Two Shades of Grey: Effect of Temperature on Seed Germination of the Escaping Ornamental Species *Lychnis Coronaria* and *Stachys Byzantina*

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Abstract

This paper describes germination and cytological variability in two popular ornamental and potentially invasive species, lamb's ear *Stachys byzantina* and rose campion *Lychnis coronaria*. Both xerophytic species have the potential to invade natural habitats across Europe and create viable naturalised populations, with subsequent impacts on native vegetation. To assess the species' invasiveness, seeds were collected from naturalised populations and germination rate recorded under different temperature regimes. Flow cytometry, used to record cytological variability, indicated that all populations of both species were cytologically homogeneous. Germination success, a key spreading factor in both species, was significantly influenced by temperature, with final germination of *L. coronaria* being extremely high at temperatures > 15/10 °C (98.5%) and extremely low at temperatures < 10/5 °C (2.9%). In comparison, final germination in *S. byzantina* highest at 22/15 °C (55.6%), reducing to 40.3% at 15/10 °C and just 0.3% at temperatures < 10/5 °C. No significant differences in germination rate were observed between escaping and non-escaping populations, though there were differences between particular populations. Our results indicate germination temperature limits between species consistent with sizes of primary distribution and distance between primary and secondary distribution borders. However, the observed germination rates allow for successful generative reproduction of both species over their secondary distribution areas, suggesting that these species are likely to become invasive species of European grasslands soon.

Introduction

Horticulture has been an integral part of human society for centuries (Relf and Dorn 1995; Zhou 1995; Kendle and Stoneham 2014). Recently, however, the increasing influence of horticulture has made it one of the major sources of alien plant species (Dehnen-Schmutz and Touza 2008; Klonner et al. 2017; van Kleunen et al. 2018; Vojík et al. 2020). These not only contribute significantly to regional floral communities (Köppler et al. 2014; Lososová et al. 2015; Pergl et al. 2016), they potentially threaten biodiversity and natural ecosystems, leading to homogenisation of the Earth's biota (Vilà et al. 2011; Price et al. 2018; Morri et al. 2019; Pyšek et al. 2020). The control and eradication of such 'invasive species' is demanding and often unfeasible (Ewel and Putz 2004; Downey et al. 2010) and, as such, it is crucial to prevent the spread of potentially invasive species before they get a hold (van Kleunen et al. 2007). Whether a species becomes invasive depends not only on the species' ecology but also the physical characteristics of the invaded habitat and the prevalent environmental conditions (Pyšek et al. 2012a). One of the most important ecological traits to assess when investigating the potential for invasion in early spreading species are their reproduction traits, i.e. whether they can be a 'master of all traits' or if they have predominant characteristics leading to their success (Pyšek 2007; Pyšek et al. 2009). Species reproducing by seed tend to be associated with traits such as light availability and temperature regime, with low temperatures limiting germination and short days influencing seedling establishment (Pepe et al. 2020). However, species that are capable of germinating at lower temperatures, with earlier timing of

germination and rapid seedling growth, will have a competitive advantage over other species (Baskin and Baskin 1998).

Recent studies focused on reproduction traits have often concentrated on variability within the genetic structure of plant populations (e.g. Saad et al. 2011). Introduced populations with high genetic or cytological diversity are likely to adapt rapidly to novel environments and, as such, it represents an important factor influencing their survival capacity (Puillandre et al. 2008; Estoup et al. 2016). If the introduced population is small, however, it may have a low level of variability, creating a bottleneck effect that could limit its invasion success (Puillandre et al. 2008). Nevertheless, some introduced species have become invasive with a low level of genetic/cytological diversity due to a so-called 'genetic paradox of invasion' (Hassan et al. 2003; Dlugosch and Parker 2008b; Estoup et al. 2016), whereby genome size and polyploidy in newly spreading alien species may be the first indication of increasing variability or ongoing evolutionary processes, which could then support invasion (Meyerson et al. 2020; Wan et al. 2020).

Other characteristics aside from reproduction traits have also been identified as significantly affecting invasion/naturalisation probability in ornamental taxa (Pyšek et al. 2019). For example, the horticultural success of certain species is mainly related to their charisma (e.g. colour, shape and size of bloom or time of flowering), which affects not only global public perception but also introduction by humans and resistance to management following their escape (McNeely 2001; Shackleton et al. 2019; Jarić et al. 2020). The invasion potential of ornamental species following introduction will then be linked to their ability to grow under low maintenance (e.g. abandoned sites) and their adaptability to local conditions (e.g. temperature, humidity), such characteristics also giving the species a greater ability to escape from horticulture (Williams et al. 2010; Flory et al. 2011; Vojík et al. 2020; Kutlvašr et al. 2020). There have also been several recent cases of ornamentals spontaneously spreading due to global warming, highlighting their potential to reflect climate change (Dullinger et al. 2017; Haeuser et al. 2018).

In this study, we describe germination and cytological variability in two popular ornamental and potentially invasive species, lamb's ear *Stachys byzantina* and rose campion *Lychnis coronaria*, both of which are naturalised in the Czech Republic and have spread into thermophilous human-made habitats. Both species are popular ornamentals worldwide due to their grey-felted leaves and easy maintenance and have been planted in a wide range of habitats, including public parks and private gardens and courtyards (Dimke et al. 2008; Wade 2010; Cullen et al. 2011; Glawe and Koike 2018). Records of both species escaping from cultivation have increased in recent years, with such escapes going on to form viable populations in both artificial and semi-natural habitats (Barošová and Baroš 2009; Kutlvašr et al. 2019; Vojík et al. 2020), including narrow and broad-leaved dry grasslands and acidophilous dry grasslands. Consequently, these species can be considered as newly emerging alien species that could easily become invasive. Moreover, in the case of *S. byzantina*, hybridisation may be possible with an endangered native species, *Stachys germanica* – *Endangered* category (C2b) in the IUCN Red List of endangered species (see Grulich 2017), recently recorded in the Czech Republic (Danihelka in prep.). Hybridisation, alongside effective generative reproduction, could significantly accelerate the invasive

success of *S. byzantina* by producing high performance genotypes that could colonise even unfavourable sites rapidly (see Buhk and Thielsch 2015).

Though both species originate in the Middle East, they differ in their geographical range of primary distribution (POWO 2019). It has been shown repeatedly that the probability of naturalisation success is affected by the size of a species' geographical range and its habitat range niche (e.g. Rejmánek 1996; Pyšek et al. 2009). With a native distribution centred on the Mediterranean and Central Asia, *L. coronaria* has a sizeable primary distribution with a broad ecological amplitude (Hejný 1997; CABI 2019; Chytrý et al. 2021) unlike *S. byzantina*, which is an endemic species to Iran (Asnaashari et al. 2010). While the basic descriptive characteristics regarding biology and ecology are known for both species (see Hejný 1997; Chytrý et al. 2021), details on population variability and their ability to spread remain largely unknown.

Study aims

In order to identify reasons for the recent spread of both species, we undertook a series of tests to assess cytological variability and *in vitro* seed germination potential. In doing so, we asked a series of questions: (1) How homogenous is their cytological variability? (2) Is the germination rate of either species affected by temperatures? (3) Does germination vary among populations and are there differences between escaping and non-escaping populations? (4) Is there a difference in the generative reproduction temperature limit between species with different ecological niches, size of primary distribution and distance between primary and secondary distribution borders?

Material And Methods

Study species

Stachys byzantina C. Koch (*Lamiaceae*) is a perennial, long-felted, 20–80 cm tall herb. It is diploid ($2n = 30$; Hejný 1997; Lindqvist and Albert 2017; Wild et al. 2019). Originally an endemic Iranian mountain species (Asnaashari et al. 2010), it has widened its native area to include north Turkey and the Crimean Peninsula (POWO 2019). It has been introduced to Central and Western Europe, North America, Canada and New Zealand (POWO 2019). Germination rates for *S. byzantina* are not available; however, generative reproduction has been observed in the field (Vojík et al. 2020).

Lychnis coronaria (L.) Desr. (*Caryophyllaceae*) is a short-lived, white felted, perennial plant, generally grown as a biennial or annual, 30–60 (–100) cm tall (Jiang et al. 2016). It is diploid ($2n = 24$; Hejný 1997; Jeelani et al. 2011; Zonneveld 2019). It originates from the Mediterranean, Middle East and the whole of Central Asia. The northern boundary of the species area reaches southern Slovakia. It is non-native in Central and Western Europe and North and South America (Brazil, Chile; Hejný 1997; POWO 2019). The germination rate of *L. coronaria* is 100% under optimal laboratory conditions, with seedling establishment reaching almost 60% (Moravcová et al. 2010).

Population genome size variability

To assess genome size homogeneity, the DNA weight per nucleus (genome size; expressed in picograms [pg]) was determined for each study population (see Online Resource table) using flow cytometry, based on a two-step procedure using Otto I and II buffers (Otto 1990). As its genome size (2C DNA = 3.38 pg) is close to that of the species studied, the common daisy *Bellis perennis* was used as an internal reference standard (Schönswetter et al. 2007). In each case, 1 cm² of fresh leaf tissue from the study species and from *B. perennis* were macerated together with a sharp blade and placed in a Petri dish containing 0.1 ml of ice-cold Otto I buffer (0.1 M citric acid monohydrate, 0.5% v/v Tween 20) for approx. 90 s, after which the suspension was filtered through a 42 µm nylon mesh. Nuclei within the filtered suspension were then stained with 1 ml of Otto II buffer (0.4M Na₂HPO₄ · 12H₂O) supplemented with 1 ml of DAPI stock solution (DAPI 10 mg dissolved in 100 ml H₂O) + 50 µl β-mercaptoethanol (2 µL/mL). Each sample was then incubated at room temperature for 10 min and analysed using a Sysmex-Partec CyFlow SL equipped with a green solid-state laser flow cytometer (532 nm, 100 mW output power; Sysmex Partec GmbH, Görlitz, Germany).

Seed collection and germination experiments

Two germination experiments were undertaken, the first assessing the effect of temperature regime on seed germination, and the second assessing differences in germination rate between escaping and non-escaping populations.

To evaluate the effects of temperature regime on seed germination, we collected seeds from fully mature plants of four *L. coronaria* populations and four *S. byzantina* populations from the Czech Republic at the end of the 2017 and 2018 vegetation seasons (see Online Resource table). The seeds were then incubated in Petri dishes on three-layers of filter paper dampened with distilled water. Seeds were kept under a simulated daily light regime consisting of 14 hours light and 10 hours dark with four different temperature regimes, 22/15°C, 15/10°C, 10/5°C and 5/3°C. The 5/3°C regime was only used for *L. coronaria* as a previous study has shown that *S. byzantina* seeds stop germinating at 10/5°C. All germinated seeds were counted and recorded at two-day intervals for 30 days.

To compare differences in germination rate between escaping and non-escaping populations, seeds were collected from mother plants (not escaping daughter plants) growing in garden bed. Eight *S. byzantina* populations were sampled, four escaping and four non-escaping, and six *L. coronaria* populations, four escaping and two non-escaping (see Online Resource table). Escaping populations were characterised by intensive spontaneous spreading into surrounding semi-natural grasslands, while non-escaping populations showed no sign of spreading, despite suitable habitats for spontaneous spreading being available.

Seeds from three distant populations were also collected – one from the primary distribution area of *L. coronaria* (LC7; Istanbul, Turkey), one from the secondary distribution area of *S. byzantina* (SB9; Dublin,

Ireland), and one from the primary distribution area of *S. byzantina* (SB10; Tehran, Iran) for basic cytological testing of genome size (see Online Resource table).

Before setting up the experiments, the seeds were dark-stratified at -16°C for six weeks to break dormancy, then surface-disinfected in 70% (v/v) ethanol for 1 min and immersed in 50% (v/v) bleach solution (< 5% sodium hypochlorite; Ślesak et al. 2017).

Three germination traits were measured: i) final germination, calculated as total number of germinated seeds at the end of the germination period, ii) differences in germination over time, calculated as the number of germinated seeds in each time interval, and iii) germination delay (hereinafter GD), calculated as the number of days until first germination recorded (Cerabolini et al. 2004; Pepe et al. 2020). Each population sample consisted of 30 seeds in three replicates for each treatment.

Differences in germination rate between escaping and non-escaping populations were tested using seeds grown in the 22/15°C regime, as this was shown to be optimal for both species during the previous experiment of this study (see above).

Data analysis

Differences in seed germination between populations and between temperature regimes were tested by comparing the observed 'final germination' counts using general linear models (GLM) with Poisson distribution. Full models were simplified using posterior comparisons and the most plausible models selected based on the Akaike information criterion (Akaike 1978; Crawley 2012), using backward selection. Differences in 'germination over time' were tested using repeated measures ANOVA (RM ANOVA), where the number of germinated seeds at a particular time was used as the response variable (Lepš and Šmilauer 2016), while observations taken over the 30 days, temperature and population were used as predictors. The effects of experimental temperatures on seed germination were assessed using a multivariate test based on Wilks' Λ statistic value, used for within-subject effects (Mardia et al. 1979).

GLMs were used to assess differences in germination rate between escaping and non-escaping populations of both species, using the number of germinated seeds as the response variable and population characteristic of escaping populations as the predictor (binomial distribution).

All analyses were performed using R software (R Development Core Team 2019), with differences considered significant at $P \leq 0.05$.

Results

Population genome size variability

Flow cytometric analysis confirmed genome size homogeneity in all populations of both species in their secondary area of distribution (Fig. 1), with *S. byzantina* populations ranging from 1.55 to 1.63 pg (2C-

values), and *L. coronaria* populations ranging from 6.40 to 6.58 pg (2C-values) (see Online Resource table).

Seed germination

Lychnis coronaria

Temperature had a highly significant impact on final germination in all populations tested ($F_{3,41} = 1766.99$, $P < 0.001$), with posteriori comparisons distinguishing two levels of significance, i.e. up to 10/5°C ($\bar{x} \pm \text{SE}$; 0.88 ± 1.83 ; SE: Standard Error of Mean) and $> 15/10^\circ\text{C}$ (29.54 ± 0.91) (average per Petri dish; Fig. 2). While germination at temperatures $< 10/5^\circ\text{C}$ was low, with final germination at 2.9% (21 of 720 seeds germinating), final germination at temperatures $> 15/10^\circ\text{C}$ was exceedingly high at 98.5% (709 of 720 seeds germinating).

While GD was significantly affected by temperature ($F_{3,41} = 93.53$, $P < 0.001$), population had no significant effect ($F_{3,41} = 0.41$, $P = 0.75$). Seeds germinated later at lower temperatures, with a GD of three days at 22/15°C, three days at 15/10°C, nineteen days at 10/5°C and no germination at 5/3°C. There was a non-significant difference in final germination between populations ($F_{3,41} = 0.97$, $P = 41.67$), the seed germination rate over the first twelve days differed significantly (RM ANOVA, $F_{126} = 9.0$, $P < 0.001$), primarily due to population LC2 having a lower germination rate at 22/15°C, though this difference later vanished (Fig. 3).

Stachys byzantina

As with *L. coronaria*, temperature had a highly significant impact on final germination in all populations tested ($F_{2,30} = 45.21$, $P < 0.001$). Likewise, germination was low at temperatures $< 10/5^\circ\text{C}$, with a final germination rate of 0.3% (1 of 360 seeds germinating), but increased with increasing temperature, with final germination at 15/10°C reaching 40.3% (145 of 360 seeds germinating) and the highest final germination being recorded at 22/15°C at 55.6% (200 of 360 seeds germinating) (Fig. 4).

There was also a significant difference in seeds germinating between populations ($F_{3,30} = 11.11$, $P < 0.001$), with no seeds (all populations) germinating at 10/5°C (apart from one seed from the SB3 population) and large differences in final germination among populations at higher temperatures, i.e. min. 6.7% (SB2) and max. 70.0% (SB4) at 15/10°C and min. 28.9% (SB3) and max. 74.4% (SB4) at 22/15°C. There were significant differences in seed germination between all populations and all temperatures over time (RM ANOVA, $F_{84} = 11.8$, $P < 0.001$), with the strongest effect observed at higher temperatures, i.e. 12.08 ± 8.21 at 15/10°C and 16.70 ± 5.93 at 22/15°C (average per Petri dish). Populations SB1 and SB4 both displayed a higher germination rate than the other populations, with almost all seeds germinating during the first ten days of the experiment (Fig. 5). GD was significantly affected by temperature ($F_{2,30} = 63.7002$, $P < 10^{-6}$), though again there was no significant effect of

population ($F_{3,30} = 1.67$, $P = 0.2$). In all populations, seeds germinated later at lower temperatures, with a GD of two days at 22/15°C, seven days at 15/10°C and just one seed germinating at 10/5°C.

Difference in germination rate between escaping and non-escaping populations

There was no significant difference in seed germination rate or average seed germination values between escaping and non-escaping populations of *L. coronaria* ($F_{1,16} = 0.29$, $P = 0.6$) or *S. byzantina* ($F_{1,22} = 0.003$, $P = 0.95$) (Fig. 6).

Discussion

Detailed studies of generative reproduction and population ecology, including studies of genetic and cytological variation, have proved to be promising tools providing important information on the determinants of species invasiveness (Moravcová et al. 2010; Suda et al. 2015; Pyšek et al. 2018). Standardised studies on the germination characteristics of plant taxa (Grime et al. 1981; Grime et al. 1988), for example, help clarify the capacity for spreading and the potential for establishment of new populations (Pyšek and Richardson 2007).

In the present study, we describe cytological variability and germination rate in two newly spreading species, *S. byzantina* and *L. coronaria*. The seeds of both species displayed optimal germination at temperatures between 15/10°C and 22/15°C, with *S. byzantina* closer to 22/15°C (Figs. 3 and 5). Hence both species, which have similar spreading potentials but different primary distribution areas, proved highly successful at germinating under temperature conditions typical of Central Europe (Iran, Ireland and Turkey were only sampled for testing cytological homogeneity). Differing environmental conditions are usually the main reason plants fail to survive or spread in their secondary distribution area (Richardson et al. 1994; Mack 1996; Richardson et al. 2000); thus, their ability to germinate successfully in this temperature range allowed them to overcome a major barrier between their primary and secondary distribution areas. Though *L. coronaria* was previously classified as non-invasive in the basic IAS screening study of Moravcová et al. (2010), the species has recently begun spreading, with multiple observations of the species escaping from plantations (Kutlvašr et al. 2019; Vojík et al. 2020). Similarly, *S. byzantina* was originally classified as casual in the study of Pyšek et al. (2012b), but has recently shown similar spreading behaviour as *L. coronaria* in the field (see Online Resource figure).

Germination, and timing of germination, depends heavily on a combination of factors, including light, temperature and dormancy (Hoyle et al. 2015), with external temperature and available light in particular regulating the enzymes directly involved in germination (Baskin et Baskin 1998). Consequently, these two key factors formed the basis of our study into the germination capabilities of *S. byzantina* and *L. coronaria*.

For *Lychnis coronaria*, a species with an extremely high germination rates at 22/15°C (almost 99% of seeds germinated; see Fig. 3), rapid germination within the first six days of the experiment, and a germination rate that does not differ between populations, our results were similar to those of Moravcová

et al. (2010). However, we disagree with their suggestion that this species does not display invasive behaviour. The species' high germination rate under conditions typical of its secondary distribution area and its rapid growth both increase its ability to invade new environments (Beerling and Perrins 1993; Gioria et al. 2016), and numerous recent recordings of localised spreading in the field (Vojík et al., in prep.) clearly indicate the high invasive potential of the species. An early and/or rapid germination strategy (Ross and Harper 1972; Gioria and Pyšek 2017) is especially typical for ornamental plants originating from arid or semi-arid environments. Indeed, the same strategy is found in a number of fast-growing invasive annuals, including Himalayan balsam *Impatiens glandulifera*, and some of the most invasive monocarpic perennials, including giant hogweed *Heracleum mantegazzianum* and Chilean rhubarb *Gunnera tinctoria* (Gioria and Osborne 2009; Gioria and Osborne 2013). The potential for *L. coronaria* to spread invasively is also supported by its high seed production, with a two-year-old plant releasing 19 000 seeds on average (counted on plants growing in experimental garden bed). Clearly, therefore, *L. coronaria* must be considered as a new and potentially dangerous invasive species in this country.

Stachys byzantina, given the species' primary distribution area and its thermophilicity, was expected to show highest germination at around 22/15°C, similar to other species of the *Lamiaceae* family with a similar primary distribution, e.g. basil *Ocimum basilicum*, which has a germination optimum of 20–30°C (Nassar et al. 2013; Elhaj 2015) or Egyptian sage *Salvia aegyptiaca*, which germinates optimally at 30°C (Gorai et al. 2011). Our results confirmed this expectation, with highest germination (55.6%) indeed occurring at 22/15°C, while almost no seeds germinated (0.3%) at temperatures < 10/5°C. Furthermore, two populations examined (SB 1, SB 4) clearly differed from each other – their germination rate at 22/15°C was 72.8% and at 15/10°C was 65.6% (Fig. 5), which is about 30% higher than the germination rate of the rest of populations. Both these localities were placed in urban sites, and urban environments and phenomena (e.g. urban heat and dry islands) are known to strongly influence species adaptation (Williams et al. 2015; McDonnell and Hahs 2015), with species changing their ecology and reproductive traits to become more invasive (Dubois and Cheptou 2017). Such phenomenon has been described in several highly invasive European species, including highly invasive common ragweed *Ambrosia artemisiifolia* (Gorton et al. 2018).

While the results of our experiment confirmed the expected characteristics of such thermophilic species (Baskin and Baskin 1988; Escudero et al. 1997), the final germination rate was relatively high and contradicts the expected reproduction limits typical for rare, often endemic, species (Pavlik et al. 1993; Kaye 1999), though these can vary between specific sites (e.g. with altitude) or with the age of the populations sampled (Giménez-Benavides et al. 2005). Until recently, it was believed that a small primary distribution area would not include many safe sites for sexual propagules (germination conditions), and that this would limit species from spreading in other part of the world (Massey and Whitson 1980). However, there are now numerous examples of rapid and heavy invasion by such species, e.g. *I. glandulifera* (Beerling and Perrins 1993), suggesting that a species' individual ecological characteristics and its ability to evolve may be all that's needed to produce invasive behaviour (Dlugosch and Parker 2008a).

We analysed also the cytological variability of both *S. byzantina* and *L. coronaria* to determine differences in ploidy levels among populations. We did not assess genetic diversity as it was expected to be high in both species due to their predominant generative reproduction mode (Crawley 1996). Nevertheless, genome size variability is a fast screening method for detecting changes in ploidy level or ongoing evolution that can affect seed germination and/or growth rate (Pegtel 1999; Schween et al. 2005; Münzbergová 2006). Polyploidy, for example, can cause differently sized seeds, changes in viability and changes in a species' ability to germinate under stressful conditions (Stevens et al. 2020). Such changes to genome size can significantly influence the ability of an alien species to spread and potentially become invasive (Suda et al. 2015; Pyšek et al. 2018). Our results indicated that both species were cytologically homogenous with no evidence of polyploidy, with all populations of either species having a similar species-specific genome size (*S. byzantina* 1.55–1.63 pg; *L. coronaria* 6.40–6.58 pg), levels similar to those given by Zonneveld (2019). Furthermore, reference samples taken from the species' primary area of distribution around Istanbul in Turkey (LC 7) and Tehran in Iran (SB10) showed the same ploidy levels as those from the secondary area (Online Resource table).

Comparison of escaping and non-escaping populations

Based on field observations (visual detection of escapes), we expected to see a difference in cytological variability and germination rate between escaping and non-escaping populations. Surprisingly, our results failed to show any significant difference in variability and germination rate for either species examined (see Fig. 6), suggesting that germination and genome size are not key factors for escape from plantings. However, spreading into surrounding habitats could be caused by changes in local site conditions, such as management or disturbance regime (Buckley et al. 2007). Both species have now been recorded escaping from urban habitats, especially parks and gardens (Hejný 1997; Barošová and Baroš 2009; Pergl et al. 2016; Kutlvašr et al. 2019; Vojík et al. 2020). While both species are currently classified as naturalised (*L. coronaria*) or casual (*S. byzantina*) (Pyšek et al. 2012b), they are likely to become problematic species as they spread into natural or semi-natural habitats, with rural semi-dry grasslands and semi-natural species-rich grasslands and steppes particularly at risk. *L. coronaria* is a particularly competitive species due to its ability to produce a huge number of viable seeds that can be spread in different ways (e.g. hydrochory, epizoochory), allowing it to spread into faraway habitats (Moravcová et al. 2010). *S. byzantina* can be spread successfully by either seed or vegetative dispersal. It is also likely that *S. byzantina* could erode the genome of an endangered native species, *S. germanica*, through hybridisation (see Introduction), process generally considered one of the most serious impacts of invasive species on native biota (Largiadèr 2008; Muhlfeld et al. 2014).

Overall, our results suggest that generative spreading of both *L. coronaria* and *S. byzantina* is directly influenced by environmental factors, such as mean and spring temperature, both of which are likely to increase due to global climate change (Hansen et al. 2006; Anderson et al. 2008). In addition, indirect phenomena such as dry and warm islands in urban habitats (Roth et al. 1989; Lokoshchenko 2017; Yang et al. 2017; Hao et al. 2018) may be increasing the likelihood that these species escape and spread

further. As such, both species may now pose a real threat to native species, particularly those in Central European mesic and xeric grasslands.

Declarations

Statements & Declarations

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Conflict of Interest

There are no potential conflicts with Ethical Standards and conflicts of interest (financial or non-financial). All financial supporting resources are listed.

Author Contribution

All persons entitled to authorship have been so named, and all authors have seen and agreed to the submitted version of the manuscript. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Animal Research

Not applicable.

Consent to Participate

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Figures

Figure 1

Flow cytometric histograms (nuclei stained with propidium iodide) for *Lychnis coronaria* and *Stachys byzantina*, with *Bellis perennis* used as an internal reference standard for both species (placed at 100 relative fluorescence for *L. coronaria* (a) and at 200 relative fluorescence for *S. byzantina* (b)). The G1 peak represents nuclei with DNA 2C, while the G2 peak represents nuclei with DNA 4C (G2 phases of the cell cycle with double the amount of DNA present)

Figure 2

Influence of temperature on final germination of *Lychnis coronaria*, showing two contrasting responses between treatment levels 5/3 °C and 10/5 °C vs. 15/10 °C and 22/15 °C. Squares = means of final germinated seeds, whiskers = min-max; different letters at the end of each treatment row indicate a statistically significant difference ($P < 0.05$)

Figure 3

Time course for *Lychnis coronaria* seed germination under different temperature regimes (a: 10/5 °C; b: 15/10 °C; c: 22/15 °C). The results for the 5/3 °C temperature regime are not shown as no seeds germinated, giving a final germination of 0%). LC1–LC4 represent particular localities of sampled populations (see Online Resource table)

Figure 4

Influence of temperature (10/5 °C, 15/10 °C, 22/15 °C) on final germination of *Stachys byzantina*. Squares = mean of germinated seeds, whiskers = min-max; different letters at the end of each treatment row indicate a statistically significant difference ($P < 0.05$)

Figure 5

Time courses of *Stachys byzantina* seed germination under different temperature regimes (a: 15/10 °C; b: 22/15 °C) (The results for the 10/5 °C temperature regime are not shown as only one seed germinated, giving a final germination of 0.3%). SB1–SB4 represent particular localities of sampled populations – see Online Resource table

Figure 6

Analysis of final seed germination between escaping and non-escaping populations of *Lychnis coronaria* (a) and *Stachys byzantina* (b). Squares = mean of germinated seeds, whiskers = min-max; the same letters at the end of the treatment row show statistically non-significant differences ($P > 0.05$)