, especially Japanese Knotweed, which can be found there. But they have escaped their natural habitat and become formidable global invaders. Because of their extremely effective spreading by rhizomes, knotweed taxa are among the most infamous invasive plants in a variety of environments across the globe (Vasekova *et al.*, 2022).

The ability to thrive in a variety of temperatures and habitats is a characteristic of knotweed taxa. They have flourished outside of their natural area thanks to their adaptability, often at the expense of local flora. Due to their ability to outcompete native flora and alter ecosystems to their advantage, knotweeds have earned a reputation for being resilient, hardy, and competitive (Clements *et al.*, 2016).

3.2 Ecological and Economic Impacts of Knotweed Invasion

The spread of knotweed has far-reaching and complex ecological and economic repercussions. These invasive species can swiftly overtake and supplant native plants because of their speedy development. As a result, they have a negative impact on biodiversity in the ecosystems they touch. Their creation of dense thickets can change the properties of the soil and cause riverbank erosion, which can affect the stability of these important ecological features (Anderson, 2012).

According to Aguilera *et al.*, (2010), Knotweed does not just grow quickly in natural settings. It is a significant obstacle for land managers and property owners in suburban and urban settings. Unbelievably, these invasive plants can penetrate and harm building foundations, asphalt, and concrete, requiring expensive and time-consuming repairs. Hocking *et al.* (2023) assumed that wide-ranging economic effects are caused by the financial strains associated with infrastructure management and property upkeep.

In another study by Beerling *et al.*, (1994), agriculture and forestry are two industries that are affected economically by knotweed infestation. A crop's output may be reduced by knotweed infestation, which would need farmers to apply more pesticides. Further compounding its

economic impact, knotweed in forests impedes natural regeneration processes and interferes with timber harvesting activities.

In sum, the genus *Fallopia*, with its invasive knotweed taxa at the forefront, offers an illustrative case study for exploring the ecological and economic consequences of invasive species. Their capacity to spread, thrive, and reshape ecosystems underscores the pressing need for research to develop effective management strategies. This study aims to contribute to this understanding by scrutinizing the germination of knotweed seeds under varying experimental conditions, offering insights into potential control and mitigation measures.

3.3 Seed Germination in Knotweed

The life cycle of many knotweed taxa, including Japanese knotweed (*Fallopia japonica*), depends heavily on seed germination. It's a procedure that starts a dormant seed into a flourishing plant. For knotweed to persist as a species and be successful in spreading throughout the environment, seed germination is essential. According to Lavoie (2017), knotweed seeds facilitate the plant's ability to reproduce and spread, allowing it to occupy previously uninhabited areas and possibly even overtake virgin ecosystems. The mechanisms underlying knotweed's quick and aggressive proliferation must be understood considering the dynamics of seed germination.

Given the ecological and economic challenges that invasive plants—in particular, knotweed species—pose, research on seed germination in these plants is crucial. Ecosystems can be disrupted, and native vegetation outcompeted by invasive plants, such as knotweed. Germination of seeds is an important step in this invasive process. Researchers can learn more about the elements that support knotweed species' establishment and spread in non-native areas by investigating their germination patterns. This information is essential for creating management plans that work and containment techniques that stop them from spreading (Jones *et al.*, 2020).

Knowledge of knotweed species' germination patterns has been enriched by numerous research. Previous studies have demonstrated that these plants respond differently to environmental stimuli, such as temperature, light, and substrate type, as they attempt to germinate (Šerá, 2011). This variation in germination behaviour offers a wealth of opportunities for additional research. To detect gaps and inconsistencies and to inform management methods targeted at reducing the impact of knotweed invasions, it is imperative that the collection of available knowledge be synthesized and evaluated.

3.4 Factors Affecting Seed Germination

A complicated web of environmental conditions interacts to control the germination of knotweed seeds. The three main factors influencing seed germination are light, substrate (soil type and content), and temperature. These elements may help or hinder knotweed germination, which in turn affects how well the plant establishes itself later. For example, various taxa of knotweed have been shown to favour light conditions, and the kind of substrate might influence the establishment and growth of seedlings (Dassonville *et al.*, 2011). It has also been demonstrated that temperature variations significantly affect the time and pace of germination.

There are notable differences in knotweed seed germination between natural and regulated settings. The term "natural conditions" refers to the environments in which knotweed is invasive, where germination patterns can be influenced by weather fluctuations and competition with native flora. On the other hand, controlled environments, such as those seen in laboratories or greenhouses, provide exact conditions that enable researchers to separate and modify components. For a thorough understanding of knotweed germination dynamics and to identify which environmental elements may be most important in various situations, it is imperative to study seed germination in both settings.

There are numerous aspects to the present understanding of how environmental factors impact the germination of knotweed seeds. Research has demonstrated that although knotweeds are hardy and versatile, they have certain germination inclinations and are sensitive to various environmental conditions. The effects of temperature range, soil composition, and light intensity on germination rates and patterns have all been studied. Furthermore, it has been observed that these impacts might differ between varied taxa of knotweed as well as depending on the ecological circumstances in the area (Delbart *et al.* 2012).

The intricate details of knotweed seed germination have been the subject of numerous research, which have helped to clarify the variables and circumstances affecting this vital phase of the life cycle of these invasive plants. These investigations have used several approaches and have produced a wide range of results that help us comprehend the dynamics of knotweed germination. In one noteworthy study, Japanese Knotweed (*Fallopia japonica*) seed germination patterns were examined in controlled laboratory settings by Lavoie (2017). The author observed the rates of germination after exposing knotweed seeds to various temperature and light regimes. According to the research, temperature and germination rates are strongly positively correlated, with higher

temperatures resulting in more successful germination. Additionally, they discovered that light conditions had a major impact on germination, with seeds exposed to constant light showing the highest rates of germination.

Delbart *et al.* (2012) looked to examine the effect of competition with natural vegetation on knotweed seed germination in a different study that involved field research. Study plots were set up in invaded environments, and the germination rates of knotweed seeds were observed with and without native plant competitors. Their results showed that germination success is negatively impacted by competition, as knotweed seeds germination rates are lower in locations with high native plant density.

Tania and Bernd (2007) examine the factors that hinder the growth and survival of two native plant species in the presence of the invasive Bohemian knotweed (*Fallopia ×bohemica*, *Polygonaceae*). The study focused on understanding the mechanisms through which the invasive species negatively impacts the native plants. The research includes an analysis of various factors such as competition for resources, allelopathy, and soil characteristics. The results contribute to a better understanding of the ecological interactions between invasive and native species, providing valuable insights for the management and conservation of native plant populations in the presence of invasive plants.

3.5 Research Justification

While previous research has made valuable contributions to our understanding of knotweed seed germination, there remain critical gaps and inconsistencies in the existing literature that warrant further investigation. These gaps and inconsistencies include:

3.5.1 Limited Research on Environmental Interactions

Prior research has frequently concentrated on a single environmental element, such as competition, light, and temperature. However, complex interactions among these elements most likely affect knotweed germination (Martin *et al.* 2020). Research on the combined effects of these factors is needed, especially in environments that are like those in which knotweed invasion happens in real life.

3.5.2 Variability Among Knotweed Species

Japanese knotweed has been the subject of most research on knotweed seed germination. Different taxa of knotweed, like gigantic and bohemian knotweed, could have different germination patterns (McHugh 2006; Parepa *et al.* 2014). Gaining a deeper understanding of this biological or ecological process can be achieved by examining the germination behaviour of several knotweed taxa.

3.5.3 Natural Versus Controlled Settings

Previous research has often occurred in controlled environments, such as laboratory or greenhouse conditions. However, the germination behaviour of knotweed seeds in natural, invaded ecosystems may differ significantly due to competition with native plants and variations in soil composition (Dassonville *et al.* 2011). Comparative research that examines germination under both controlled and natural conditions is needed.

3.5.4 Management Implications

Existing studies have primarily focused on understanding germination patterns. However, to effectively manage knotweed invasions, research should also explore how germination findings can be applied to develop targeted management strategies and containment measures.

The justification for this research, which attempts to investigate knotweed seed germination under various experimental conditions and provide insightful information about the factors influencing germination and their implications for knotweed management and control strategies, is strengthened by these gaps and inconsistencies in the literature that have been identified.

3.6 Experimental Approaches in Germination Studies

Studying seed germination, particularly in the context of invasive species like knotweed, requires a robust experimental approach. Common methodologies and approaches employed in seed germination studies include:

3.6.1 Laboratory Germination Tests

In the laboratory surroundings, these controlled experiments enable researchers to adjust variables like substrate, light, and temperature. Usually, controlled circumstances are used to sow seeds, and timing and germination rates are noted. According to McNair *et al.* (2012), laboratory testing offers accurate control over environmental conditions and is helpful in analysing the basic prerequisites for germination. In this type of experiment, there is precise control over environmental variables, facilitating the isolation of specific factors influencing germination and replicable and standardized conditions for comparing results across studies. However, there is limited ecological authenticity and does not account for competition, soil interactions, or other complex field conditions.

3.6.2 Greenhouse Experiments

Studies conducted in greenhouses provide a link between natural ecosystems and well monitored laboratory settings. They incorporate fluctuations in substrate, light, and temperature to create a scenario that is more realistic. Examining germination patterns in semi-natural settings through greenhouse experiments can shed light on how invasive species, such as knotweed, might act in the wild (Gillies *et al.*, 2016). This experiment is still controlled, which may not fully replicate the conditions of natural ecosystems. However, it provides insights into the semi-natural conditions in which invasive species often thrive.

3.6.3 Field-Based Studies

The real environments where invasive organisms flourish are the sites of field experiments. Within invaded areas, researchers set up study plots and track seed germination in situ. The benefit of field studies is that they can investigate germination patterns in an actual setting, taking into consideration native plant competition and organic soil characteristics. They shed light on potential differences in germination dynamics when intricate ecological interactions are present (Kovarova *et al.*, 2010). It accounts for competition and natural soil conditions. It also offers insights into germination behaviour under complex ecological interactions. However, it can be difficult to control and replicate, making it challenging to isolate specific factors.

3.6.4 Comparative Germination Trials

In comparative research, the germination of seeds is subjected to varying environmental factors, including temperature ranges, light exposure, and soil types. Researchers can learn more about the variables that most strongly affect seed germination in invasive species like knotweed by examining germination rates and patterns under each of these circumstances (Martin *et al.*, 2020). It allows for the study of multiple factors simultaneously and their interactions. Can help identify key drivers of seed germination. Results may not be directly applicable to natural conditions, as they may not precisely mimic specific field situations.

Combining different approaches can provide a more comprehensive understanding of seed germination patterns in invasive species like knotweed.

This study makes a significant contribution to the broader fields of invasive species management, ecology, conservation, restoration, and sustainable agriculture in addition to addressing the unique germination patterns of knotweed seeds. Through the study of invasive knotweed taxa, new perspectives on their germination behaviour may be gained. This could lead to more efficient methods of reducing the negative ecological and economic effects of invasive plants, protecting natural ecosystems, and advancing sustainable land management techniques.

4. METHODOLOGY

4.1 Study Species

Fallopia japonica (Japanese knotweed), a well-known knotweed taxon, is the subject of the study. It was selected due to its prevalence in the study area and well-established invasive traits. This taxon was chosen because of the difficulties they present to native ecosystems, their enormous ecological impact, and their quick spread.

The structural features of the leaves and flowers, such as the presence of trichomes, the size and shape of the petiole and apex, and the presence of buds with small, flat, empty anthers with stigma longer than the perianth, were used to identify the *F. japonica* plants (Beerling *et al.*, 1994). In the study area, Japanese knotweed is widespread and exhibits a broad distribution and habitat adaptability. It serves as the primary species for invasive species management due to its prevalence along roadside ditches, riverbanks, and disturbed regions.

The invasive characteristic of Japanese knotweed is its fast growth, which results in dense thickets that outcompete native plants. Its ability to spread quickly through rhizomatous growth makes it a dangerous invader in both urban and natural settings.

According to Kadlecová (2024), it is noteworthy that *Fallopia* taxa possess the ability to establish considerable and long-lasting clonal stands, yielding viable seeds via processes like hybridization, intercrossing, and backcrossing. As a result, seedlings may be established in favourable habitats, resulting in the emergence of genetically varied populations.

F. japonica is represented by octoploid ($2n = 88$) (Bailey & Stace, 1992; Mandák *et al.*, 2003). Since *F. japonica* pollen is lacking, the female clone of this taxa may only generate viable seeds through hybridization (Bailey *et al.*, 2009; Saad *et al.*, 2011; Tiébré *et al.*, 2007).

4.2 Experimental Conditions

4.2.1 Light Intensity

Plant germination is significantly impacted by light intensity, which also affects photomorphogenic responses and subsequent growth (Patterson *et al.*, 1977). The goal is to find out how the germination patterns of *Fallopia japonica* seeds are impacted by varying light intensity. The experimental strategy entails subjecting seedlings to a range of light intensities to replicate environmental circumstances typical of several different ecosystems and landscapes impacted by human activity.

Experimental conditions with varying light intensities were set up during the project. This was achieved using controlled light sources in the laboratory and by placing the experiments in location with different light exposures (full sunlight, partial shade, and shade).

In the laboratory, experiments were placed in a climabox (equipped with adjustable light fixtures) with artificial light sources, allowing for precise manipulation of light intensity and adjusted to mimic natural day-night cycles.

In the greenhouse, experiments were implemented to introduce a more realistic setting compared to the controlled laboratory conditions. It provides a controlled yet dynamic environment with exposure to natural light variations. Seeds were allowed to experience fluctuations in natural light conditions, including changes in sunlight intensity due to weather conditions and seasonal variations. This approach aims to bridge the gap between laboratory-controlled and natural settings.

In the field-based scenarios, seeds were exposed to the complexities of natural environmental conditions. Sites where knotweed taxa are prevalent were selected, considering variations in light exposure due to canopy cover and topographical features. Field experiments were conducted during different seasons (spring and summer) to capture seasonal variations in light intensity and its impact on seed germination.

This study intends to further understand the complex responses of *Fallopia japonica* to various light environments by methodically investigating the impact of light intensity on knotweed seed germination under controlled laboratory, greenhouse, and field-based conditions. This research will provide important insights into the broader understanding of knotweed invasiveness.

4.2.2 Substrate Types

The physical and chemical environment in which seeds develop is determined by the substrate selection, which has a substantial impact on seed germination. Examining the germination habits of *Fallopia japonica* on various substrate type sheds light on these invasive species' capacity for adaptation (Šerá, 2011). The experimental strategy includes exposing seeds to a range of substrates to replicate the diversity of soils that both invaded and non-invaded areas of knotweed may experience.

To simulate the variety of soil conditions that knotweed taxa might experience in their native habitats, certain substrates have been chosen, such as garden soil, sandy soil, natural soil with knotweed leaves, and natural soil without knotweed leaves.

A well-fertilized and nutrient-rich substrate that is frequently seen in landscapes that are managed is garden soil. Certain varieties of knotweed are known to grow well in disturbed environments, such as gardens and farmed areas [Jones, 2022]. The experiment's inclusion of garden soil enables an investigation of knotweed germination behaviour under circumstances with relatively high nutrient availability.

Low capacity to retain water and nutrients is a characteristic of sandy soil (Bruand *et al.* 2005). This type of substrate was selected to mimic the soil conditions found in coastal regions and riverbanks, which have well-draining soils. Certain environments are common places for knotweed taxa to establish, and researching germination in these conditions can reveal how adaptable knotweed is to various soil textures.

The addition of knotweed leaves to natural soil gives the substrate a new level of complexity. The organic matter released by decomposing knotweed leaves affects the soil's microbial activity and nutrient content (Šerá, 2011). This substrate provides a more accurate representation of the soil environment in invaded areas by imitating conditions where knotweed has established.

As a control condition, the natural soil without knotweed leaves mimic the organic soil environment free from the impact of knotweed leaf litter. The experiment aims to determine whether the presence of decomposing knotweed leaves has an observable effect on seed germination by comparing germination outcomes in natural soil with and without knotweed leaves. This control aids in identifying the exact effect that knotweed leaf litter has on the process of germination.

The selection of these substrates reflects a thoughtful consideration of the ecological ecosystems where knotweed species commonly establish. The experiment is designed to assess how knotweed seeds respond to different soil types, each representing a distinct aspect of their natural habitat.

4.2.3 Temperature Ranges

Controlled temperature regimes were implemented to simulate conditions knotweed seeds may experience during different seasons. In the laboratory, the climabox temperature was set at 22°C for fourteen hours (14hrs) during the day and 15°C for ten hours (10hrs) during the night.

Temperature in the greenhouse is more stable and ranged between 8-19°C. Because the greenhouse experiment was conducted during mid spring, an air conditioner was provided to maintain the above temperature range whenever it exceeds 20°C.

However, the temperature in the field was subjected to the current weather/climate. Temperature in the field follows the regular mid spring /summer temperature range.

4.3 Environmental Settings

Experiments were conducted in three places/environments viz: climabox in the laboratory, greenhouse, knotweed stand under the canopy of the maternal plant and outside the canopy of maternal plants. These environments were chosen to capture a spectrum of ecological habitats along with a controlled environment.

4.4 Experimental Setup

Seeds were collected in the Czech Republic (Central Bohemia region –Fig.1). The following are the site's climatic features according to Vašátko (2023): 205 metres above sea level and 9°C is the average yearly temperature there. The average summer temperature is 18.3°C, and the average winter temperature is 0.07°C. There is 583 mm of precipitation on average each year at the study site.



Fig.1 Study area in Central Bohemia region ($49^{\circ}54'13.6''N$, $14^{\circ}23'46.3''E$) for collecting the seeds and where the experimental plots for EXP3 were placed.

Seed preparation began by cleaning and removing any debris or non-viable seeds. The process of storage also helps in stratifying the seeds to break dormancy, mimicking natural conditions. Thirty (30) seeds were deposited into a small plastic tray filled with the different substrate types, i.e., 30 seeds in a plastic tray filled with garden soil, 30 seeds in a plastic tray filled with sandy soil, 30 seeds in a plastic tray filled with natural soil with knotweed leaves and 30 seeds in a plastic tray filled with natural soil without knotweed leaves. Each tray was properly labelled and covered with a thin plastic film to retain moisture (climabox experiment). Seeds in the field were covered with a thin net to avoid wind/insect/bird disturbance while seeds in the greenhouse were not covered. In the field, a small plot was selected, and plastic trays were perforated below to allow for root growth, the ground was dug few inches deep, and trays placed inside partially covered.

Each experiment was replicated thrice to enhance the robustness and reliability of the results, increasing statistical power. Therefore, there were 30 seeds deposited into each substrate type as mentioned above bringing the total number of seeds to 30 x 3 samples for each substrate type.

Three germination experiments were conducted: Experiment 1 evaluated the impact of various substrate types on seed germination in a climate box; Experiment 2 evaluated the impact of various substrate types and temperature on seed germination in a greenhouse; and Experiment 3, which examined the variations in germination between various substrate types and light conditions both close and far from *F. japonica* stands.

4.4.1 Experiment 1:

The seeds were incubated in dishes (18 × 10 × 4.5 cm, volume: 0.5l) at 14 h light at 22 °C/ 10 h dark at 15 °C with four different substrate types (S1-S4; Table 1) in a climabox and watered regularly with distilled water. Each population sample consisted of 30 seeds in three replicates for each substrate type (i.e., 12 dishes were tested). All germinated seeds were counted and recorded at two-day intervals for 17 days. The experiments were performed in complete randomised blocks.

4.4.2 Experiment 2:

Following a similar procedure as experiment 1, dishes were placed in the greenhouse and watered regularly with distilled water as well. Each population sample consisted of 30 seeds in three replicates for each substrate type (i.e., 12 dishes were tested). However, temperature was recorded (minimum, maximum and average), germinated seeds were counted and recorded at two-day interval for 36 days.

Table 1: The experimental design with used treatments. The treatments are described, and complete factorial design of treatments was used in the study. The number of repeats per treatment combination is shown

Treatment	Description	Experiment location	Number of seeds
S1	Soil taken from beneath maternal plants	Climabox	90
S2	Soil taken from beneath maternal plants covered by <i>F. japonica</i> leaves	Climabox	90
S3	Universal garden soil	Climabox	90
S4	Sand	Climabox	90
S1	Soil taken from beneath maternal plants	Greenhouse	90
S2	Soil taken from beneath maternal plants covered by <i>F. japonica</i> leaves	Greenhouse	90
S3	Universal garden soil	Greenhouse	90
S4	Sand	Greenhouse	90
L1	Shade	Under the mother plant	360
L2	Semi-shade	Partial coverage by the mother plant	360
L3	Full sunlight	Away from the mother plant	360

4.4.3 Experiment 3:

Follows the methodology of experiment 1, but the dishes with seeds were placed at the study site of *F. japonica*, where the seeds were collected. The location of the experiment was chosen to ensure that 12 dishes were in shade from the mother plant (L1), 12 dishes in semi-shade (i.e., about 50% of full sunlight) (L2), and 12 dishes in full sunlight (L3) (the experimental design and the numbers of seed were combined in Table 1). Dishes were covered with a fine mesh to prevent further seeds from falling into it and were placed in the soil so that their upper edges were level with the soil surface.

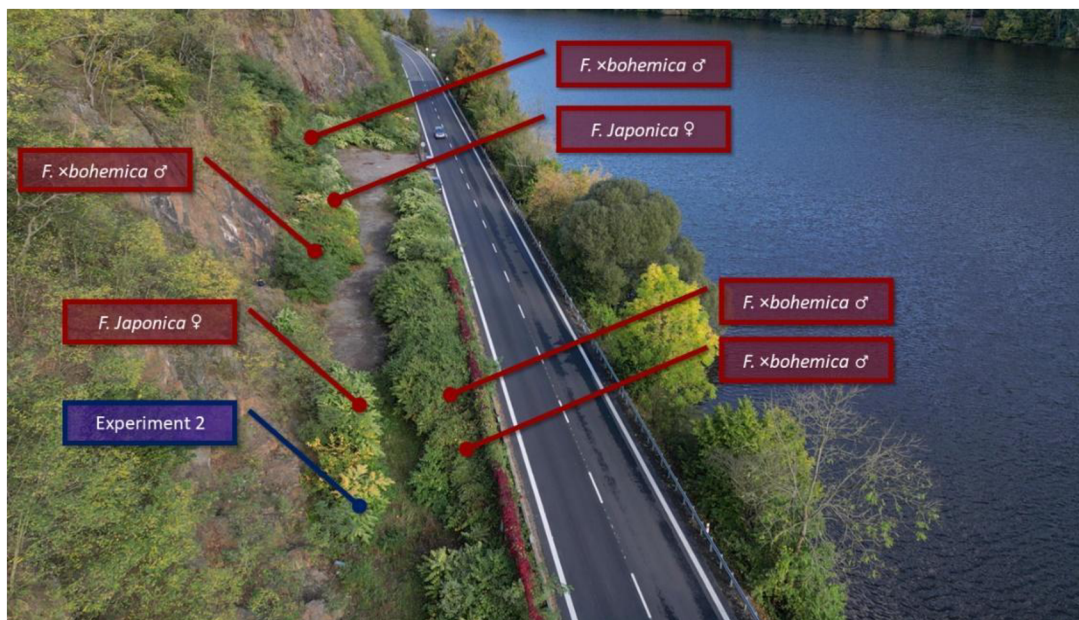


Fig. 2 Study site for field experiment (EXP3)

4.5 Data Collection

Monitoring started immediately after sowing seeds. For experiment 1, it was monitored every two days, and the number of germinations was recorded. All germinated seeds were transplanted to avoid repetitive counting. Experiment 2 follows the same as the climabox monitoring while Experiment 3 was monitored once a week.

Data were recorded in a table containing date, substrate type, temperature variations, number of seeds germinated and the total number of germinated seeds.

4.6 Statistical Analysis

In experiments 1 and 2, differences in final germination according to different temperatures and substrates were tested by generalised linear models (GLM) for the negative binomial family. Substrates were included as a fixed factor and the number of germinated seeds as the response variable.

In experiment 3, differences in final germination according to different substrates and different light availability (L1-4) were tested by generalised linear models (GLM). Substrate, and light regime were included as a fixed factor in all and the number of germinated seeds as the response variable.

The statistical software R (R Development Core Team, 2019) with relevant libraries and Statistica 13 (TIBCO, 2017) were used for conducting analyses efficiently and accurately. A Generalized Linear Model (GLM) was carried out to analyse the different data types and distributions. A separate GLM test was conducted for each experimental factor (substrate types, temperature ranges) to evaluate their individual impact on germination.

Also, interactions between different experimental factors were investigated to understand whether their combined effects on germination outcomes are significant. This analysis helps determine whether the impact of one experimental factor is dependent on the levels of another factor.

To compare specific pairs of experimental conditions, Posterior Comparisons Method was carried out. This provides a more detailed understanding of which conditions lead to significant differences in germination rates.

Depending on the distribution and variance of the data, data transformation techniques (e.g., log transformation) was considered to meet the assumptions of statistical tests, such as normality and homogeneity of variance. This was done to ensure a reliable result.

A predetermined significance level of 0.05 or 5% was set to determine statistical significance and the confidence interval was noted as well.

5. RESULTS

5.1 Experiment 1

Significant differences were not seen in the germination rates across different substrate types (GLM; $z_{2,87} = 1.7$ $p > 0.05$). The type of substrate had no significant influence on final germination.

Substrate Type	Mean Germination (%)	Standard Deviation
S1	70	5
S2	60	7
S3	85	6
S4	50	10

*Table 2 summarizes germination outcomes under different substrate types, including S1: soil taken from beneath under the maternal plants, S2: soil taken from beneath under the maternal plants covered with *F. japonica* leaves, S3: universal garden soil, S4: sand.*

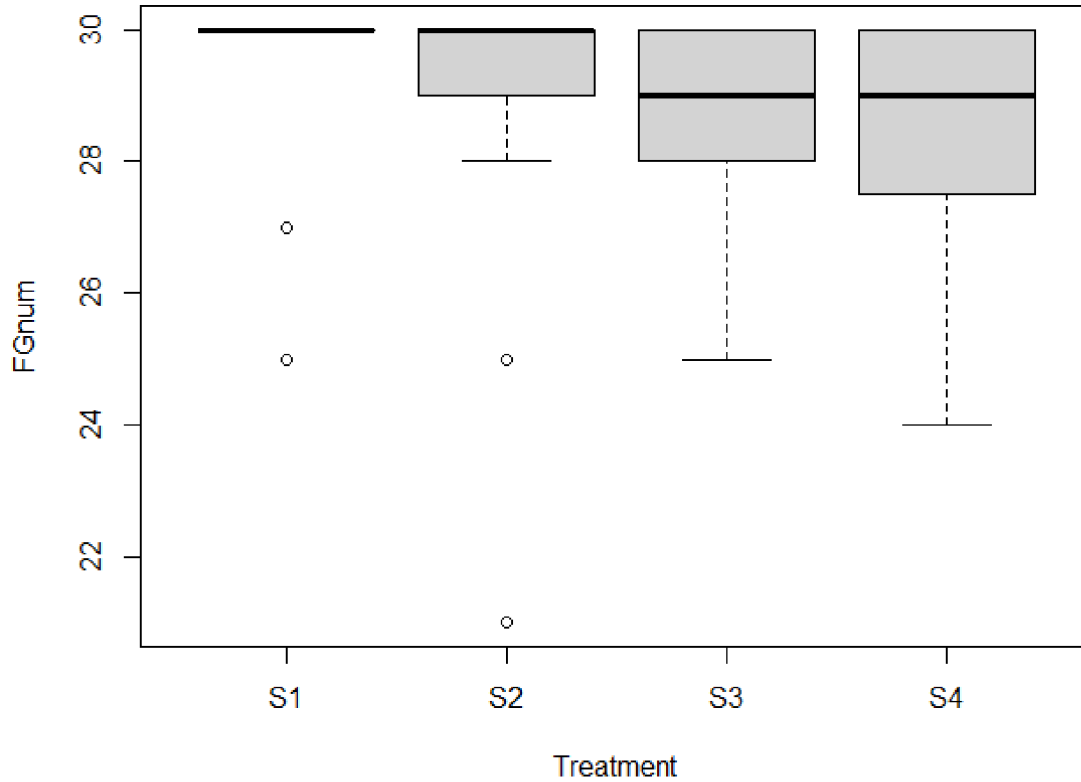


Fig. 3 Final germination of *F. japonica* seeds of EXP1. Four different substrate types (S1: soil taken from beneath under the maternal plants, S2: soil taken from beneath under the maternal plants covered with *F. japonica* leaves, S3: universal garden soil, S4: sand)

5.2 Experiment 2

5.2.1 Substrate

Like experiment 1, soil substrates played no significant part in final germination. Significant differences were not seen in the germination rates across different substrate types (GLM; $z_{2,30} = 3.3$ $p > 0.05$).

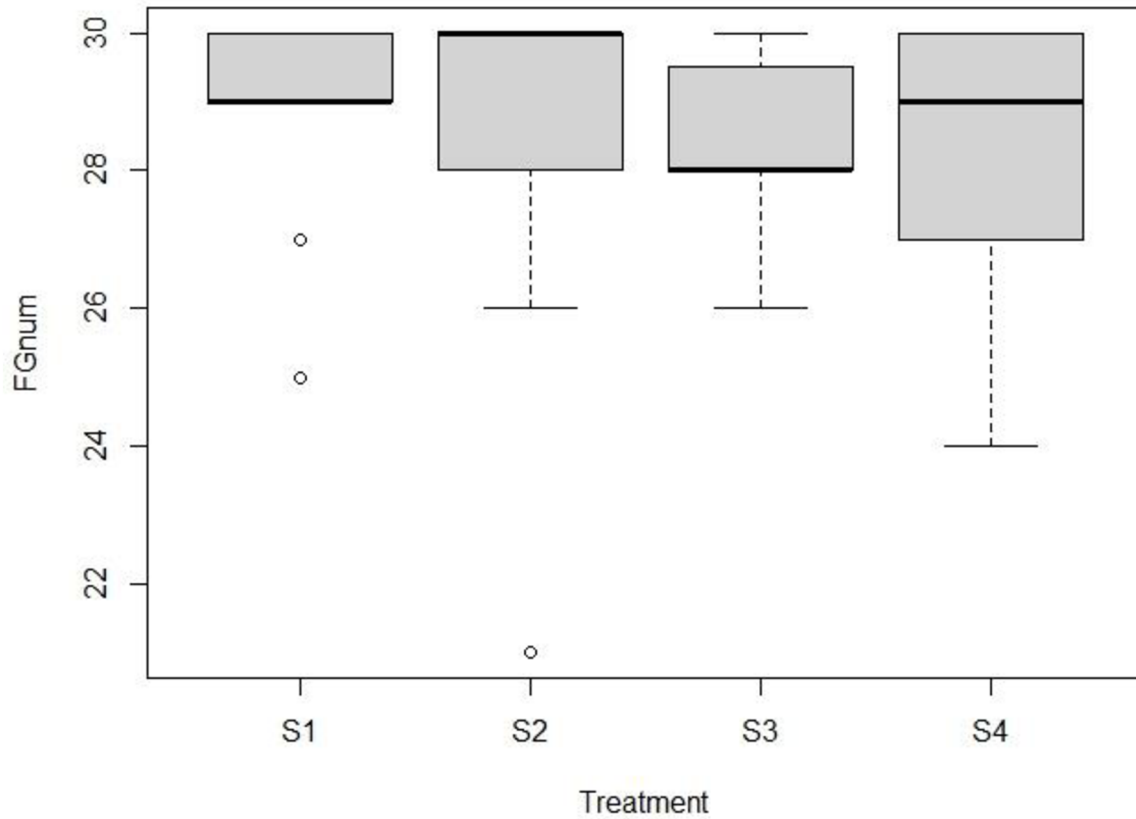


Fig. 4 Final germination of *F. japonica* seeds of EXP2. Four different substrate types (S1: soil taken from beneath under the maternal plants, S2: soil taken from beneath under the maternal plants covered with *F. japonica* leaves, S3: universal garden soil, S4: sand)

Germination was successful across all substrate type although there was delay in germination time as most seeds especially in the S4 and S2 substrate type germinated later than the rest.

Substrate Type	Mean Germination (%)	Standard Deviation
S1	73	5
S2	70	3
S3	80	6
S4	60	3

Table 3 summarizes germination outcomes under different substrate types, including S1: soil taken from beneath under the maternal plants, S2: soil taken from beneath under the maternal plants covered with *F. japonica* leaves, S3: universal garden soil, S4: sand.

5.2.2 Temperature

Temperature in the greenhouse fell between 8-18°C and thus were grouped into three ranges for the purpose of determining the mean germination rate.

Temperature Range (°C)	Mean Germination (%)
8-10	65
10-12	75
12-18	68

Table 4 presents germination rates under different temperature ranges, allowing for an assessment of the impact of temperature on seed germination.

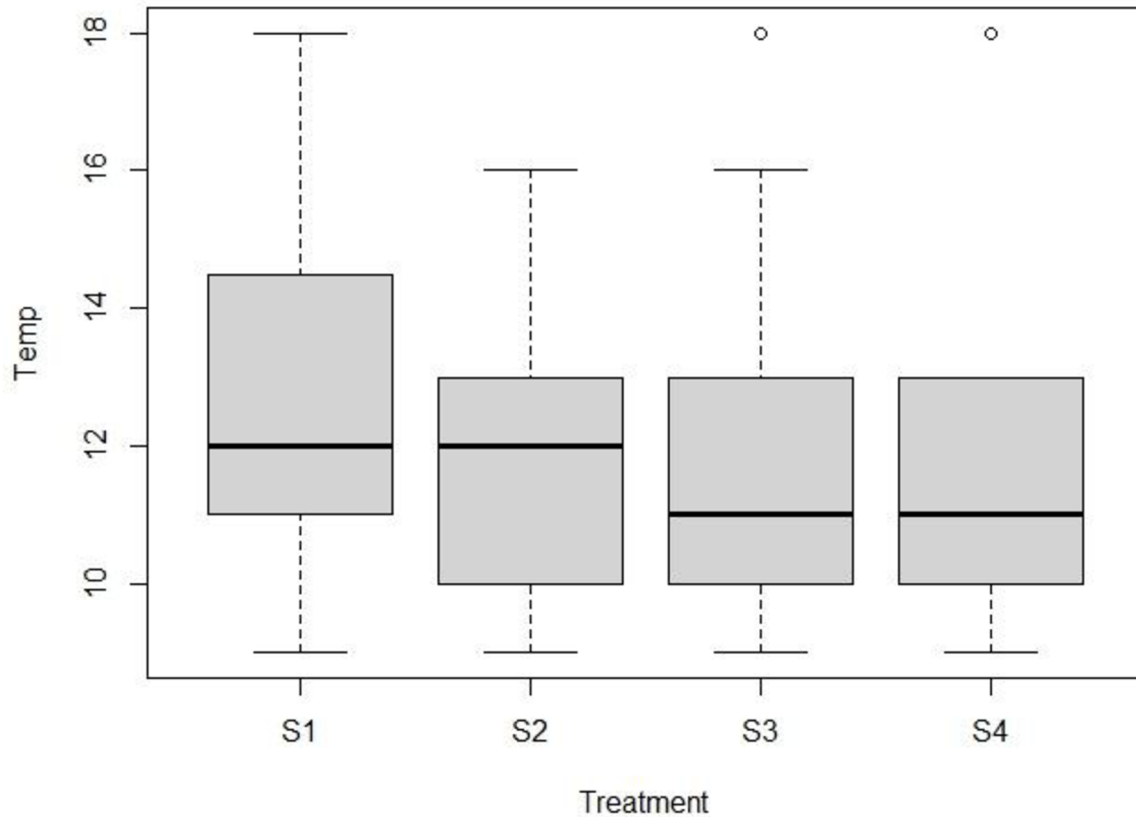


Fig. 5 Final germination of *F. japonica* seeds of EXP2. Four different substrate types (S1: soil taken from beneath under the maternal plants, S2: soil taken from beneath under the maternal plants covered with *F. japonica* leaves, S3: universal garden soil, S4: sand)

Germination is not significantly affected by temperature alone, as indicated by the main impact of temperature, which is not statistically significant (GLM; $z_{2,30} = 0.141$, $p = 0.888$). As mentioned above also, the main effects of the different soil treatments (S1-S4) are not statistically significant.

The interaction between temperature and soil type on seed germination were also found to be statistically insignificant.

Conclusively, the germination rate remains largely unaffected by temperature or treatment levels, either separately or in combination.

5.3 Experiment 3

5.3.1 Light Intensity

Germination rates were highest in the shade (L1), followed by the partial shade (L2), and significantly lower in the full sunlight (L3). This data suggests a positive correlation between light intensity and germination rates, indicating that knotweed species exhibit higher seedling emergence in partially illuminated conditions and do not particularly favour the direct sunlight. Different availability of light also had a significant impact on the final germination of seeds (GLM; $z_{2,30} = -4.041$, $P < 0.001$). The highest germination was in shade (L1: 7.4 ± 4.9) compared to semi-shade (L2: 2.7 ± 4.1) and full sunlight (L3: 3.9 ± 1.9) (Fig. 6).

There was a noticeable differential in the germination rates under different light levels. The germination rates under the shade of the mother plant were found to be substantially greater ($p < 0.001$) than those under partial shade and full sunlight, according to posterior comparisons method. This supports the idea that knotweed species have photoblastic germination traits by indicating a significant positive correlation between light intensity and germination.

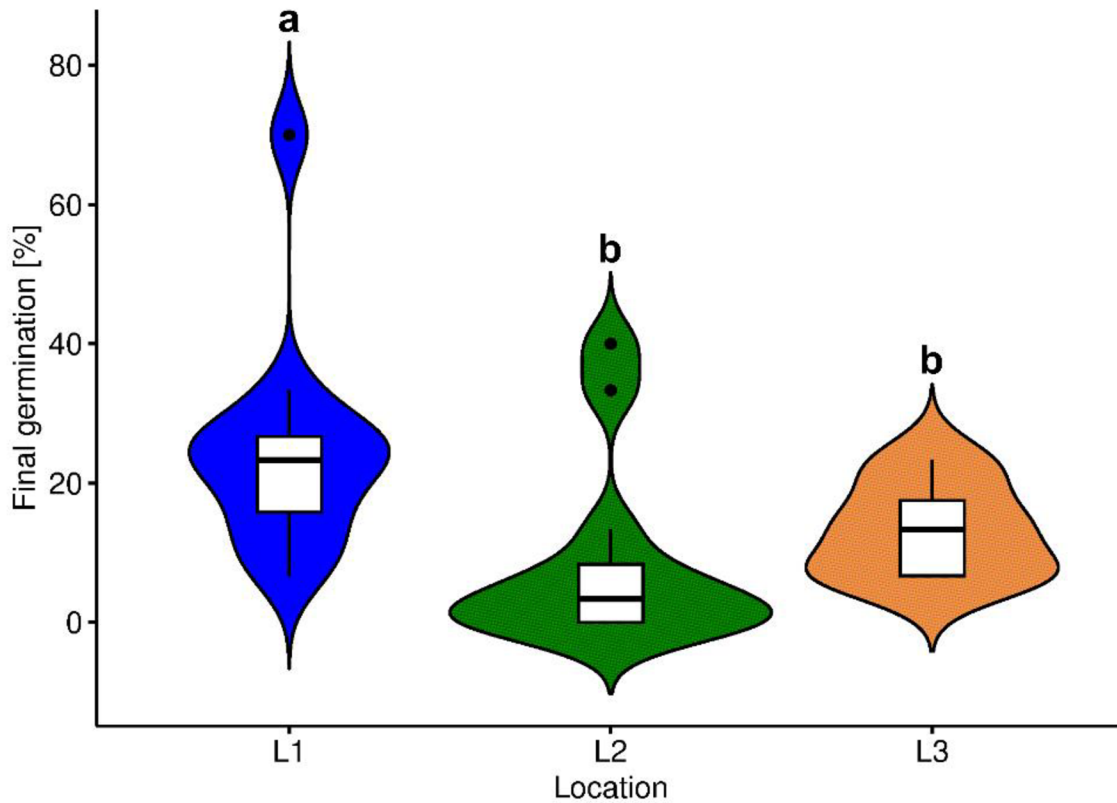


Fig. 6 Final germination of *F. japonica* seeds in EXP3 under different light conditions (L1: shade, L2: semi-shade, L3: full sunlight); kernel density plot (distribution of observations in a dataset): black lines = means of final germinated seeds, boxes = the range between the first (lower) quartile (Q1) and the third (upper) quartile (Q3), whiskers = min. – max.; different letters indicate a statistically significant difference ($P < 0.05$) identified through the posterior comparisons method.

5.3.2 Substrate Types

In experiment 3, the type of substrate had a significant impact on the final germination of *F. japonica* seeds (GLM; $z_{2,30} = 1.600$, $P < 0.01$) (Fig. 7), which is a different outcome compared to experiment 1 where the substrate type had no significant influence on final germination. Among the different substrates tested, S3 exhibited the highest germination rate ($\bar{x} \pm SE$; 7.2 ± 3.3), while S1, S2 and S4 demonstrated considerably lower germination rates in comparison. The lowest germination was at S1 (3.1 ± 2.9) (Fig. 7).

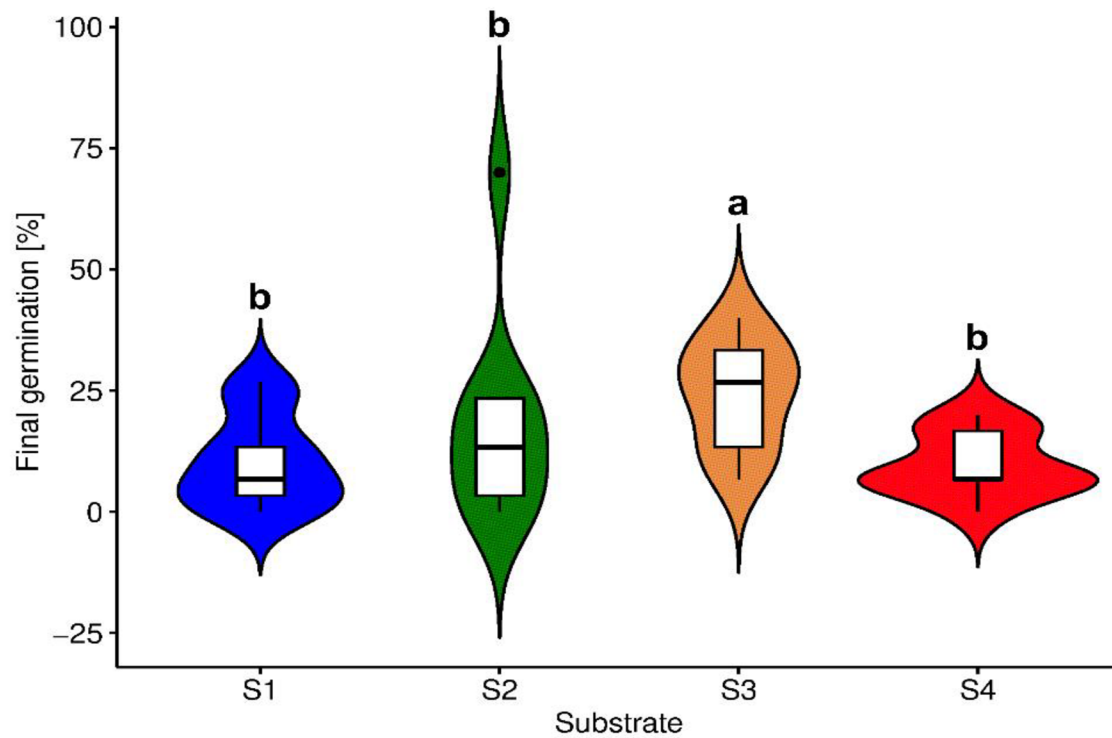


Fig. 7 Final germination of *F. japonica* seeds of EXP3. Four different substrate types (S1: soil taken from beneath under the maternal plants, S2: soil taken from beneath under the maternal plants covered with *F. japonica* leaves, S3: universal garden soil, S4: sand) were tested. Kernel density plot (distribution of observations in a dataset): black lines = means of final germinated seeds, boxes = the range between the first (lower) quartile (Q1) and the third (upper) quartile (Q3), whiskers = min. – max.; different letters indicate a statistically significant difference ($P < 0.05$) identified through the posterior comparisons' method.

6. DISCUSSION

Three main crucial influencing factors were studied to understand the germination pattern of *Fallopia japonica*. These factors include: the effects of temperature, soil substrate types and light intensity and the interactions between these factors on the germination rate of the taxa.

For a more comprehensive insight into the dynamics of spread and germination, experiments were carried out in three (3) designs: Experiment 1 was carried out in the laboratory, experiment 2 was carried out in the greenhouse while experiment 3 was a field-based design.

All these findings are linked and discussed in the following chapters.

6.1 Experiment 1

The objective of this experiment was to examine the germination patterns of *Fallopia japonica*, a taxon of knotweed, using several experimental designs. Emphasis was placed on the effects of different substrate types. Experiment 1 investigated whether germination rates would be affected by variations in substrate composition using a climabox.

The study revealed no significant differences in the germination rates of the different substrate types (GLM; $z_{2,87} = 1.7$, $p > 0.05$), as can be seen in Fig. 3.

Interestingly, moderate germination rates were observed in natural soil devoid of knotweed leaves, indicating that knotweed seeds are adaptable to a variety of soil conditions even in the absence of knotweed leaf litter. Furthermore, the capacity of knotweed seeds to grow in natural soil devoid of knotweed leaves begs the question of what part leaf litter plays in the germination process (Jones, 2022).

Significant effects for managing invasive species and ecological restoration initiatives result from these findings, which shed light on the germination ecology of knotweed species. In all treatments, the germination rates were reasonably constant despite the diversity in substrate composition, including soil types and the presence of knotweed leaves.

The absence of observable variations shows that knotweed seeds are somewhat adaptable and able to adjust to various substrate conditions. According to Kadlecová *et al.* (2022), knotweed species may have a competitive advantage due to their versatility, which allows them to colonize a variety of habitats and endure in a range of environmental circumstances. It also calls into

question the effectiveness of manipulating the substrate as a tactic to prevent knotweed invasion in natural environments.

While the primary focus of experiment 1 was on substrate types, next experiments examined other variables like light intensity, and temperature regimes, that may affect knotweed germination. Knowing how these variables combine to affect germination patterns may help to clarify the ecological tactics used by knotweed species and guide the development of more focused control techniques.

6.2 Experiment 2

The objective of experiment 2 was to evaluate how temperature ranges and soil substrates affected *Fallopia japonica* seed germination. As in experiment 1, the results showed that soil substrates had no significant impact on final germination rates (GLM; $z_{2,30} = 3.3$, $p > 0.05$). Germination rates were constant in all treatments, regardless of the types of substrates used, such as sand, soil from beneath maternal plants, soil covered with *F. japonica* leaves, and universal garden soil (see Fig. 4).

Given that all substrate types showed effective germination, it appears that *F. japonica* seeds are very adaptable to a variety of soil conditions (Engler *et al.* 2011). The fact that there was a delay in germination time is significant, especially in substrates S4 (sand) and S2 (soil covered in *F. japonica* leaves), where most seeds germinated six (6) days later than in the other substrates.

Furthermore, temperature alone had no observable effect on seed germination rates (GLM; $z_{2,30} = 0.141$, $p = 0.888$). As can be shown in fig. 5 and table 4, mean germination rates were largely constant throughout all temperature ranges, even though changes in greenhouse temperatures fell between 8-18°C, divided into three ranges (8-10°C, 10-12°C, and 12-18°C).

Given that temperature had little impact on germination, *F. japonica* seeds might be somewhat temperature tolerant, allowing for effective germination in a variety of greenhouse-typical temperature ranges. This is quite like a study conducted by Engler *et al.* (2011) which stated that soil temperature had to be between 15-25°C.

The results highlight the susceptibility of knotweed germination to temperature variations and point to a preference for mild temperatures.

Additionally, it was discovered that the relationship between soil type and temperature and seed germination was statistically insignificant. This suggests that the results of *F. japonica* seed

germination were not considerably impacted by the combined effects of temperature and soil substrates.

Finally, experiment 2 showed that temperature ranges and differences in soil substrates, alone or in combination, have no effect on the germination rates of *F. japonica* seeds. These findings highlight the resilience of *F. japonica* seeds to a variety of environmental situations and advance our understanding of the ecological tactics used by these seeds (Kolar & Lodge 2001). To further understand the ecological mechanisms driving knotweed invasion, future research could examine additional parameters like light intensity, soil moisture levels, and competition with other plant species that may influence germination dynamics.

6.3 Experiment 3

Experiment 3 investigated the effects of light intensity and substrate types on the germination of *Fallopia japonica* seeds. The results revealed distinct patterns in germination rates in response to varying light conditions and substrate types.

According to the results, germination rates were considerably lower in full sunlight (L3), comparatively lower in partial shade (L2), and highest in shade (L1). With knotweed species showing stronger seedling emergence in partially lighted settings as opposed to direct sunlight, this pattern shows a favourable link between light intensity and germination rates. Additional statistical analysis (GLM; $z_{2,30} = -4.041$, $p < 0.001$) confirmed the substantial effect of light intensity on seed germination. This result contradicts previous research by Shaw (2013), which suggested that *Fallopia japonica* requires relatively high light levels.

According to Engler *et al.* (2011), knotweed taxa exhibit photoblastic germination features, wherein seeds prefer darkened circumstances. This is highlighted by the differential germination rates under different light levels. This biological adaptation may provide knotweed species a competitive edge in shaded habitats, enabling them to flourish and multiply in partially shaded areas or beneath tree canopies.

Unlike experiments 1 and 2, experiment 3's substrate type significantly affected the *F. japonica* seeds' ultimate germination (GLM; $z_{2,30} = 1.600$, $p < 0.01$). Sand (S4) and soil extracted from beneath mother plants (S1 and S2) showed significantly lower germination rates than universal garden soil (S3), which had the greatest germination rate among the substrates examined.

The observed differences in germination rates between substrate types highlight how crucial substrate composition is in determining how seedlings emerge. When compared to other substrates, universal garden soil seems to offer the best circumstances for seed germination because of its nutrient-rich and well-aerated qualities. On the other hand, poorer germination may have resulted from soils removed from beneath mother plants because they may have held inhibitory substances or lacked vital nutrients for seedling establishment (Šerá, 2011).

The germination rates in full sunlight were notably lower, even though the experimental conditions typically matched theoretical expectations. This surprising finding raises questions about possible relationships between light and other environmental elements that require more research.

The results of experiment 3 demonstrate how important it is to take environmental variables like substrate type and light intensity into account when attempting to understand the germination ecology of knotweed species. These observations can help direct efforts to restore natural ecosystems and to manage invasive species.

The results show the ecological subtleties of knotweed germination, offering insightful information for managing invasive species and emphasizing the significance of considering a variety of environmental conditions when analysing seedling emergence in relation to knotweed invasion.

The study's results on the ecology of knotweed germination (*Fallopia japonica*) are consistent with and add to the body of knowledge already available on invasive plant species and the dynamics of seed germination. Numerous research endeavours have examined the variables impacting the germination patterns of knotweed species, providing insights into their ecological tactics and potential for invasion.

These results support earlier studies by showing a positive relationship between light intensity and knotweed species' rates of germination. For instance, knotweed seeds showed higher rates of germination in shaded settings than in full sunlight, suggesting a preference for partially lit environments. Similarly, Gardner *et al.* (2001) found that plant species had higher seedling emergence in shady and understory areas of forests, emphasizing the advantageous adaptation of photoblastic germination features.

The significant influence of substrate types on seed germination that our research revealed is consistent with results from other studies. Variations in soil conditions were found to affect

knotweed seed germination success in a study by Madalin *et al.* (2012), with nutrient-rich soils supporting higher germination rates.

In summary, although the experiment's results did not reveal a significant effect of substrate types on knotweed germination rates, they do add to our knowledge of the ecological factors that drive knotweed invasion and highlight the necessity of comprehensive management approaches that consider various environmental factors.

6.4 Implications and Significance of Findings

This study's observations on the germination of knotweed seeds under various experimental settings have significance for managing invasive species, restoring ecosystems, and understanding the ecology of plant invasions.

6.4.1 Invasive Species Management

It is essential to understand the ecology of knotweed germination to create management plans that work. According to the findings, knotweed seeds may adjust to a range of environmental factors, such as substrate type and light intensity. Because of their ability to adapt, knotweed species may be more successful in spreading in a variety of ecosystems.

The environmental elements affecting seed germination should be considered in management initiatives aimed at stopping knotweed invasion. These findings, for instance, imply that increasing light availability may prevent seed germination and the establishment of seedlings, suggesting the possible effectiveness of no shading or canopy cover in preventing knotweed growth.

6.4.2 Ecological Restoration

The study's findings can guide ecological restoration strategies meant to lessen the effects of knotweed invasion on native plant populations. Restoration practitioners can encourage the regeneration of native vegetation by using focused restoration techniques (Stella *et al.* 2021) by knowing the environmental variables that are conducive to knotweed germination.

In areas overrun by knotweed species, revegetation initiatives can, for example, concentrate on increasing light availability and although no significant influence on soil substrate,

altering soil substrates to make circumstances less conducive to knotweed germination and establishment such as reducing or eliminating soil moisture. Restoration initiatives may also benefit from the addition of native plant species that compete with knotweed to inhibit knotweed growth and promote ecosystem recovery.

6.4.3 Plant Invasion Ecology

This research advances an understanding of the ecological methods used by invasive knotweed species, which benefits the larger field of plant invasion ecology. The relationship between germination rates and light intensity, and the effect of substrate types on seedling emergence provide information about the ecological requirements and adaptive characteristics of knotweed species during invasion.

To completely understand the mechanisms underlying knotweed invasion, more investigation is required into the interactions between various environmental conditions and knotweed germination and mainly seedling establishment. Field research, modelling techniques, and long-term monitoring [Chenggang & Allison, 2018] studies can all be combined in future studies to give a thorough understanding of the intricate relationships invasive plants have with their habitats.

6.5 Study Limitations

Although the study offers insightful information about the ecology of knotweed germination, there are several limitations to be aware of that could impact how our findings are interpreted and applied in broader contexts.

Because the study was carried out in a controlled greenhouse, it's possible that the intricate environmental conditions knotweed seeds encounter in natural habitats were not entirely replicated. In field settings, the dynamics of seed germination may be affected differentially by variations in variables such as soil moisture, microbial populations, and competition with other plant species.

Particularly studied was the *Fallopia japonica*, sometimes called Japanese knotweed. Although these species are important invasive plants in many areas, the results might not apply to other species of knotweed or to other places where environmental factors might differ. The seeds

had a hybrid origin in the study, and the pollen donor is unknown. This fact could also affect the results; however, the germination rate was high, and the seeds showed no chromosomal or other genotype limitations (but see Bailey *et al.*, 2009).

The study may not have had a large enough sample size or enough replication of experimental units, which could have compromised the validity and dependability of our findings. The statistical power and validity of the conclusions would be improved by larger sample sizes and more replication [McNair *et al.* 2012].

The study, which focused mostly on seed germination rates, was carried out over a brief period. On the other hand, certain knotweed species are long-lived perennials with intricate life cycles that include methods for dispersal and vegetative reproduction. Long-term monitoring programs should be considered in future study to evaluate the permanent and temporal dispersion of knotweed populations [Mandak *et al.* 2004].

The interactions with biotic elements that could affect the dynamics of knotweed seed germination, such as herbivory, mutualistic relationships, or allelopathic effects [Madalin *et al.* 2012], were not specifically considered in this study. Future studies on knotweed invasion ecology may be able to gain a deeper understanding by including these biotic interactions. Future studies should examine other variables including soil pH, moisture content, and the presence of allelopathic substances that may interact with light intensity and substrate types to affect germination dynamics. Researchers can create more effective plans for preventing the spread of invasive knotweed species and encouraging the reestablishment of native plant communities by clarifying the intricate relationships that exist between environmental conditions and seed germination.

Studies that address these shortcomings will be able to control invasive knotweed species in natural habitats more effectively and advance our understanding of the ecology of knotweed germination.

7. CONCLUSION

The germination ecology of knotweed species, especially *Fallopia japonica*, has been better understood because of this research. Key patterns and trends in knotweed seed germination dynamics have been established through controlled experiments examining the influence of environmental conditions such as light intensity, substrate kinds, and temperature ranges.

Knotweed seeds prefer shadow or partial shade to full sunshine, and they germinate more quickly in partially lighted environments. This preference toward shady areas points to a photoblastic germination response, which could help knotweed establish itself in a variety of habitats.

While some experiments (1 & 2) showed that substrate types had no significant effect on germination rates, whereas another experiment (3) showed that soil composition had a major effect on seedling emergence. When compared to soils collected from beneath maternal plants or sandy substrates, nutrient-rich soils—like universal garden soil—supported higher germination rates.

In this study, temperature had no significant impact on the germination rates of knotweed seeds. But more research that examines how temperature interacts with other environmental factors could reveal more details about the ecology of knotweed germination.

The management of invasive species, ecological restoration, and general ecological understanding are all significantly impacted by these findings. It helps to design targeted management measures to limit knotweed invasion and promote native plant community restoration by clarifying the environmental parameters influencing knotweed germination dynamics. Furthermore, these findings broaden our knowledge of the ecological strategies used by invasive plants and how they interact with native biodiversity.

Firstly, the study highlights the environmental variables—such as temperature ranges, substrate types, and light intensity—that affect the nature of knotweed seed germination. It sheds light on the ecological strategies used by knotweed species to colonize a variety of habitats and endure in a range of environmental circumstances by identifying these important factors.

In addition, the outcomes have applications in the field of managing invasive species. It helps to design focused management measures to minimize the ecological implications of knotweed invasion and control it by identifying the circumstances that favour knotweed seed

germination. These tactics could involve changing the habitat, applying herbicides selectively, and working on restoration projects that support the diversity of native plants.

Furthermore, this study advances the ecological knowledge of the processes of plant invasion. We can better understand the ecological mechanisms behind the spread of invasive species and get insight into managing other invasive plant species by examining the germination ecology of knotweed.

To sum up, this research makes a substantial addition to ecology's theoretical and applied fields. It contributes to the greater understanding of plant invasion dynamics in natural ecosystems and supports evidence-based management methods for reducing invasive species by filling in important information gaps in the ecology of knotweed germination.

In summary, the study's results show that temperature ranges, substrate types, and light intensity all have a big impact on the nature of knotweed seed germination. To be more precise, the germination of knotweed seeds grows faster in shady areas and nutrient-rich soils; germination is not greatly impacted by temperature alone. These findings improve our understanding of the ecology of knotweed germination and have significance for controlling invasive species and ecological restoration projects.

REFERENCES

- Aguilera, A. G., Alpert, P., Dukes, J. S., & Harrington, R. (2010). Impacts of the invasive plant *Fallopia japonica* (Houtt.) on plant communities and ecosystem processes. *Biological Invasions*, 12, 1243-1252.
- Alpert, P. (2006). The advantages and disadvantages of being introduced. *Biological Invasions*, 8, 1523-1534.
- Anderson, H. (2012). Invasive Japanese knotweed (*Fallopia japonica* (Houtt.)) best management practices in Ontario. Ontario Invasive Plant Council: Peterborough, ON, USA.
- Bailey, J.P., Bímová, K., Mandák, B., 2009. Asexual spread versus sexual reproduction and evolution in Japanese Knotweed s.l. sets the stage for the “battle of the Clones.” *Biol. Invasions* 11, 1189–1203. <https://doi.org/10.1007/s10530-008-9381-4>
- Bailey, J.P., Stace, C.A., 1992. Chromosome number, morphology, pairing, and DNA values of species and hybrids in the genus *Fallopia* (Polygonaceae). *Plant Syst. Evol.* 180, 29–52. <https://doi.org/10.1007/BF00940396>.
- Berling, D. J., Bailey, J. P., & Conolly, A. P. (1994). *Fallopia japonica* (Houtt.) ronse decaene. *Journal of Ecology*, 82(4), 959-979.
- Clements, D. R., Larsen, T., & Grenz, J. (2016). Knotweed management strategies in North America with the advent of widespread hybrid Bohemian knotweed, regional differences, and the potential for biocontrol via the psyllid *Aphalara itadori* Shinji. *Invasive Plant Science and Management*, 9(1), 60-70.
- Cuthbert, R. N., Bartlett, A. C., Turbelin, A. J., Haubrock, P. J., Diagne, C., Pattison, Z., ... & Catford, J. A. (2021). Economic costs of biological invasions in the United Kingdom. *NeoBiota*, 67, 299-328.
- Dassonville, N., Guillaumaud, N., Piola, F., Meerts, P., & Poly, F. (2011). Niche construction by the invasive Asian knotweeds (species complex *Fallopia*): impact on activity, abundance and community structure of denitrifiers and nitrifiers. *Biological Invasions*, 13, 1115-1133.
- Dassonville, N., Vanderhoeven, S., Gruber, W., & Meerts, P. (2007). Invasion by *Fallopia japonica* increases topsoil mineral nutrient concentrations. *Ecoscience*, 14(2), 230-240.

- Delbart, E., Mahy, G., Weickmans, B., Henriët, F., Crémer, S., Pieret, N., ... & Monty, A. (2012). Can land managers control Japanese knotweed? Lessons from control tests in Belgium. *Environmental management*, 50, 1089-1097.
- Desjardins SD, Bailey JP, Zhang B, Zhao K, Schwarzacher T (2023) New insights into the phylogenetic relationships of Japanese knotweed (*Reynoutria japonica*) and allied taxa in subtribe Reynoutriinae (Polygonaceae). *PhytoKeys* 220: 83–108. <https://doi.org/10.3897/phytokeys.220.96922>
- Engler, J., Abt, K., & Buhk, C. (2011). Seed characteristics and germination limitations in the highly invasive *Fallopia japonica* s.l. (Polygonaceae). *Ecological Research*, 26, 555-562.
- Forman, J., & Kesseli, R. V. (2003). Sexual reproduction in the invasive species *Fallopia japonica* (Polygonaceae). *American journal of botany*, 90(4), 586-592.
- Gillies, S., Clements, D. R., & Grenz, J. (2016). Knotweed (*Fallopia* spp.) invasion of North America utilizes hybridization, epigenetics, seed dispersal (unexpectedly), and an arsenal of physiological tactics. *Invasive Plant Science and Management*, 9(1), 71-80.
- Hocking, S., Toop, T., Jones, D., Graham, I., & Eastwood, D. (2023). Assessing the relative impacts and economic costs of Japanese knotweed management methods. *Scientific Reports*, 13(1), 3872.
- Jones, V. (2022). Understanding plant-soil-management interactions of *Reynoutria* spp (knotweeds) to inform ecological restoration (Doctoral dissertation, University of British Columbia).
- Kadlecová, M (2024) *Population structure of invasive Fallopia taxa in the secondary distribution range* [Unpublished doctoral dissertation]. Czech University of Life Sciences, Prague.
- Kadlecová, M., Vojík, M., Kutlvašr, J. & Berchová Bímová, K. (2022) Time to kill the beast - Importance of taxa, concentration, and timing during application of glyphosate to knotweeds. *Weed Res.*, 62(1), 215– 223
- Kolar, C. S., & Lodge, D. M. (2001). Progress in invasion biology: predicting invaders. *Trends in ecology & evolution*, 16(4), 199-204.
- Kovářová, M., Bartůňková, K., Frantík, T., Koblihová, H., Prchalová, K., & Vosátka, M. (2010). Factors influencing the production of stilbenes by the knotweed, *Reynoutria bohemica*. *BMC Plant Biology*, 10(1), 1-16.

- Lavoie, C. (2017). The impact of invasive knotweed species (*Reynoutria* spp.) on the environment: review and research perspectives. *Biological Invasions*, 19(8), 2319-2337.
- Mandák, B., Pyšek, P., Lysák, M., Suda, J., Krahulcová, A., Bímová, K., 2003. Variation in DNA-ploidy levels of *Reynoutria* taxa in the Czech Republic. *Ann. Bot.* 92, 265–272. <https://doi.org/10.1093/aob/mcg141>
- Martin, F. M., Dommanget, F., Lavallée, F., & Evette, A. (2020). Clonal growth strategies of *Reynoutria japonica* in response to light, shade, and mowing, and perspectives for management. *NeoBiota*, 56, 89-110.
- McHugh, J. M., & West Haven, V. T. (2006). A review of literature and field practices focused on the management and control of invasive knotweed. The Nature Conservancy: West Haven, CT, USA.
- McNair, J. N., Sunkara, A., & Frobish, D. (2012). How to analyse seed germination data using statistical time-to-event analysis: non-parametric and semi-parametric methods. *Seed Science Research*, 22(2), 77-95.
- Parepa, M., Fischer, M., Krebs, C., & Bossdorf, O. (2014). Hybridization increases invasive knotweed success. *Evolutionary applications*, 7(3), 413-420.
- Patterson, D. T., Longstreth, D. J., & Peet, M. M. (1977). Photosynthetic adaptation to light intensity in Sakhalin knotweed (*Polygonum sachalinense*). *Weed Science*, 25(4), 319-323.
- Saad, L., Tiébré, M.S., Hardy, O.J., Mahy, G., Vanderhoeven, S., 2011. Patterns of hybridization and hybrid survival in the invasive alien *Fallopia* complex (Polygonaceae). *Plant Ecol. Evol.* 144, 12–18. <https://doi.org/10.5091/plecevo.2011.444>
- Šerá, B. (2011) Effects of Soil Substrate Contaminated by Knotweed Leaves on Seed Development. *Polish Journal of Environmental Studies*, 21(3), 713-717.
- Shaw, D. (2013). *Fallopia japonica* (Japanese knotweed). *Invasive Species Compendium*, (23875).
- Siemens, T. J., & Blossey, B. (2007). An evaluation of mechanisms preventing growth and survival of two native species in invasive Bohemian knotweed (*Fallopia* × *bohemica*, Polygonaceae). *American Journal of Botany*, 94(5), 776-783.
- Tiébré, M.S., Bizoux, J.P., Hardy, O.J., Bailey, J.P., Mahy, G., 2007. Hybridization and morphogenetic variation in the invasive alien *Fallopia* (Polygonaceae) complex in Belgium. *Am. J. Bot.* 94, 1900–1910. <https://doi.org/10.3732/ajb.94.11.1900>

- R Development Core Team (2020). R: A language and environment for statistical computing, R Foundation for Statistical Computing.
- TIBCO (2017). TIBCO Statistica. TIBCO Stat. ©Computer Program.
- Vašátko, T., 2023. Elektronický digitální povodňový portál [Electronic digital flood portal] [WWW Document]. URL <https://www.edpp.cz/>
- Vaseková, B., Majorošová, M., Belčáková, I., & Slobodník, B. (2022). Distribution and management of *Fallopia japonica* in riparian biotopes in Slovakia and Austria. *Biosystems Diversity*, 30(4), 442-452.