Does Local Adaptation Facilitate the Success of Plant Invasions?: A Case Study on Buddleja Davidii

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Does local adaptation facilitate the success of plant invasions? – A case study on *Buddleja davidii*

Susan Ebeling

Does local adaptation facilitate the success of plant invasions?
A case study on *Buddleja davidii*

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Susan K. Ebeling

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Erstgutachter: Prof. Dr. Isabell Hensen

Zweitgutachter: Dr. Harald Auge

Drittgutachter: Prof. Dr. Ingo Kowarik

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Buddleja davidii **Franch.**
Sommerflieder

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General introduction

The worldwide exchange of species is considered as one of the greatest threats to global biodiversity (Sala *et al.* 2000). The number of species translocated by humans, either deliberately or by accident, has dramatically increased (Simberloff *et al.* 2005) as a result of the rapid expansion in international travel and trade (Elton 1958, Brandl *et al.* 2001). Thus, biological invasions may exceed natural dispersal processes in terms of dimension, speed, range and consequences on ecological, economic and social levels (Kowarik 2003). Biological invasions are defined as the overcoming of biogeographical barriers of species caused by humans and the subsequent spread beyond their native distribution area. In general the invasion process in the new range consists of three stages: introduction, naturalization and invasion per se (Lodge 1993, Richardson *et al.* 2000b). In this thesis, the term "invasive" is used to describe species that are not native to an ecosystem (i.e. nonindigenous), that reproduce consistently and sustain populations over many life cycles without direct intervention by humans. Depending on the region and environmental conditions, an invasive species can change an ecosystem and thereby alter its function (e.g. Gordon 1998), biodiversity (Richardson *et al.* 1996) and the regional gene pool (e.g. Ellstrand & Schierenbeck 2000, Vila *et al.* 2000). In addition, biological invasions may result in high economic costs. The cost of control of invasive species and the loss of native species in the United States amounts to $ 137 billion annually (Pimentel *et al.* 2000). In Germany the management of just 20 problematic species costs approximately € 167 million per year (BMU 2003).

The effects of invasive species on global biodiversity were specifically addressed in the Convention of Biodiversity, an international treaty, adopted in Rio de Janeiro, Brazil in 1992 (CBD2007). Governments from around the world have committed to prevent the introduction of invasive species and to control or eradicate them, if they threaten ecosystems, habitats or native species (BMU 1992, CBD2007). Consequently, a goal of prospective invasion biology should be to investigate mechanisms responsible for biological invasions with particular emphasis on how humans facilitate the invasive processes. Additionally, another aim should be the mitigation of the effects of already established species by prevention, eradication and control.

Mechanisms: Phenotypic plasticity and local adaptation

A central question in invasion biology has been identifying the traits, that predispose a species to be invasive or not (Richardson & Pyšek 2006). The features which should characterize some plant species as "ideal weeds" (Baker 1974) have been also applied to the "ideal invader." Successful invaders should have a wide ecological niche, a short life cycle, high growth rates and great longevity. Furthermore, they are self-compatible and able to reproduce vegetatively and sexually with high output of propagules or seeds that are easily dispersed. However, research has determined that there really is no consistency of life history and reproduction across all weeds or introduced plants (Williamson 1993, Williamson & Fitter 1996). A comparative review by Pyšek and Richardson (2007) revealed that height, vegetative growth, early and extended flowering and attractiveness to humans are those traits that are associated with invasiveness. Although these traits are obviously advantageous to an introduced species, their presence does not automatically imply the species will be a successful invader. It has rather been shown that the invasion process is stage- and habitat-specific (Pyšek & Richardson 2007). The tens rule (Williamson & Fitter 1996) predicts that 10% of all introduced species will escape to become casuals (i.e. species that reproduce but fail to sustain over a longer period) and that about 10% of the casuals become naturalized (i.e. successful reproduction and maintenance of population over a longer period). Only about 10% of the naturalized species will be able to become a problematic species (sensu Pyšek *et al.* 2004a). Even if we accept the more probable estimation of 5-20% of naturalized species (Pyšek *et al.* 2004a) that will become a pest, then mechanisms other than traits must be responsible for the success of invasive species. In this context, it is often assumed that invasive species usually grow more vigorously and perform better than their conspecifics in the native range (Crawley 1986, Blossey & Nötzold 1995). Increased growth and fecundity, together with decreased levels of herbivory in the invaded range have been shown for e.g. *Solidago gigantea* (Jakobs *et al.* 2004) and *Senecio inaequidens* (Prati & Bossdorf 2004). However it is difficult to imagine that nonindigenous plants will perform better in an environment

in which they have not evolved. How can this paradox been explained (Allendorf & Lundquist 2003)?

Plants have two options to achieve a higher fitness to successfully invade a new area: Either by phenotypic plasticity or by undergoing genetic differentiation resulting in local adaptation. Being able to alter the physiology or the morphology in response to environmental changes, i.e. to be phenotypically plastic (Schlichting 1986), should be advantageous for the invasion success of alien plants (Barrett 1992) which has been revealed in several species (e.g. Parker *et al.* 2003, Dybdahl & Kane 2005).

On the other hand, the time lag between the introduction and an extensive spread of plant species (Kowarik 1995, Mack *et al.* 2000, Mooney & Cleland 2001) which can last up to 200 years (Kowarik 1995) has lead to the suggestion that invasiveness evolves after colonization due to altered selection pressure (Blossey & Nötzold 1995, Ellstrand & Schierenbeck 2000, Lee 2002, Müller-Schärer & Steinger 2004). These changes may produce locally adapted populations that would have a higher fitness in their local habitat than genotypes originating from other habitats. Thus, the ability for rapid evolution in invasive plant species is likely to facilitate their spread and establishment in new habitats and results in an expansion of area (Rice & Mack 1991a). Probably, evolutionary processes are crucial to whether an invasion will occur or not (Sakai *et al.* 2001). In this context, biological invasions represent 'natural experiments' that allow the study of rapid evolution in action.

Investigations on local adaptation in plant populations require a comparison of different genotypes under the same environmental conditions. This can be realized by common garden experiments that provide the opportunity to estimate whether phenotypic differences between populations result from environmental variation or whether they are caused by genetic differentiation (Sakai *et al.* 2001, Kawecki & Ebert 2004). Ideally, genotypes should be reciprocally transplanted between the habitats from where the genotypes were collected (Kawecki & Ebert 2004).

One prerequisite for evolutionary adjustments is that the species or the population is genetically diverse (Caroll & Dingle 1996). The negative consequences associated with the loss of genetic variability are well documented in rare species

(Matthies 2000, Fischer *et al.* 2000, Luijten *et al.* 2000, Durka 2000). Populations with only a few individuals may be affected by the loss of genetic diversity (e.g. founder effects), inbreeding depression and reduced evolutionary potential (Müller-Schärer *et al.* 2004). Hence, one would expect for invasive species that the establishment and adaptive evolution in a new area is impeded by genetic bottlenecks (Sakai *et al.* 2001). However, multiple introduction of a species from different geographic locations may counteract this genetic bottleneck (Müller-Schärer & Steinger 2004, Bossdorf *et al.* 2005). In addition, hybridisation, either interspecific or between previously isolated populations of the same species, can be an important stimulus for evolution of invasiveness (Ellstrand & Schierenbeck 2000). The study of genetic variation in invasive species is therefore important for their management (Barrett 1992).

Climate is a primary influence on plant fitness and, thus an important ecological filter limiting the distribution of plants. This was demonstrated, for example, by the clinal variations that occur with latitude in the invasive plant species *Solidago altissima* und *S. gigantea* in Europe (Weber 1997, Weber & Schmid 1998). However, next to abiotic filters (e.g. climate, nutrient availability, disturbance), adaptation to biotic conditions (e.g. competition, herbivory, pathogens) are also crucial for a species to expand into new geographic locations. For instance, the absence of herbivores and pathogens may also lead to evolutionary adaptation that may be expressed as increased competitive ability (EICA hypothesis; Blossey & Nötzold 1995, Joshi & Vrieling 2005). Moreover, invasive species may have impact on interactions between species within or between trophic levels in the new range (e.g. Richardson *et al.* 2000a). So, the invasive species may alter the succession process in new, unoccupied or disturbed habitats (Smale 1990) or influence existing plant pollinator-interactions (Chittka & Schurkens 2001). So far, little is known about the evolutionary consequences of plant invasion and consequences to the native plant community. This thesis addresses the evolutionary processes associated with invasiveness using the invasive shrub *Buddleja davidii* as the model system.

The study system: Buddleja davidii Franch.

Buddleja is a cosmopolitan genus of the family Scrophulariaceae (Angiosperm Phylogeny Group II 2003) consisting of about 100 species in the tropical and temperate zones (Leeuwenberg 1979). Within the distribution centre of Asia, more than 90% of the Buddleja species are distributed in the Sino-Himalayan region (Leeuwenberg 1979).

One of these species is *Buddleja davidii*, commonly called butterfly bush. It is native to at least nine provinces in China (Lauener 1996) at altitudes between 800 m and 3000 m (Wu & Raven 1996). This multi-stemmed shrub reaches a height of 2 - 5 m (Lauener 1996). The deciduous or semi-evergreen plant (Owen & Whiteway 1980) is relatively short-lived up to an age of 30 years (Bellingham *et al.* 2005).

Buddleja davidii usually begins flowering and fruiting one year after germination, although some individuals may flower within the first year (Kreh 1952). Many fragrant flowers appear at the terminal end of branches arranged in panicles that are up to 30 cm long (Figure 1). The hermaphroditic flowers produce nectar attracting mostly butterflies, but also bees and other insects are frequent. The shrub has a large annual seed production of up to several million sees per plant (Kreh 1952, Campbell 1984). The fruit is a two-valved capsule that may release between 28 and 75 seeds (Kreh 1952, Brown 1990) that are 3 mm long with linear wings at both ends (Wu & Raven 1996) facilitating the dispersal by wind or even water (Campbell 1984).

Figure 1.   *Buddleja davidii* Franch. (from: Rothmaler 2002).

Buddleja davidii was one of several plant species that was introduced from Asia to Europe in the 1890s for ornamental reasons. It is now one of the most popular woody species in gardens (Kay 2002), with more than 90 cultivars differing in growth form, flower colour and length of inflorescences as well as leaf shape and colour (Krüssmann 1976, Stuart 2006). *Buddleja davidii* can tolerate a wide range of soil conditions (Kreh 1952, Humphries & Guarino 1987, Webb *et al.* 1988). Although originally intended for cultivation, the species naturalized outside of gardens in the bombed, devasted areas of many cities (Kreh 1952, Kunick 1970, Owen & Whiteway 1980, Pfitzner 1983, Schmitz 1991). It rapidly colonizes bare, disturbed soils (Smale 1990) found on railroad embankments, quarries, roadsides and riparian areas (Randall & Marinelli 1996) and has also been found growing on walls and rock faces (Segal 1969).

    Buddleja davidii has naturalized within sub-oceanic climates in the temperate and sub-mediterranean zones in most European countries as well as in Africa, New Zealand, Australia and in North America (Tutin 1972, Leeuwenberg 1979, Webb *et al.* 1988, Reichard & Hamilton 1997, Csurshes & Edwards 1998). It is listed on several countries´ plant watch list because *B. davidii* may form dense stands that may displace native plant species. Due to the plants´ popularity and its tolerance of a broad range of environmental conditions, high seed production and fast development to maturity, *B. davidii* has the potential to become a serious problem species in Europe as it has been reported in New Zealand (Kay & Smale 1990).

Aim and structure of this thesis

Biological invasion can be considered as unintended experiments, providing the opportunity to investigate biogeographic, ecological and evolutionary mechanisms (Vermeij 1991, Lodge 1993, Sakai *et al.* 2001). There have been several studies that have explored the patterns of plant invasions, invasibility of communities, differences between phylogenetic groups and strategies of invasive plants and their control (Drake *et al.* 1989, Vitousek 1990, Pyšek 1995, Auge & Brandl 1997, Pyšek 1998, Alpert *et al.* 2000, Willis & Hulme 2002, Woitke & Dietz 2002). However, only a few studies have

attributed local adaptations as a reason for the success of plant invasions (e.g. Thompson 1998, Galloway & Fenster 2000, Parker *et al.* 2003). The aim of this thesis is to improve the understanding of how local adaptations contribute to the success of plant invasions using *Buddleja davidii* as the model system. The results of this research will contribute to the understanding of risks associated with the naturalization of *B. davidii* and development of best management strategies. This thesis specifically addresses different aspects of the potential rapid evolution in *Buddleja davidii.* First, it has to be determined whether plants differ in their performance between their native and invasive range. **Chapter 2** describes how the growth, reproduction and impact of herbivory differ among native and invasive populations of *B. davidii* in the field. This step is a prerequisite for testing different hypotheses (e.g. Enemy Release Hypothesis or Evolution of Increased Competitive Ability hypothesis) concerning the success of invasive plant species.

Chapters 3 and 4 test genetic differentiation between invasive *B. davidii* populations in the new range. Specifically, the adaptation of populations to climatic conditions is addressed in **Chapter 3**. For this purpose, common garden experiments were carried out and replicated in three central European regions, ranging from an oceanic to a sub-continental climate. The replication of common garden studies represents an approach that has been rarely realised. It allows both, investigations of genetic variation and local adaptation. For the latter, the integration of a reciprocal transplant experiment on populations originated from the three regions hosting the common gardens allowed to test for local adaptation. Genetic variation in response to different environments was investigated using various growth and fitness-related plant traits. Additionally, clipping treatments were used to simulate disturbance (Smale 1990) and frost damage to determine if there are differences among populations in re-sprouting ability and tolerance to damage.

Chapter 4 further explores the local adaptation to frost in invasive *B. davidii* populations, specifically the physiological sensitivity to frost and response to frost damage. This study is based on the assumption that the geographic distribution of invasive *B. davidii* populations in Europe is restricted to the oceanic or sub-oceanic

climate due to sensitivity to frost. This research combines an eco-physiological approach and an ecological niche model to investigate whether evolutionary adjustments may exacerbate the prediction of spatial distribution of *B. davidii* and whether frost is actually limiting its further spread.

Buddleja davidii is an ecological specialist, able to colonise new coarse-textured, nitrogen-deficient surfaces (Humphries *et al.* 1982). In Europe railway embankments are typical habitats where large numbers of *B. davidii* have naturalized. There, plants have to be removed for safety reasons, mostly using the herbicide glyphosate. While the application of herbicides may exert a strong selection pressure on populations coincidentally, this may lead to evolution of herbicide resistance, which in turn, may hamper the management of weeds and invasive plants (Culliney 2005). **Chapter 5** investigates the susceptibility to the herbicide in populations of three different habitat types, that either has been treated with glyphosate or not. The potential for evolution of herbicide resistance will have far-reaching consequences for the successful and efficient management of invasive *Buddleja davidii* populations.

Overall, this thesis examines the genetic differences between invasive populations of *Buddleja davidii* in Europe with respect to different environmental influences and their importance for the invasion success of this species.

Chapter 2

The invasive shrub *Buddleja davidii* performs better in its introduced range

with Isabell Hensen & Harald Auge

Abstract

It is commonly assumed that invasive plants grow more vigorously in their introduced than in their native range, which is then attributed to release from natural enemies or to microevolutionary changes, or both. However, few studies have tested this assumption by comparing the performance of invasive species in their native versus introduced ranges. Here, we studied abundance, growth, reproduction and herbivory in ten native Chinese and ten invasive German populations of the invasive shrub *Buddleja davidii* (Scrophulariaceae; Butterfly Bush). We found strong evidence for increased plant vigour in the introduced range: plants in invasive populations were significantly taller, had thicker stems, larger inflorescences and heavier seeds than plants in native populations. These differences in plant performance could not be explained by a more benign climate in the introduced range. Since leaf herbivory was substantially reduced in invasive populations, our data rather suggest that escape from natural enemies, associated with increased plant growth and reproduction, contributes to the invasion success of *B. davidii* in Central Europe.

Introduction

Biological invasions are one of the major threats to global biodiversity (see Vitousek *et al.* 1997, Sala *et al.* 2000) and may result in high economic costs (Pimentel *et al.* 2000). Much research has been carried out in search of the general mechanisms that allow non-indigenous plants to become invasive (Richardson *et al.* 2000b, Levine *et al.* 2003, Bossdorf *et al.* 2005). A common assumption in many of these studies is that invasive species perform better in their introduced ranges than in their native ranges (Crawley 1986, Blossey & Nötzold 1995). Based on this assumption, two major hypotheses for the success of invasive plants have been proposed: the Enemy Release Hypothesis (ERH) posits that a lack of natural enemies in the new area results in an increase of abundance of the invader (Keane & Crawley 2002). The Evolution of Increased Competitive Ability Hypothesis (EICA) states that this lack of natural enemies should lead to a selection for increased competitive ability at the expense of defence mechanisms (Blossey & Nötzold 1995). While many studies have tested the predictions of these two hypotheses, very few have investigated the underlying assumption of different performance in the field (Bossdorf *et al.* 2005). Although demographic comparisons on a biogeographic scale cannot distinguish between phenotypic plasticity and genetic differentiation, they are crucial to understanding plant invasions in general (Hierro *et al.* 2005).

Previous comparative studies on invasive plants were mostly conducted with herbaceous species (but see e.g. Siemann & Rogers 2001, Buckley *et al.* 2003, Dewalt *et al.* 2004). Although the study of woody plants provides useful insights on central issues of invasion ecology for a variety of reasons (Richardson *et al.* 2004), manipulative experiments with long-lived woody species are difficult and inevitably restricted to short periods of their whole life-cycle (Harper 1977). Moreover, quantitative-genetic investigations require pre-cultivation of plants to control for maternal effects (Roach & Wulff 1987) and to carry out controlled crosses (Falconer & MacKay 1996), and are therefore rather elaborate. Nevertheless, the study of biological invasions should not neglect woody plants because results obtained from herbs cannot be generalized.

Hence, more case studies are needed, and even field comparisons focussed on one particular life stage of woody species may substantially contribute to our general understanding of plant invasions (e.g. Buckley *et al.* 2003, Prati & Bossdorf 2004).

The Butterfly Bush (*Buddleja davidii* Franch.) is a shrub native to China and was introduced to Europe and other continents for ornamental reasons around 1900 (Kunick 1970). It has become naturalized or invasive (*sensu* Richardson *et al.* 2000) in several regions of the world and may also have impact on native ecosystems. In New Zealand for instance, the species invades riversides and forest plantations, where it out-competes native vegetation (Smale 1990), and causes significant economic problems (Kay & Smale 1990). In some European countries and some regions of the United States, *B. davidii* is now recognized as a potential pest plant and can be found on plant watch lists (O'Neill, K. 1998 cit. in Starr *et al.*2003, SKEW2008). However, we still have poor knowledge of the species' potential to spread successfully and to become a problematic weed on a larger scale. As a first step to exploring the ecological and evolutionary processes underlying the successful spread of *B. davidii* in Europe, we compared plant abundance, growth and reproduction, as well as leaf herbivory between ten native populations of *B. davidii* in China and ten invasive populations in Germany. In particular, we hypothesized that in the invasive range (1) population size and density of *B. davidii* would be larger, (2) plant individuals would be more vigorous in terms of growth and reproduction, and (3) plants would suffer less from herbivory than in the native range.

Methods

Study species

Buddleja davidii (Scrophulariaceae), is a multi-stemmed, 0.5 m to 5 m tall shrub (Wu & Raven 1996) with either deciduous (Tutin 1972) or evergreen leaves (Kunick 1970) and with a life span of approximately 40 years. It usually starts flowering and fruiting in the second year after germination, but sometimes even within the first year (Esler 1988a). The terminal inflorescences appear at current-year stems or branches, and are

thyrsoid panicles of 4 to 30 cm in length (Leeuwenberg 1979, Wu & Raven 1996). A medium-sized individual may produce 300 to 350 inflorescences, each of them bearing from fewer than 100 to more than 1700 capsules (Kreh 1952, Brown 1990). The average number of seeds per capsule is reported to range from 28 seeds (Brown 1990) to 75 seeds (Kreh 1952). Thus, *B. davidii* can have an enormous seed production of up to several millions of seeds per plant (Kreh 1952, Campbell 1984). The small seeds are long-winged at both ends and are dispersed by wind or water (Campbell 1984).

The species is native to central and south-western China where it grows on mountain slopes at altitudes between 800 m and 3000 m (Wu & Raven 1996). Since its introduction to Europe in the 1890s, *B. davidii* has been cultivated as an ornamental garden plant with colourful and fragrant flowers which mainly attract butterflies (http://www.hear.org/starr/hiplants/reports/html/buddleia_davidii.htm). It escaped from gardens and has become naturalized or invasive (sensu Richardson *et al.* 2000b) in Europe, North America, Africa, Australia and New Zealand (Tutin 1972, Leeuwenberg 1979, Webb *et al.* 1988, Csurshes & Edwards 1998). In both its native and invaded range, *B. davidii* prefers naturally or anthropogenically disturbed sites along roads, river banks and railways (Kunick 1970, Randall & Marinelli 1996). It also survives in walls and on rock faces and can tolerate a wide range of soil conditions (Kreh 1952, Webb *et al.* 1988). Thus, the first spread in Europe appeared in bombed, devastated areas of many cities after World War II (Kreh 1952). *Buddleja davidii* populations in Europe are restricted to oceanic and sub-oceanic climates in the temperate and sub-mediterranean zones. The eastern range margin currently crosses Germany (http://www.floraweb.de/neoflora/handbuch/buddlejadavidii.html). It has been suggested that its further spread is limited by a lack of frost tolerance (Kunick 1970, Krivanek *et al.* 2006). Because of the broad environmental tolerance, high seed production, fast development to maturity, as well as the vigorous re-sprouting after damage, *B. davidii* has the potential to become a serious problem species, even in areas where it is not yet recognized as an environmental weed.

Field data

We collected field data from ten populations in China and ten populations in Europe between December 2005 and February 2006 (Figure 1). For our study, we defined a population as a group of *B. davidii* individuals in a given habitat with a distance of at least 500 m to another group of *B. davidii* individuals. In each of the two ranges, the populations were chosen randomly concerning habitat, population size and population age. However, we tried to avoid a spatial clumping of populations in order to ensure a representative sampling across each range.
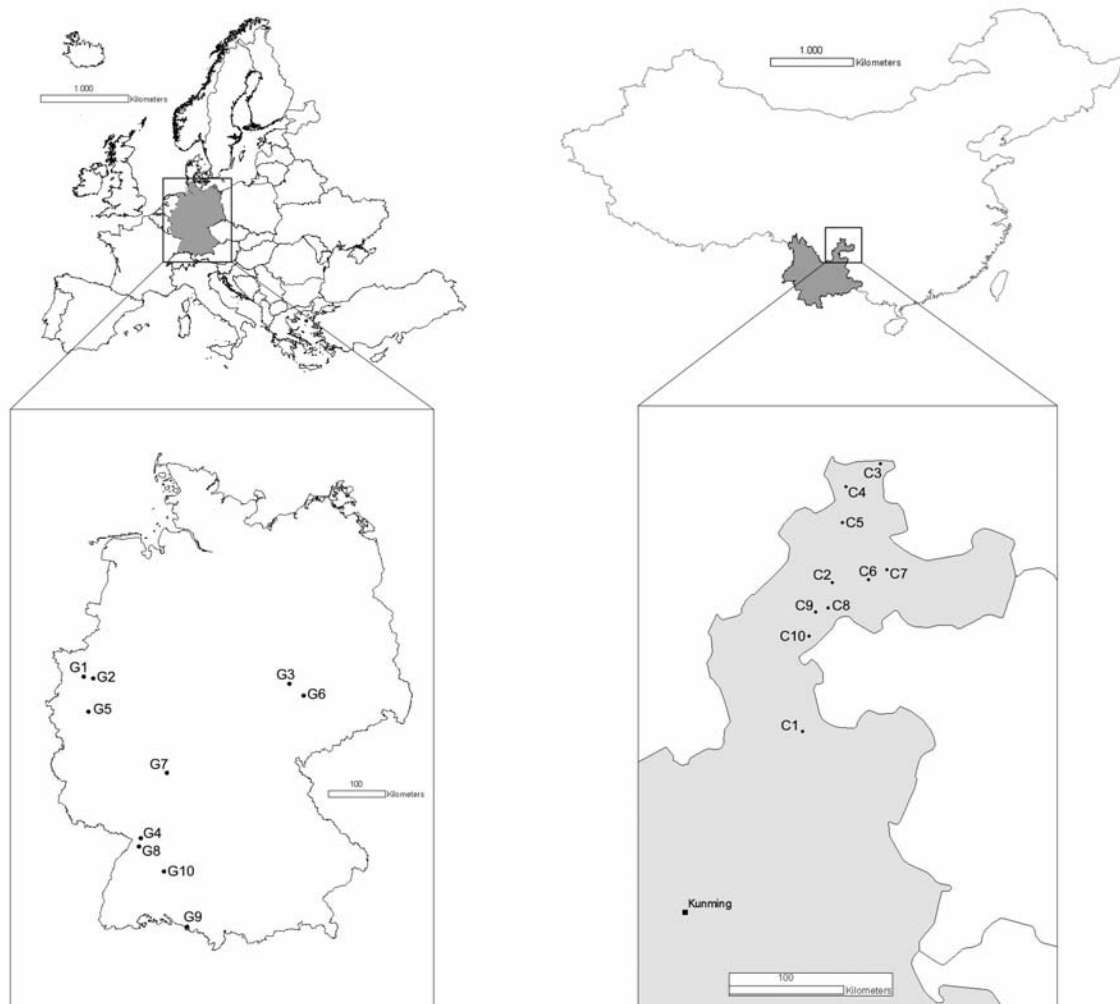

Figure 1. Maps of sampling areas, (a) introduced range: Europe (Germany) and (b) native range: China, province Yunnan highlighted.

Unfortunately, comprehensive range maps of *B. davidii* are not available yet for the native and introduced ranges (E. Welk, pers. comm.). In China, the occurrences of *B. davidii* are well documented only in the province Yunnan, where it grows in the Zhaotong district in the north-eastern part of the province (W. Sun, pers. comm.). We therefore performed our sampling in this particular region but likely did cover the whole native range. In Europe, we sampled populations across Germany, from the western border of the country to the current range margin of *B. davidii* in eastern Germany. This sampling scheme did not include more western parts of the introduced range in Europe, but was representative for Central European populations and covered quite different climatic zones ranging from oceanic to sub-continental climates. Furthermore, our sampling in the native and introduced range comprised areas of similar size, with distances among populations being only slightly smaller in China (12 km to 243 km) than in Germany (17 km to 465 km) (Table1).

Table 1. List of sampled populations of *Buddleja davidii* in its native (China / Asia) and invaded (Germany/ Europe) range.

| Population | coordinates | | Altitude a.s.l. [m] |
	latitude	longitide	
Europe			
G1	51.4827N	6.7855E	31
G2	51.4652N	7.0266E	116
G3	51.4811N	11.9708E	87
G4	48.9957N	8.4155E	118
G5	50.9316N	6.9647E	38
G6	51.2972N	12.3286E	113
G7	50.0402N	8.9680E	110
G8	48.8630N	8.3711E	112
G9	47.6336N	9.5847E	472
G10	48.4841N	8.9997E	338
Asia			
C1	26.4866N	103.6226E	2230
C2	27.6664N	103.8567E	1200
C3	28.6097N	104.2368E	580
C4	28.4274N	103.9647E	1370
C5	28.1427N	103.9349E	1050
C6	27.6920N	104.1435E	1370
C7	27.7711N	104.2884E	1840
C8	27.4673N	103.8268E	2215
C9	27.4350N	103.7266E	2070
C10	27.2396N	103.6735E	1940

To compare the climatic conditions of the two regions, we obtained the following climatic variables for each study site from http://www.worldclim.org (Hijmans *et al.* 2005): annual mean temperature, mean maximum temperature of the warmest month, mean minimum temperature of the coldest month, and mean annual precipitation. We estimated the length of the growing season, defined as the number of days with a mean temperature above 5°C, by fitting generalized additive models to mean monthly temperature values of six weather stations near the sampled populations in Germany (http://www.dwd.de). For the Chinese populations, data of only one station, Zhaotong, was available (www.ncdc.noaa.gov). This station (27.32 E, 103.72 E, 1 950 m a.s.l.) is located at a relatively high elevation, but within the altitudinal range of the sampled populations.

In small populations, we determined population size by counting all individuals. In large populations, we counted all plant individuals in a randomly chosen 10 m x 10 m plot and estimated population size by multiplying this number by the total area of the population. Population density was calculated by dividing the size of each population by the area covered by it. In each population, ten individuals were randomly chosen for further measurements. On each, we counted the number of stems, measured the basal diameter of the thickest stem and the length of five randomly selected stems. Furthermore, we determined the length of 20 randomly chosen inflorescences per individual. Because of the positive correlations between length of inflorescences and number of capsules (Brown 1990), and between number of stems and number of inflorescences ($r^2 = 0.21$, n = 283, p < 0.01; S. Ebeling, unpublished data), we considered number of stems and length of inflorescences as surrogates of reproductive success.

From each sampled plant we collected one mature inflorescence and determined mean seed mass by weighing a bulk sample of either 500 or 1,000 seeds. Seeds were stored in paper bags at room temperature. Since the plants still had green leaves, we were able to quantify leaf herbivory by visually estimating the percent area destroyed of 20 randomly chosen leaves of each plant.

Germination rate

In June 2006, three samples of 30 seeds from each of five individuals per population (in total 90 seeds per plant and 450 seeds per population, with the exception of two Chinese populations, that had only two and three fruiting individuals) were placed on moist filter paper in Petri dishes. Previous germination trials revealed that no stratification is necessary for *B. davidii* and that seeds germinate at a high percentage with 12 / 12h light / dark cycle at 10 / 20°C (data not shown). Therefore, we placed the Petri dishes in a climate chamber with these same conditions. Petri dishes were checked every second day, and moistened regularly using distilled water. After 36 days, when there was no new germination for ten days, we determined the proportion of germinated seeds.

Data Analyses

We carried out analysis of variance (ANOVA) to test for differences in population size and density between the ranges using the statistical software SAS (procedure GLM, SAS Institute 9.1). For the other traits we used a nested ANOVA to test for differences between the ranges and among the populations nested within range. For the analyses of leaf herbivory, length of inflorescences and length of stems, we averaged the data for each individual.

Populations within ranges and individuals within populations (for the analysis of germination rate) were considered as random factors. Accordingly, range effects were tested against population effects, and in the case of germination rate, population effects were tested against individuals within populations. Since the data were unbalanced, we used type III sum of squares (Shaw & Mitchell-Olds 1993). Stem diameter, number of stems, and length of stems were log-transformed, length of inflorescences was square-root transformed, and percentage of herbivory and percentage of germinated seeds were arcsine-transformed to normalize their distribution prior to analysis. We did not apply sequential Bonferroni tests to account for multiple comparisons because we intended to investigate differences between native and invasive populations in

more detail without inflating the likelihood of type II errors (Cabin & Mitchell 2000). Instead, we calculated the probability to find a significant result by chance and compared this with the observed frequency of significant results among the six plant traits related to growth and reproduction (Moran 2003).

To investigate whether observed differences in plant vigour are caused by climatic differences between the two ranges, we compared the six plant traits after correcting for climatic variables with ANCOVA (procedure GLM, type I sum of squares). Since we considered mean annual temperature as redundant to mean minimum and maximum temperatures (as reflected also by their strong correlation within both ranges), we only included mean minimum temperature, mean maximum temperature, and precipitation as covariates in the model.

Results

We found large variation among *B. davidii* populations within each range concerning size and density: population size varied from 12 to 15,000 individuals in the native range in China, and from 9 to 860 individuals in the invasive range in Germany (Table 2). Population density ranged from 150 to 4,200 ha^{-1} in China, and from 38 to 12,700 ha^{-1} in Germany (Table 2). The differences between the ranges were not statistically significant (Table 2). There was highly significant variation among populations within ranges for all measured traits. We found strong evidence for increased plant growth and reproduction in invasive populations in two of three traits related to plant growth (Table 2): length of stems was 79% larger (Figure 2a), and mean diameter of thickest stem was 73% larger than in native populations. European plants had on average twice as many stems as Chinese plants. This difference was marginally significant (p = 0.055).

Table 2. Mean values of population traits and traits of growth and reproduction reflecting vigour and leaf herbivory of plants in native (China) and invasive populations (Germany) of *Buddleja davidii* and summary of ANOVAs on these traits between and among the populations (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

	Mean ± s.e.		Continent		Population within	
	China	Germany	d.f.	F ratio	d.f.	F ratio
Population						
Population size	1593 ± 1490	86 ± 35	1, 18	1.02		
Population density [ha^{-1}]	3037 ± 1028	2011 ± 1211	1, 18	0.42		
Plant vigour						
Growth traits						
Number of stems	3.8 ± 0.4	6.3 ± 0.4	1, 18	4.20	18, 175	3.44 ***
Stem diameter [mm]	15.1 ± 0.9	26.1 ± 3.6	1, 18	5.93 *	18, 175	4.90 ***
Stem length [cm]	97.5 ± 3.6	174.7 ± 3.6	1, 18	13.21 **	18, 175	6.96 ***
Reproductive traits						
Inflorescence length [cm]	10.3 ± 0.1	12.7 ± 0.2	1, 18	12.58 **	18, 168	2.26 **
Seed mass [ng]	29.1 ± 1.7	36.4 ± 1.2	1, 18	8.53 **	18, 135	3.53 ***
Seed germination [%]	32 ± 6.0	42 ± 8.0	1, 18	0.84	18, 75	3.22 ***
Leaf herbivory [%]	14.6 ± 0.7	0.5 ± 0.7	1, 18	16.95 ***	18, 175	20.25 ***

Furthermore, inflorescences were 23% longer in European populations (Table 2) compared to Chinese populations. Since the number of stems predicts the number of inflorescences (S. Ebeling, unpubl. data), and inflorescence length predicts the number of seed capsules (Brown 1990), our data indicate increased seed production in invasive populations. In addition, seed mass differed significantly between ranges: seeds in Europe were 25% heavier than in Asia (Table 2, Figure 2b), but did not germinate significantly better (Table 2). Leaf herbivory was more pronounced in *B. davidii* populations in the native range. Here, about 15% of leaf area was removed, as opposed to plants in the invasive range that showed almost no signs of herbivory (Table 2, Figure 2c).

For testing our hypothesis of increased plant vigour, we investigated six traits related to plant growth and reproduction. In a table with six statistical tests of the same hypothesis, the probability to find a significant effect by chance alone is $1-0.95^6 = 0.265$ (Moran 2003). Since we found significant differences between native and invasive populations in four out of six traits (Table 2), we are confident that the observed differences are not a product of chance.

Altitude of sampled populations differed remarkably between the two ranges (Table 1). Although populations in China were located at higher altitudes, they still experienced a much longer growing season (314 days at 1 950 m a.s.l.) than populations in Germany (242 - 283 days). Probably due to the greater altitudinal variation among Chinese populations, the variances in two out of four climatic variables were larger in China than in Germany, indicating a steeper environmental gradient in the native range (annual mean temperature: $F_{9,9} = 6.13$, $p < 0.05$; mean maximum temperature: $F_{9,9} = 7.92$, $p < 0.01$, mean minimum temperature: $F_{9,9} = 2.58$, $p = 0.17$; precipitation: $F_{9,9} = 3.45$, $p = 0.08$).

Despite much overlap between the two ranges, annual mean temperatures (U-test, $p < 0.001$), mean maximum temperatures ($p < 0.05$), and mean annual precipitation ($p < 0.05$) were higher in the Chinese populations compared to the German ones, while mean minimum temperatures were similar ($p = 0.14$). When adjusted for the variation in climatic variables using an ANCOVA, the differences in plant traits among native and invasive populations remained nearly unaffected compared to the unadjusted analyses (number of stems: $F_{1,15} = 3.71$, $p = 0.07$; stem length: $F_{1,15} = 22.94$, $p < 0.001$; stem diameter: $F_{1,15} = 7.24$, $p < 0.05$; inflorescence length: $F_{1,15} = 13.85$, $p < 0.01$; germination rate: $F_{1,15} = 0.02$, $p = 0.89$), except for seed mass ($F_{1,15} = 3.14$, $p = 0.10$). For illustrative reasons, we show the relationship between stem length and mean maximum temperature in Figure 3: while stem length increases significantly with temperature in China, no such relationship is evident in Germany. Across the range of overlapping temperatures, however, native populations posses much shorter stems than the invasive populations.
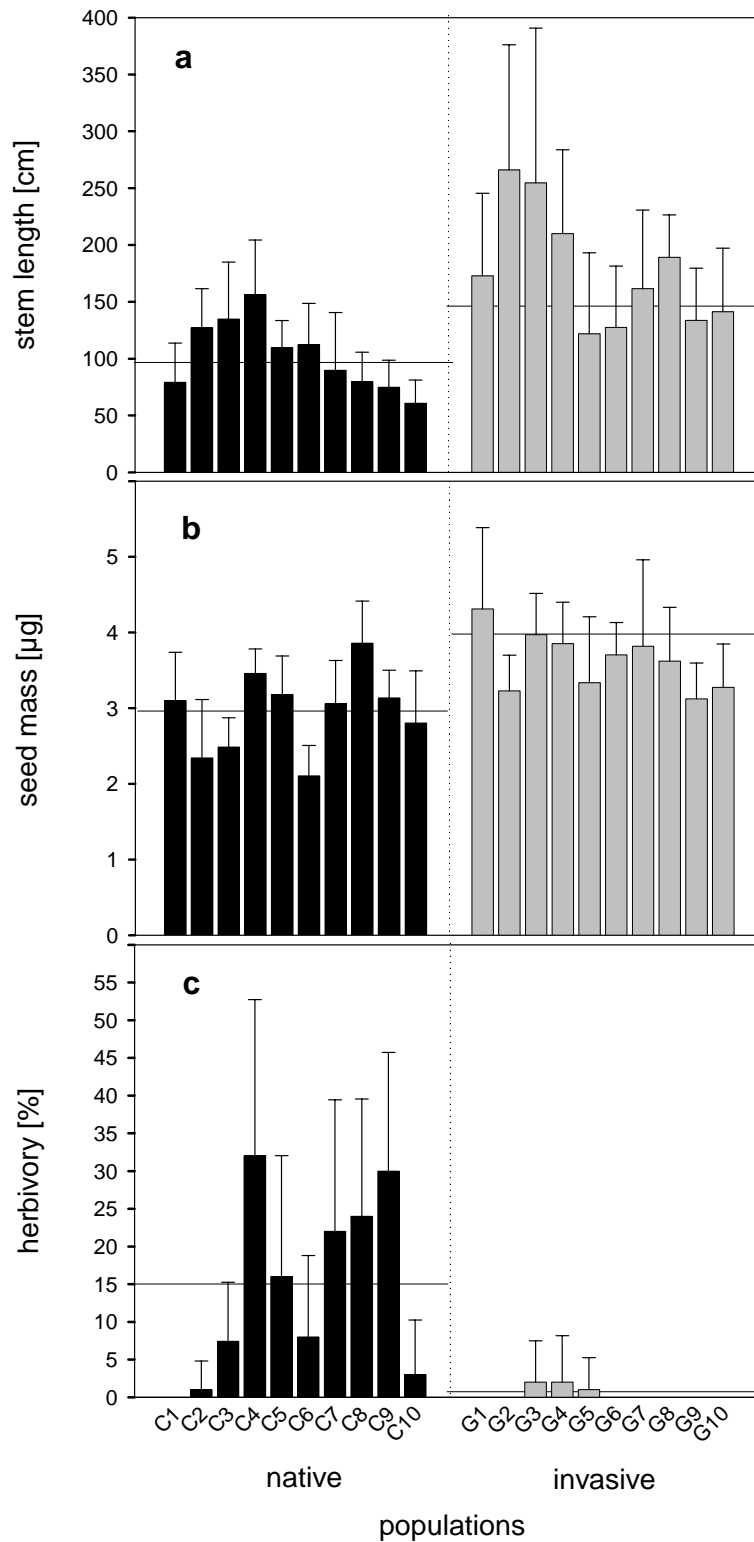
Figure 2. Variation between and among native (black columns) and invasive (grey columns) populations of *Buddleja davidii* in (a) length of stems, (b) seed mass and (c) estimated percentage of leaf damage by herbivores (columns represent population means + s.e., and horizontal lines are continent means).
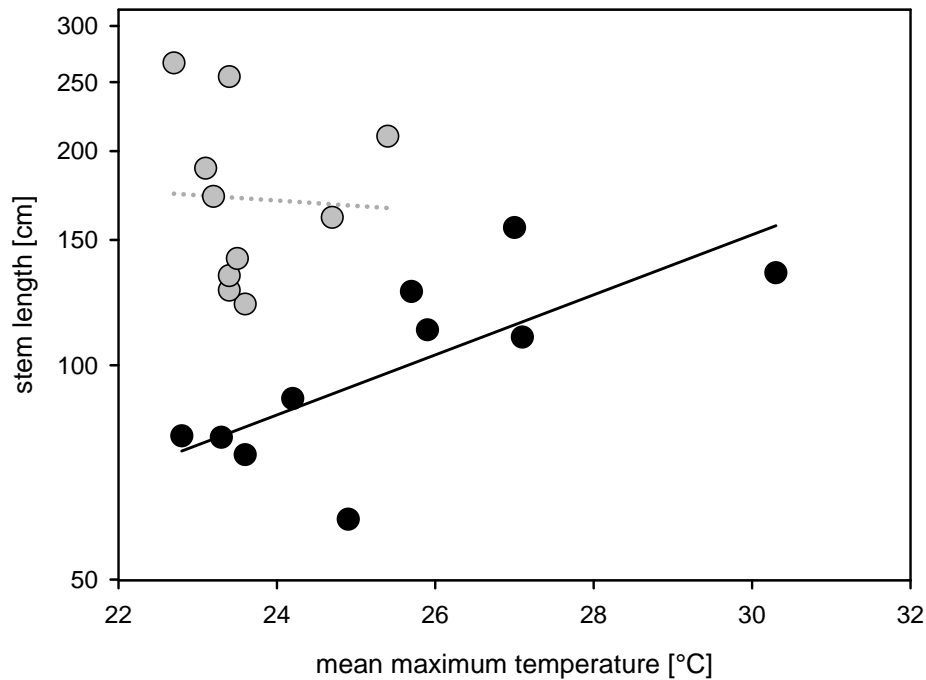
Figure 3. Relationship between mean maximum temperatures of the warmest month, and mean stem length among *B. davidii* populations in the native range (China, black symbols) and the invasive range (Germany, grey symbols). The slope is only significant in the native range ($b = 0.0422$, $t = 3.02$, $p < 0.05$, 8 d.f.) but not in the invasive range ($b = -0.0075$, $t = -0.14$, $p = 0.89$, 8 d.f.). After correcting for mean maximum temperature, stem length of German populations is still larger than stem length of Chinese populations (ANCOVA: $F_{1,16} = 28.09$, $p < 0.0001$).

Discussion

Our comparative field study on the performance of *B. davidii* provides evidence that in the invaded range, individuals are bigger, have higher fecundity and seed mass, and suffer much less from herbivory than in the native range. Increased growth and fecundity, together with decreased levels of herbivory in the invaded range were also shown for the perennials *Solidago gigantea* (Jakobs *et al.* 2004) and *Senecio inaequidens* (Prati & Bossdorf 2004). Our data are also in line with Buckley *et al.* (2003), who reported heavier seeds of *Cytisus scoparius* in the invaded range.

A straightforward explanation for the larger plant size and fecundity in our invasive *B. davidii* populations would be that these individuals are simply older compared to the native populations sampled. Unfortunately, we do not have 'hard' data on population age, in particular on the age of native populations in China. Yet invasion history and the type of invaded habitats give some hints about the age of invasive populations. The first spread of *B. davidii* in Central Europe occurred after World War II (Kreh 1952); hence, neither of our invasive populations is likely older than 60 years. Furthermore, the invasive as well as the native *B. davidii* populations grew on disturbed sites, suggesting a rather young population age in both ranges. Available information on some of our invasive populations indicates that they are between 10 and 25 years old: Populations G1 and G2 were founded around 1980 (G. H. Loos, personal communication), G4 before 1988 and G8 in the 1990s (T. Breuning, pers. comm.), and G6 as well as G9 after 1990 (S. Ebeling, pers. obs.). These arguments strongly suggest that our invasive populations were not older than the native ones, and that the larger plant size and fecundity reflects increased vigour of *B. davidii* populations in the invasive range. Nevertheless, the possibility that differences in size or abundance may generally reflect different population ages highlight the need to include dendro- or herb-chronological measurements in future comparative studies of invasive plants (see Dietz *et al.* 1999, Pergl *et al.* 2006).

Another explanation for the observed increased vigour of invasive *B. davidii* populations would be a plastic response to a more 'benign' abiotic or biotic environment (Crawley 1987, Willis & Blossey 1999). While in both ranges *B. davidii* grows in disturbed habitats such as riverbanks and roadsides, it is still possible that invasive populations may benefit from more favourable climatic conditions. For instance, populations in the invasive range were located at much lower altitudes than native populations which might result in better growing conditions. However, differences between native and invasive populations remained significant, even after we corrected for differences in climate (except of seed mass). On average, Chinese populations experienced higher temperatures and precipitation as well as a longer growing season than German populations. A plastic response to benign climatic

conditions is, therefore, unlikely to be responsible for the increased vigour of *B. davidii* in its invasive range. Instead, our data suggest that low levels of herbivory may contribute to the increased size and fecundity of invasive *B. davidii* populations.

As argued by the ERH, a competitive advantage may be caused by release from natural enemies, such as pathogens (Reinhart *et al.* 2005) and specialized herbivores (Maron & Vila 2001, Keane & Crawley 2002). In our study, we found evidence for release from herbivory: while leaves in German populations remained nearly untouched by herbivores, about 15% of leaf area was damaged in Chinese populations. Decreased herbivore attack in the invasive range has also been revealed by several other studies (Memmott *et al.* 2000, Wolfe 2002, Prati & Bossdorf 2004, Dewalt *et al.* 2004, Vilà *et al.* 2005). It should be noted, however, that attack rate or biomass loss is not equivalent to the populations' response to herbivory or to the release from it (Keane & Crawley 2002). We suggest that one reason for decreased herbivory in invasive *B. davidii* populations is the lack of related native plant species in Europe, which makes a pre-adaptation of generalists (Keane & Crawley 2002) and a host range extension or host switch of specialists unlikely (cf. Soldaat & Auge 1998, Frenzel & Brandl 2003). Another reason might be that no herbivores from the native range are present in Europe.

To our knowledge, the two native specialist insects, *Cleopus japonicus* and *Mecysolobus erro*, that were tested for biological control of *B. davidii* in New Zealand (Kay & Smale 1990, Brockerhoff *et al.* 1999), have not been introduced in Europe so far.

Besides phenotypic plasticity, genetic differentiation might be a second mechanism underlying the increased vigour of invasive *B. davidii* populations, as result of evolutionary changes toward increased plant growth (Bossdorf *et al.* 2005). There is increasing evidence that the ability for evolutionary adjustments to novel environments of exotic species encounter is a key feature of successful invasions (Sakai *et al.* 2001). However, genetic differentiation may also be due to artificial selection, since *B. davidii* was introduced to and cultivated in Europe and other continents as an ornamental plant. Selection for desirable cultivars may lead to changes in traits related to growth and reproduction thus enhancing the invasive potential (Kitajima *et al.* 2006).

Hence, plant breeding has to be taken into account when assessing the evolutionary ecology of plant invaders (Kitajima *et al.* 2006). Traits that might be related to invasion success have been found by several authors in some of 70 existing cultivars of *B. davidii* (Anisko & Im 2001, Wilson *et al.* 2004a, Wilson *et al.* 2004b). It is likely that cultivars rather than native genotypes are the source of the *B. davidii* invasion, thus selection by breeders may be one reason for the differences in plant traits among native and invasive populations revealed in our study. Nevertheless, this does not preclude the possibility that adaptive evolution by natural selection imposed by the novel environment may play a role in this species. The reduced herbivory in the new range, in particular the lack of specialist herbivores, may have led to the evolution of increased plant size and fecundity in *B. davidii* as proposed by the EICA hypothesis (Blossey & Nötzold 1995, Müller-Schärer *et al.* 2004, Joshi & Vrieling 2005).

An important prerequisite for this process would be a genetic trade-off between fitness in the absence of herbivores, and defence, but studies published so far have provided rather inconsistent evidence for this hypothesis (Bossdorf *et al.* 2005).

While the observed differences in plant size and reproduction among native and invasive *B. davidii* populations are striking, they are not reflected in terms of population size and density. Instead, we found enormous variation among populations in both ranges. The results of other comparative studies are also rather inconsistent and include cases where abundance was not increased compared with the native range (Bossdorf *et al.* 2005). We suggest that the variable and often limited size of the habitats available for *B. davidii* (i.e. naturally or anthropogenically disturbed sites) in the native and the invaded range to be responsible for the large variation of abundance within regions and the lack of a difference between them. Although we found a strongly increased vigour of invasive *B. davidii* populations, paralleled by decreased herbivory, our field comparison cannot reveal to what extent phenotypic plasticity or genetic differentiation is responsible for the observed differences. Hence, there is a need for common garden experiments to reveal the role of competition, herbivory, and adaptive evolution for the successful invasion of *B. davidii.*

Chapter 3

Multiple common gardens suggest lack of local adaptation in a successful plant invader

with Jürg Stöcklin, Isabell Hensen & Harald Auge

Abstract

Climatic conditions are widely recognized as important factors shaping distributional ranges of plant species, and driving genetic differentiation between populations resulting in local adaptation. Using the invasive shrub *Buddleja davidii* as study species, we performed a multiple common garden experiment in three central European regions, ranging from an oceanic to a sub-continental climate. Progeny from 20 invasive populations from central and west Europe were raised in each common garden to investigate genetic differentiation among these populations in response to the growing conditions of each garden. In addition, we incorporated a reciprocal transplantation experiment to test for local adaptation, by including one population from each of the three places harboring the common gardens. In contrast to our expectation, the results of our common garden experiments do not provide evidence for local adaptation of invasive *B. davidii* populations in Europe. All populations responded remarkably similar to the different environments, and trait values were not correlated to climatic conditions or the geographic coordinates of their home site. In addition, we did not detect a home-site advantage for the populations reciprocally transplanted between the common gardens, and did not detect differences in compensatory ability to mechanical damage. Assuming that invasive populations originate from cultivated individuals, we suggest that large-scale mixing of genotypes due to horticultural trade across Europe, and continuous gene flow from planted individuals, may have prevented local adaptation so far. Nevertheless, our data also indicate that large phenotypic plasticity enables invasive *B. davidii* populations to grow under various climatic and soil conditions, thus contributing to their geographic spread.

Introduction

Invasive species offer the opportunity to study evolutionary responses to a new environment in a contemporary time frame (Weber & Schmid 1998). To successfully invade a new area, non-native species have to cope with novel abiotic or biotic environments they are usually not adapted to (Allendorf & Lundquist 2003). Moreover, it has repeatedly shown that invasive species perform better in the new range compared with their native range (Prati & Bossdorf 2004, Jakobs *et al.* 2004, Ebeling *et al.* 2008b). The successful invasion of novel environments and the increased vigour in the new range may be traced to two, not mutually exclusive mechanisms: adaptive evolution and phenotypic plasticity (e.g. Bossdorf *et al.* 2005).

Local adaptation to different habitats is apparently a common feature of many plant species (e.g. Linhart & Grant 1996, Joshi *et al.* 2001, Becker *et al.* 2006). Moreover, adaptive evolution in response to novel environments is considered to play a major role also in successful plant invasions (Sakai *et al.* 2001, Mooney & Cleland 2001). Evolutionary adjustments may thus be an explanation why many species become invasive only after a time lag (Kowarik 1995, Williamson & Fitter 1996). Introduced species are usually released from competitors, parasites, herbivores or pathogens (Elton 1958, Maron & Vila 2001, Keane & Crawley 2002) which may lead to adaptive divergence between the native and the invasive range (Blossey & Nötzold 1995). Within the introduced range, geographic gradients in abiotic conditions can impose divergent selection and promote genetic differentiation among introduced populations (Maron *et al.* 2004). Climate is one of the main driving forces of natural selection in plants (Clausen *et al.* 1940), and does often explain the distribution of plant species (Woodward & Williams 1987). Clinal variation along climatic gradients in the new range has been detected in several invasive plants, e.g. in *Impatiens glandulifera (Kollmann & Banuelos 2004)*, *Solidago altissima* and *S. gigantea* (Weber & Schmid 1998), indicating that adaptive evolution in response to climate has contributed to the geographic spread of these species.

Besides local adaptation, phenotypic plasticity enables species to persist under a wide range of environmental conditions, and is considered to be a key characteristic of colonizing species (Frazer 1965, Baker 1974) Accordingly, phenotypic plasticity has been found to contribute to the successful spread of several invasive plant species (e.g. Novak *et al.* 1991, Sexton *et al.* 2002, Parker *et al.* 2003, Ross *et al.* 2008). Phenotypic plasticity itself has a genetic basis and is therefore subject to natural selection (Schlichting 1986). Based on this, it has been suggested that invasive plant populations may have evolved increased phenotypic plasticity (Richards *et al.* 2006). Studies comparing native and introduced populations of invasive plants found either no difference between them, or indeed a greater plasticity in introduced populations (Bossdorf *et al.* 2005, Cano *et al.* 2008).

Local adaptation as result of divergent selection is characterized by higher fitness of resident genotypes compared to genotypes from other habitats ('local vs. foreign' criterion, Kawecki & Ebert 2004). Ideally, this particular kind of genotype x environment interaction should be investigated using reciprocal transplantation experiments between the different habitats or regions. Such transplantation experiments have rarely been conducted on invasive plant species, but see Maron *et al.* (2004) and Williams *et al.* (2008) for reciprocal common gardens between continents, or Rice & Mack (1991b) and Ross (2008) for transplantations within the invaded region. Alternatively, local adaptation can also be inferred from correlative evidence, if genetic differentiation detected in common garden experiments can be related to the environmental conditions of the populations' home site (e.g. Anderson *et al.* 1996, Weber & Schmid 1998, Kollmann & Banuelos 2004). The advantage of common garden experiments is that a large number of populations or genotypes can be compared, at the disadvantage that the 'local vs. foreign' effect cannot be rigorously tested (see Kawecki & Ebert 2004). Previous studies investigating genetic differentiation among populations of invasive species usually compared plants in only one environment (see Bossdorf *et al.* 2005). However, using just one common garden may produce misleading results because it ignores that genetic and environmental factors may interactively affect plant phenotype (Williams *et al.* 2008). Although tests for local

adaptation require the comparison of genotypes in several environments, studies on invasive species using more than one common garden are still rare.

In this paper, we examine genetic differentiation and local adaptation between invasive populations of the Butterfly Bush (*Buddleja davidii*), a shrub which is invasive in Europe, Australia, New Zealand and parts of North America. The species is native to China and was introduced to Europe and the other regions for ornamental reasons. Invasive European *B. davidii* populations perform better than native populations (Ebeling *et al.* 2008b), and occur predominantly in oceanic to sub-continental regions from the northern temperate to the Mediterranean climate zone. We hypothesized that the successful spread of *B. davidii* across different climatic zones in Europe has been facilitated by local adaptation to climatic conditions. To test this hypothesis, we compared offspring from 20 invasive populations sampled across western and central Europe in a common garden experiment. We replicated the common gardens in three central European regions, ranging from an oceanic to a sub-continental climate, and incorporated a reciprocal transplantation experiment among the three populations that originate from the places hosting the common gardens. This innovative experimental design allowed us (1) to investigate genetic differentiation of a large number of populations, (2) to explicitly consider genotype x environment interactions among all populations, and (3) to test the 'local vs. foreign' effect among the reciprocally transplanted populations. Moreover, we included a stem clipping treatment to compare compensatory ability of populations, because tolerance to damage is known to have a heritable basis and can evolve in plant populations (Strauss & Agrawal 1999, e.g. Agrawal *et al.* 2004), and because *B. davidii* is able to recover from damage caused e.g. by frost or floods, by producing adventitious shoots and roots (Smale 1990).

In particular, we asked following questions:

(1) Is there evidence for genetic differentiation between invasive *B. davidii* populations in traits of growth and reproduction, and are these differences related to the climatic conditions of the populations' home site?

(2) Do the populations respond differently to the different transplant sites, indicating local adaptation to climatic conditions? In particular, do transplanted populations perform better at their home site than 'foreign' populations?

(3) How does clipping of aboveground biomass affect plant growth and reproduction, and does the effect of clipping vary between populations and among common gardens?

Methods

Study species

The Butterfly Bush (*Buddleja davidii* Franch., Scrophulariaceae) is a 3-5m tall multi-stemmed shrub. Height and basal diameter growth follows an exponential pattern indicating rapid early growth over the first 15 years, but declining later (Smale 1990). *B. davidii* is native to China and was introduced about 1890 for ornamental reasons to Europe. It usually flowers one year after germination (Esler 1988b), but sometimes already in the first year, usually from July to September. The terminal inflorescences are thyrsoid panicles appearing at current-year stems or branches and are up to 30 cm in length (Leeuwenberg 1979, Wu & Raven 1996). *B. davidii* is butterfly pollinated and mainly outcrossing (Susanne Schreiter, unpublished data). Each panicle may produce 100 to more than 1700 capsules (Kreh 1952, Brown 1990), and the small seeds are dispersed by wind or water (Campbell 1984). Both in its native and invasive range, *B. davidii* prefers naturally or anthropogenically disturbed areas, such as river banks, roadsides, and railways (Kunick 1970, Randall & Marinelli 1996), It tolerates a wide range of climatic and soil conditions (Kreh 1952, Webb *et al.* 1988). A first spread of the species occurred after World War II in bombed and wasted areas of several European cities. The species escaped from gardens also in North America, Africa, Australia and New Zealand, has become naturalized or invasive and can now be found on several plant watching lists (Tutin 1972, Leeuwenberg 1979, Webb *et al.* 1988, Csurshes & Edwards 1998, SKEW2008).

Experimental design

We carried out a common garden experiment replicated in three central European regions ranging from an oceanic to a sub-continental climate (Oldenburg and Halle in Germany, and Basel in Switzerland; Table 1). We used plants derived from seeds sampled in 20 invasive populations across western and central Europe (Figure 1, Table 1). The latitudinal gradient of sampling ranged from Northern Spain (43°) to Central England (53°) and the longitudinal gradient ranged from 3°W (Northern Spain) to 12°E (Eastern Germany), thus reflecting most of the European range of the species. In April 2005, seeds from five individuals per population were germinated separately on potting soil in a greenhouse. In June 2005, when plants were about 10 cm in height, two progeny of each of the five individuals were planted randomly in each of five blocks established in each common garden. Individuals were separated by a distance of 50 cm. To simulate loss of biomass that can be caused by floods and frost, we applied a clipping treatment. In April 2006, we clipped all stems 5 cm above ground of one of the two individuals per maternal plant in each garden using pruning shears. Within this common garden experiment, we implemented a reciprocal transplantation experiment in order to test the 'local vs. foreign' criterion (Kawecki & Ebert 2004). For this purpose, one population originating from each of the three places harboring the common gardens was included (Table 1).

In summer 2005, we quantified flowering phenology by monitoring the beginning and the duration of flowering of each plant every other day in the common garden in Halle. In January 2007, we measured several traits concerning plant size (number of stems, diameter of the thickest stem, and length of five randomly selected stems per individual) and reproduction (number of inflorescences per individual, length of 20 randomly chosen inflorescences per individual), and harvested all above-ground plant parts. We determined reproductive biomass (biomass of inflorescences) and vegetative biomass separately by weighing it after drying at 60 °C. We calculated total above-ground biomass as the sum of reproductive and vegetative biomass, and reproductive effort as the ratio of reproductive biomass to total biomass.

Table 1.   List of 20 sampled populations of *Buddleja davidii* in Europe with climatic data according to Hijmans *et al.* (2005).

Population	coordinates		Altitude a.s.l. [m]	Mean annual temperature [°C]	Mean maximum temperature of the warmest month [°C]	Mean minimum temperature of the coldest month [°C]	Mean annual precipitation [mm]
	latitude	longitude					
France / St. Malo	48.6122 N	2.0525 W	40	11.23	21.0	2.8	737
Germany / Cologne	50.9627 N	6.9647 E	46	10.13	24.5	-0.8	770
Germany / Darmstadt	49.8894 N	8.6380 E	127	10.13	24.5	-2.4	642
Germany / Duisburg	51.4827 N	6.7856 E	30	9.72	23.2	-0.5	804
Germany / Essen	51.4652 N	7.0266 E	37	10.12	22.7	-0.6	834
Germany / Halle	51.4811 N	11.9683 E	99	9.91	23.4	-2.2	483
Germany / Oldenburg	53.1438 N	8.2138 E	68	9.10	22.8	-0.6	829
Germany / Seligenstadt	50.0402 N	8.9681 E	114	9.95	24.7	-2.0	639
Germany / Sulzbach	48.8630 N	8.3711 E	470	9.99	23.1	-3.1	867
Germany / Tettnang	47.6300 N	9.5847 E	447	8.76	23.4	-3.7	1063
Italy / Merano	46.6667 N	11.1666 E	402	10.90	27.3	-4.2	795
Spain / Leioa	43.3277 N	2.9869 W	77	12.66	22.9	6.2	1203
Switzerland / Basel	47.5472 N	7.5892 E	280	14.03	24.6	-0.8	782
Switzerland / Geneve	46.2083 N	6.1428 E	387	10.04	25.9	-1.8	934
UK / Eastleigh	50.9666 N	1.3506 W	15	10.29	21.9	1.3	764
UK / Egham	51.4305 N	0.5467 W	17	10.51	23.0	0.0	651
UK / Manchester	53.4777 N	2.2456 W	50	10.25	21.8	1.6	932
UK / Reading	51.4527 N	0.9631 W	42	10.50	21.9	0.0	701
UK / Wallingford	51.6000 N	1.1258 W	48	9.96	21.4	0.5	657
UK / Yarmouth	50.7027 N	1.4967 W	1	10.57	21.8	1.8	787

Figure 1.   Map of the location of the 20 sampled invasive populations in Europe.

Seed mass was assessed by weighing a bulk sample of 100 seeds for each plant, and dividing this value by 100.

In order to assess the influence of the different soil conditions in the three common gardens on plant growth, we carried out an additional greenhouse experiment. We sampled the upper soil layer at five randomly chosen locations within each garden, and mixed these samples for each garden. In April 2006, we sowed seeds from five individuals per population into 1L plastic pots filled with soil of each garden. After germination, we reduced offspring to one seedling per maternal plant and kept the total of 300 pots in an unheated greenhouse. During winter, plants were held in a greenhouse at 10°C. In July 2007, we harvested the aboveground biomass and dried it for 4 days at 60°C. In addition, we determined chemical characteristics of the three soils: pH values in 0.1 N KCl solution using a Calimatic pH meter Typ 765 (Knick elektronische Messgeräte GmbH & Co, Berlin, Germany), C and N concentrations by dry combustion and subsequent gas analysis using an Element Analyser Vario EL

(Elementar Analysesysteme GmbH, Hanau, Germany), and conductivity (TetraCon 325 and WTW Cond 315i, Wissenschaftlich-Technische Werkstätten GmbH & Co. KG, Weilheim, Germany).

Statistical Analyses

We compared the probability of survival, and the probability of flowering between gardens and populations using a generalized linear model with binomial error distribution and logit link function (procedure GENMOD, SAS version 9.1). All other data from the common garden experiments were analyzed with general mixed effect models (procedure MIXED, REML method), with garden and clipping as fixed effects, and block, population as wall as their interactions as random effects. The length of the longest leaf at the beginning of the experiment was included as a covariate to account for initial seedling size. Random effects were assessed by comparing the full model with the reduced model using a likelihood ratio test (Littell *et al.* 1996). The same model was applied to the reduced data set of the three reciprocally transplanted populations (Basel, Halle and Oldenburg). Since the data were unbalanced due to different mortality between gardens, we used type III sum of squares (Shaw & Mitchell-Olds 1993). In case of length of stem and length of inflorescence, we used the mean value of each plant for statistical analysis to avoid pseudo-replication. While reproduction effort was arcsine square-root transformed, the other dependent variables and the covariate were log-transformed to normalize their distribution prior to analysis. P-values from the model were corrected by Benjamini and Hochberg FDR procedure (Verhoeven *et al.* 2005) to account for multiple comparisons without inflating the likelihood of type II errors (Cabin & Mitchell 2000).

Since flowering phenology was only measured in the common garden in Halle and before the clipping treatment took place, we used the procedure MIXED as described above, but without garden and clipping as fixed factors. To compare the effects of the different soils in the additional greenhouse experiment, we used the

procedure GLM with origin of soil as fixed factor. The same procedure was applied to compare soil chemical properties between the three common gardens.

To investigate whether there is a relationship between plant traits, and climatic conditions of the populations' home sites, we calculated the population means of each plant trait across the three common gardens, and correlated these values with climatic variables, geographic coordinates, and altitude of sampling locations using Pearson's product-moment correlation. As climatic variables we extracted mean annual temperature, mean maximum temperature of the warmest month, mean minimum temperature of the coldest month, and mean annual precipitation from http://www.worldclim.org (Hijmans *et al.* 2005). Since the 'local vs. foreign' criterion could only be tested for the three reciprocally transplanted populations (see above), we used the correlation between plant fitness and the geographic distance of the common garden from the populations' home site as a proxy for the 'home vs. away' effect. However, it has to be kept in mind that the 'home vs. away' criterion confounds the effects of divergent selection with intrinsic differences in habitat quality, and is thus not as diagnostic for the pattern of local adaptation as the 'local vs. foreign' is (Kawecki & Ebert 2004). In addition, we analysed whether differentiation among populations in growth and reproductive traits are simply due to isolation by distance: We correlated all pairwise phenotypic distances and geographic distances among populations, and used Mantel's t-test based on Pearson's product-moment correlation to test for statistical significance. Since we intended to consider the whole set of plant traits in this analysis, we did not use Q_{ST} values to characterize population differences in quantitative traits (Storz 2002) but Euclidian distance among populations based on all measured traits after standardization.

Results

Mortality of *Buddleja davidii* differed significantly between the three common gardens (log likelihood ratio = 34.19, p < 0.001, d.f. = 2), but not among populations (log likelihood ratio = 27.38, p = 0.096, d.f. = 19). After 19 months, mortality was highest in the common garden in Basel (28%), lowest in Halle (6.5%), and intermediate in Oldenburg (16%). The population x garden interaction was not significant.

Across the three common gardens, 20.7% of all plants flowered already in 2005, i.e. within the first year after germination. The probability of flowering in the first year was significantly different between the gardens (log likelihood ratio = 183.56, p < 0.001, d.f. = 2) and was largest in Halle (52.5%), intermediate in Oldenburg (7%) and lowest in Basel (0.05%). Moreover, populations also differed significantly in their probability of flowering (log likelihood ratio = 59.05, p < 0.001, d.f. = 19). The garden x population interaction was not significant. For plants in Halle, the beginning of flowering was on average 133.78 ± 2.2 days (mean ± s.e.) after germination and differed significantly between the populations ($F_{19,83} = 1.90$, p = 0.025) while there was no difference in the duration of flowering ($F_{19,84} = 1.42$, p = 0.141).

Plant size at the beginning of our experiment (measured as length of the longest leaf) significantly influenced biomass and number of inflorescences at final harvest (Table 2). Our study populations differed significantly in all growth and reproductive traits among the gardens, as growth conditions were apparently best in the common garden in Halle and worst in Basel. For example, plants in Halle and Oldenburg produced on average 85% and 73% more biomass than plants growing in Basel (Figure 2). Similar differences were detected in reproductive traits, i.e. plants in Halle and Oldenburg developed more (78% and 58%), larger (31% and 20%) and heavier (89% and 78%) inflorescences than plants in Basel.

Clipping of stems in spring was not completely compensated during the following growing period, as total biomass, stem diameter and reproductive biomass were significantly smaller in clipped plants compared to untreated plants (Figure 3).

Table 2. Summary of mixed model ANCOVAs conducted for 20 invasive *Buddleja davidii* populations, planted in three common gardens in Oldenburg, Halle and Basel. Plant size at the beginning of the experiment was used as covariate. Numbers in brackets indicate error degrees of freedom for reproductive traits. F-values are given for fixed effects, while log likelihood ratios are given for random effects (levels of significance: * $\alpha < 0.05$, ** $\alpha < 0.01$, *** $\alpha < 0.001$, after applying the Benjamini-Hochberg procedure to growth-related and reproductive traits in order to account for multiple comparisons).

|	Df / error df	F - value (for fixed effects) or log likelihood ratio (for random effects)								
		Growth-related traits				Reproductive traits				
		total biomass	no. of stems	length of stems	diameter of stems	reprod. biomass	reprod. effort	no. of inflores-cences	length of inflores-cences	seed mass
Fixed effects										
garden	2 / 12	52.95 ***	15.28 ***	92.21 ***	60.92 ***	37.01 ***	15.84 ***	15.85 ***	25.91 ***	8.37 **
clipping	1 / 19	38.61 ***	4.00	4.34	60.11 ***	19.24 **	0.04	4.02	0.03	1.53
garden x clipping	2 / 38 (35)	4.12 *	1.29	4.36 *	11.45 ***	6.28	1.10	1.13	2.28	0.06
Random effects										
covariate	1	31.60 ***	3.70	2.80	0.90	4.60 ***	0	6.40 *	0	0
block (garden)	1	34.20 ***	0	0	0	7.70 ***	0	0	0	4.10
population	1	3.80	4.00 ***	0.30	0	2.10 ***	10.60 ***	3.60	3.60	3.50
garden x population	1	0	0	0	0	0.30	0	0.60	0.40	0
clipping x population	1	0	0.20	0	0	0.50	1.70	0.60	0.60	0
garden x clipping x population	1	24.20 ***	0	0	0	0	0.60	0	0	0
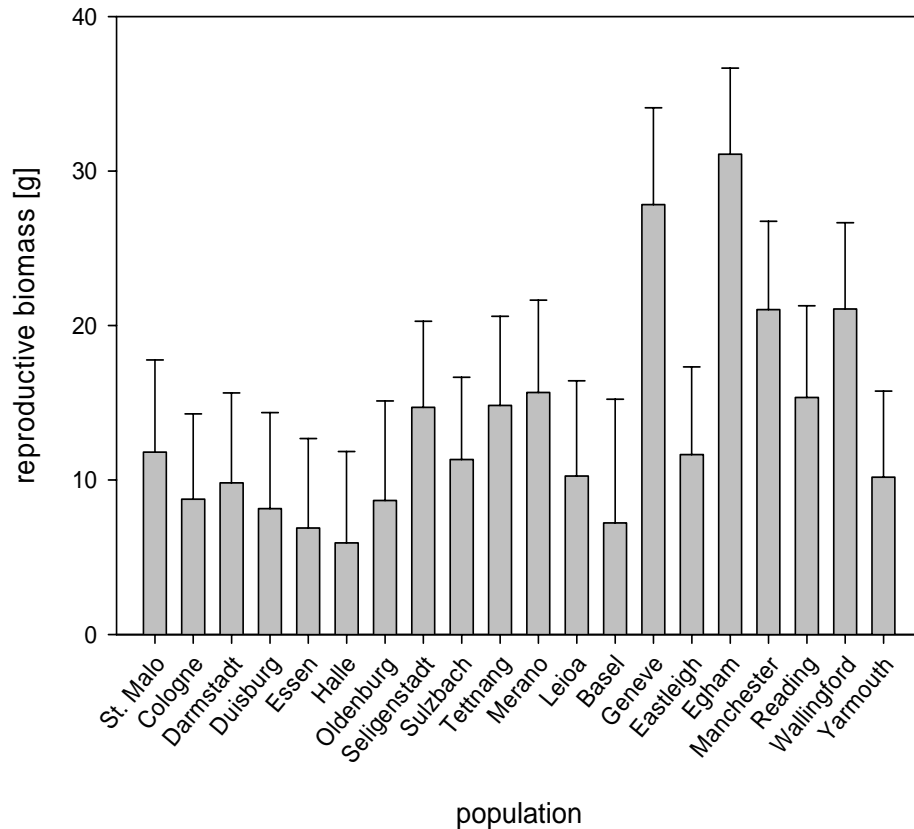
Figure 2.   Differences in reproductive biomass between 20 invasive *Buddleja davidii* populations, across all three common garden experiments conducted in Oldenburg, Halle and Basel (least square means ± s.e.). The order of the populations corresponds to Table 1.

Although the other growth and reproductive traits (except seed mass) showed the same tendency, the differences were not significant. Surprisingly, albeit also not significant, seed mass increased after clipping in all three common gardens. The significant garden x clipping interactions in growth-related traits indicate that compensatory ability of plants was strongly dependent on growing conditions of each site. The difference between unclipped and clipped plants in biomass and stem diameter was smallest in Basel (10% and 46% reduction, respectively), intermediate in Halle (30% and 50% reduction) and largest in Oldenburg (39% and 59% reduction). Hence, there was obviously no simple relationship between "habitat quality" of the common gardens (as indicated by growth of unclipped plants) and the amount of regrowth.
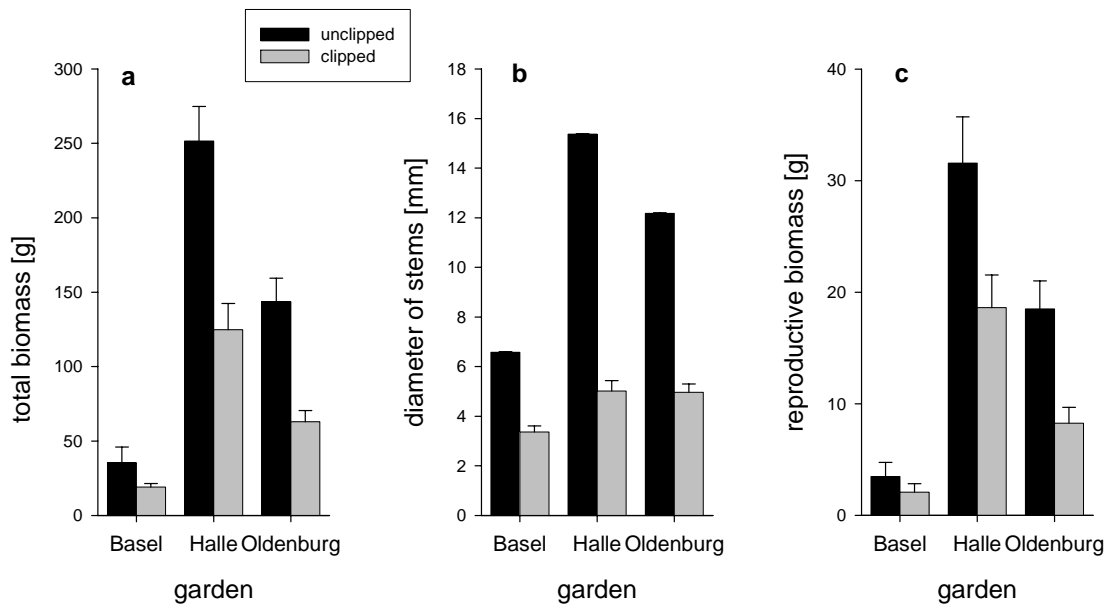
Figure 3.   Comparison of (a) total biomass, (b) diameter of stems and (c) reproductive biomass (mean + s.e.) between unclipped and clipped plants of 20 invasive *Buddleja davidii* populations from Europe growing in common gardens at three different sites.

Populations differed significantly in the number of stems, reproductive biomass and reproductive effort whereas there was no difference among populations in all other traits measured (Figure 2, Table 2). There was also no difference in the response of the 20 populations to the different common gardens, as indicated by the non-significant garden x population interaction. However, populations responded significantly differently to the clipping treatment in each garden in terms of total biomass (garden x clipping x population interaction, Table 2). We did not find significant correlations between any traits of growth or reproduction, and climatic variables or geographic coordinates of the populations' home sites ($-0.41 < r < 0.43$, n = 20). There was also no significant relationship between phenotypic distance and geographic distance, indicating that any variation in plant traits between populations did not result from isolation by distance ($r = 0.17$, $p = 0.095$, Mantel's t-test).

Table 3. Summary of mixed model ANCOVAs conducted for the invasive *Buddleja davidii* populations from Oldenburg, Halle and Basel that were reciprocally transplanted between the three common gardens. Plant size at the beginning of the experiment was used as covariate. F-values are given for fixed effects, while log likelihood ratios are given for random effects (levels of significance: * $\alpha < 0.05$, ** $\alpha < 0.01$, *** $\alpha < 0.001$, after applying the Benjamini-Hochberg procedure to growth-related and reproductive traits in order to account for multiple comparisons).

	Df / error df	F - value (for fixed effects) or log likelihood ratio (for random effects)								
		Growth-related traits				Reproductive traits				
		total biomass	no. of stems	length of stems	diameter of stems	reprod. biomass	reprod. effort	no. of inflores-cences	length of inflores-cences	seed mass
Fixed effects										
garden	2 / 4	13.79 *	1.67	19.08 *	22.08 *	22.14 *	1.64	7.71	16.05 *	7.98
clipping	1 / 2	8.59	0.42	3.19	10.78	5.01	1.45	4.22	4.26	0.05
garden x clipping	2 / 4	1.09	1.86	0.19	0.42	1.89	1.30	1.5	0.72	1.54
Random effects										
covariate	1	2.9	0	1.4	0.6	0	0.8	0.1	0	0
block (garden)	1	2.9	0	1.4	0.6	0	0.1	0.1	0	0
population	1	0.4	1.2	0.1	0.1	0	0	0.2	0	0
garden x population	1	0	0	0	0	0	0.1	0.1	0	0
clipping x population	1	0	0.1	0	0	0.1	0	0.4	0	0.1
garden x clipping x population	1	0	0.2	0	0	0	0	0	0	0

We used the relationship between average plant fitness (in terms of reproductive biomass) and distance of the common garden from the populations' home site as proxy for the 'home vs. away' effect, but we could not reveal a significant correlation (for Basel: r = 0.174, p = 0.075, for Halle: r = -0.028, p = 0.487, for Oldenburg: r = 0.067, p = 0.235; Mantel's test).

Considering the three populations reciprocally transplanted between the three places, we found significant differences between the gardens in total biomass, reproductive biomass, length of stems, diameter of stems and length of inflorescences. However, there were no significant garden x population interactions (Table 3) that would be expected in case of local adaptation of these three populations (i.e. if the 'local vs. foreign' effect would be significant).

In the greenhouse experiment, soil conditions significantly affected plant performance, especially biomass ($F_{2,3} = 20.18$, $p < 0.001$). Plant biomass was, on average, lowest in the soil from Oldenburg (5.18 g ± 0.42 g, mean ± s.e.), intermediate in the soil from Halle (8.04 g ± 0.35 g), and highest in the soil from Basel (8.48 g ± 0.42 g). This contrasts the results of the common garden, as non-clipped plants growing in Halle and Oldenburg produced much biomass (251.52 g ± 23.27 g and 143.68 g ± 15.76 g) than plants in Basel (35.38 g ± 10.61 g). Our soil analysis showed that there are significant differences between sites in terms of pH values, C_{total} and N_{total} (Table 4). All three soils show a small C/N ratio indicating a high availability of nitrogen. The conductivity varied to some extent between gardens, but the differences were not significant.

Table 4.   Comparison of soil chemical properties of the three common gardens (degrees of freedom for F-tests: 2, 3; levels of significance: ** $\alpha < 0.01$, *** $\alpha < 0.001$).

garden	pH (in KCl)	C total	N total	C:N	conductivity [µS]
Basel	8.16 ± 0.08	1.24 ± 0.06	0.08 ± 0.00	16.34 ± 3.99	323 ± 47
Halle	7.84 ± 0.13	1.99 ± 0.04	0.16 ± 0.02	12.28 ± 2.29	359 ± 34
Oldenburg	5.92 ± 0.60	3.64 ± 0.16	0.27 ± 0.01	13.59 ± 0.46	241 ± 38
F-value	107.06 ***	58.38 ***	86.22 ***	5.10**	6.83**

Discussion

The results of our study provide evidence for genetic differentiation in ecological relevant, phenotypic traits of invasive populations of *B. davidii* of central and west Europe indicated by a significant population effect and a significant environment x clipping x population interaction. Among the gardens, growth and reproductive traits differed highly significantly but all study populations responded in a similar way to the respective physical environment. Moreover, our expectation that plant fitness would decrease with increasing geographic and climatic distance to the home site did not met. We found no relationship between plant traits and the location of the home site, neither in growth and reproduction traits nor in flower phenology, although such patterns of latitudinal population differentiation has been found in several plant species (Weber & Schmid 1998, Kollmann & Banuelos 2004, Maron *et al.* 2004, e.g. Becker *et al.* 2006). Even the reciprocal transplant experiment, which was incorporated in the common gardens, revealed no evidence for the 'local vs. foreign' criterion. Altogether, these findings provide no support for our hypothesis, that local adaptation to climatic conditions has facilitated the spread of invasive *B. davidii* populations in Europe.

The evolution of locally adapted genotypes requires sufficient genetic variation in ecologically relevant traits, which can be impaired by founder effects and genetic drift after introduction (Novak & Mack 1993, Falconer & MacKay 1996, Allendorf & Lundquist 2003). At least among our invasive *B. davidii* populations, we found substantial genetic variation in some plant traits, and their response to the combination of growing conditions and clipping. Although invasive plant species were often introduced in small numbers and invasive populations often represent only a small proportion of native genotypes, genetic diversity may increase due to multiple introductions, hybridisation and introgression as well as artificial selections (Ellstrand & Schierenbeck 2000, Mooney & Cleland 2001, Allendorf & Lundquist 2003, Ross *et al.* 2008). Plant breeding probably has an important impact on invasion success of non-native species by promoting genetic differentiation between populations (Kitajima *et al.*

2006). *Buddleja davidii* was introduced as an ornamental plant and breeders focused on leaf and flower colour, inflorescence morphology, growth and frost hardiness (Albrecht 2004, Wilson *et al.* 2004a) resulting in more than 90 cultivars (Stuart 2006). Thus, it is likely, that invasive populations consist of progeny of cultivars rather than of native genotypes of *B. davidii*. Depending on frequency and residence time of cultivars provided on market, the probability of escape from cultivation rises (Dehnen-Schmutz *et al.* 2007). Thus, variation among invasive *B. davidii* populations may simply reflect genetic differences between cultivars haphazardly planted at each location and that gave rise to the invasion. Furthermore, gene flow may constrain local adaptation as argued by Kawecki & Ebert (2004). If we assume that especially populations at range margins evolve in response to particularly harsh climatic conditions, then genetic swamping from central populations and/or from planted cultivars would lead to maladapted populations and might prevent local adaptation (Bridle & Vines 2007). The popularity of *B. davidii* and their cultivars causes national and international trade and transport, wherefore genotypes were frequently mixed. This may explain why populations are obviously not isolated by distance, and at the same time, why local adaptation did fail so far.

The absence of significant results may also be attributed to the short time period since the first successful spread of *B. davidii* in Europe. The plant was introduced about 1890, while its first spread started after World War II. Several studies demonstrated that plants can evolve in response to recent environmental conditions within few generations (Davison & Reiling 1995, Thompson 1998, Bone & Farres 2001). For *B. davidii,* the response to selection may be masked by changes in environmental conditions at the new site that take place at the same time (Cooke *et al.* 1990). Perhaps, the selection regime for invasive populations of *B. davidii* imposed by climate is not as strong as in other well-known studies of local adaptation, for example in response to heavy metals or herbicide application (Bone & Farres 2001). However, this would not explain why local adaptation along climatic gradients has been found in other native and invasive plant species (Becker *et al.* 2006, Kollmann & Banuelos 2004, Weber & Schmid 1998).

We also compared the performance of unclipped and clipped plants and their compensatory ability. The artificial clipping was designed to mimic heavy damage by frost, wind or water, which *B. davidii* is apparently able to tolerate by regrowth of new stems (Smale 1990). Moreover, gardeners recommend severe pruning of the bush in spring and removal of old wood to enhance growth of new basal stems, and to produce denser and larger inflorescences (Miller 1984, Armitage & Dirr 1995, Ream 2006). Our results demonstrated that clipped plants do not produce more stems, but stems of smaller diameter. Although plants nearly fully compensate the length of stems, this is neither mirrored by reproductive biomass nor by total biomass which is nearly reduced by the half. Surprisingly, seed mass was positively influenced albeit not significantly: clipped individuals produced slightly heavier seeds. These results are contrasting to Lennartsson *et al.* (1997, 1998), who found significantly increased fruit production while number of seeds per fruit and seed mass were not effected by clipping in *Gentianella campestris*. One explanation could be the timing of clipping, because damage early in the season might be associated with high tolerance (Strauss & Agrawal 1999): e.g. *Gentianella campestris* only overcompensated during a restricted time period, but did not before or after this period (Lennartsson *et al.* 1998). This might be due to the availability of resources that can be mobilized for re-growth (Chapin & McNaughton 1989, Trumble *et al.* 1993, Rosenthal & Kotanen 1994, Lennartsson *et al.* 1998, Hochwender *et al.* 2000). Another reason for overcompensation after damage has been revealed by Paige & Whitham (1987) who explain increased fruit and seed set by a change in plant architecture as a result of growth of secondary shoots from basal meristems. This is conceivable for *B. davidii* which can recover by producing adventitious shoots and roots (Smale 1990), but despite early-season clipping we found no overcompensation.

Apart from climatic effects, the significant differences in plant growth which we have revealed between the three common gardens, might also be produced by soil differences, that we could not exclude from our experiment. The three gardens showed differences in soil characteristics, which may have been mirrored by the performance of *B. davidii* plants. To disentangle the effects of soil conditions we conducted an

additional greenhouse experiment using soils from the different gardens. However, plant growth in the common gardens was not correlated with growth in the different soils in the greenhouse; moreover, the rank order of the three sites or soils, respectively, was reversed. Thus, we feel confident that the differences among the common gardens are not simply the result of the different soils but reflect differences between the whole set of abiotic and biotic factors.

In conclusion, our study revealed no hints for local adaptation to climatic conditions among invasive European *B. davidii* populations. Our data rather suggest that high phenotypic plasticity of these populations allows them to grow across a wide range of climatic and soil conditions, thus contributing to the successful spread of the species. The importance of phenotypic plasticity for invasion success has also been demonstrated by other studies (e.g. Sexton *et al.* 2002, Parker *et al.* 2003, Ross & Auge 2008)Further studies on the importance of local adaptation and phenotypic plasticity for invasion success should incorporate multiple common garden experiments on different spatial scales, both across continents and across different places within the native and the invasive range. In addition, molecular studies on invasive populations and traded cultivars would provide more information about the impact of plant breeding on genetic differentiation between and among populations and on the potential for rapid evolution in *B. davidii*. Furthermore, it can be assumed that the species does not have filled out its potential distribution range, and that the invasion process will proceed (Ebeling *et al.* 2008a, accepted). Hence, we should watch out the further spread and impact of this species, especially with respect to the negative experiences of New Zealand (Smale 1990, Richardson *et al.* 1999).

Chapter 4

Predicting the spread of an invasive plant: Combining experiments and ecological niche model

with Erik Welk, Harald Auge and Helge Bruelheide

Abstract

Rapid evolutionary adjustments to novel environments may contribute to the successful spread of invasive species, and can lead to niche shifts making range dynamics unpredictable. These effects might be intensified by artificial selection in the course of breeding efforts, since many successful plant invaders were deliberately introduced and cultivated as ornamentals. We hypothesized that the invasion success of *Buddleja davidii*, the ornamental Butterfly Bush, is facilitated by local adaptation to minimum temperatures and thus, exhibits unpredictable range dynamics. To assess the potential effects of adaptive evolution and artificial selection on the spread of *B. davidii*, we combined a common garden experiment investigating local adaptation to frost, with ecological niche modelling of the species' native and invasive ranges. We expected that populations naturalized in sub-continental climate are less susceptible to frost than populations from oceanic climate, and that the invasive range does not match predictions based on climatic data from the native range. Indeed, we revealed significant variation among invasive *B. davidii* populations in frost resistance. However, frost hardiness was not related to geographic location or climatic variables of the populations' home site, suggesting that invasive *B. davidii* populations are not locally adapted to minimum temperatures. This is in line with results of our ecological niche model that did not detect a niche shift between the species' native range in China, and its invasive range in Europe and North America. Furthermore, our niche model showed that the potential invasive range of *B. davidii* is still not completely occupied. Together with the frost resistance data obtained in our experiment, the results indicate that climatic conditions are currently not limiting the further spread of the species in Europe and North America.

Introduction

Spatial distributions of species are determined by intrinsic factors, such as population dynamics and genetic constitution, as well as by historical and recent environmental filters acting on several spatial and temporal scales, e.g. by physical barriers, resources, and biotic interactions (e.g. Woodward & Williams 1987). On large spatial scales, climatic conditions are assumed to be the critical environmental control for the distribution of the majority of species. In the temperate zone, frost usually acts as distribution barrier (Franklin 1995), limiting the geographical range of many herbaceous and woody species (e.g. Sakai & Wardle 1978, Bannister & Polwart 2001, Bruelheide 2002). Predicting the geographical ecology of invasive species is of special importance because biological invasions are regarded as one of the most serious threats to global biodiversity and may cause economic and ecological problems (Sala *et al.* 2000). The application of species distribution models to biological invasions is therefore a promising area of applied ecology (Willis & Hulme 2002, e.g. Welk *et al.* 2002). Predictions of the potential range of invasive species are usually based on climatic variables obtained in the species' native range, thus assuming niche conservatism (see Wiens & Graham 2005). Niche shifts during biological invasions may therefore limit the predictability of range dynamics (Broennimann *et al.* 2007, Urban *et al.* 2007).

Several ecological and evolutionary processes can lead to a shift of the realized niche in the introduced range. First, invasive populations are often regulated by other abiotic or biotic factors compared with populations in their native range (Hierro *et al.* 2005). For instance, they may escape from biological constraints on growth and reproduction (EICA hypothesis, Blossey & Nötzold 1995, Mooney & Cleland 2001, Thebaud & Simberloff 2001, Keane & Crawley 2002) and, in consequence, grow much more vigorously (e.g. Ebeling *et al.* 2008b).

Second, spread of invasive species may not only be facilitated by certain pre-adapted traits (Callaway & Aschehough 2000), but also by evolutionary changes after introduction (Mack *et al.* 2000, Leger & Rice 2003, Parker *et al.* 2003). For instance, local adaptation to climatic conditions may increase the invaders' distribution (Parker *et al.*

2003), which has been revealed for several invasive plant species (Kollmann & Banuelos 2004,Weber & Schmid 1998). Changes in the ecological or climatic tolerances of invasive species can thus greatly contribute to our general understanding of how evolutionary processes contribute to range dynamics (Bridle & Vines 2007). Furthermore, a significant proportion of invasive plant species was introduced as ornamentals (Weber 2003), and has therefore been subject to artificial selection which often changes ecologically relevant traits (Kitajima *et al.* 2006). One focus in plant breeding is an increase in survival, e.g. by improving frost hardiness in the temperate zone (Albrecht 2004). It can therefore be hypothesized that evolutionary changes, including the results of plant breeding, affect niche properties, thereby leading to unpredictable range dynamics.

We tested this hypothesis using the butterfly bush, *Buddleja davidii*, as study system. This shrub species is native to China and was introduced to Europe for ornamental reasons about 1890. Today, it is a successful invader of anthropogenic and natural habitats in Europe, North America and New Zealand. In Europe, invasive *B. davidii* populations grow predominantly in oceanic regions of the temperate and Mediterranean climate zone, while their spread to continental regions seems to be limited by a lack of frost tolerance (Krivanek & Pyšek (2006). Nevertheless, there is some evidence for variation in frost hardiness between *B. davidii* cultivars (Albrecht 2004), which may provide the basis for local adaptation to winter temperatures. We combined an experimental approach and ecological niche modelling to investigate the following questions:

(1)   Are invasive *B. davidii* populations locally adapted to minimum temperatures?

(2)   Is there evidence for a niche shift which impairs the prediction of the potential invasive range based on the native range?

(3) Is frost effectively limiting the further spread of this species across Europe and North America?

Common garden and reciprocal transplantation experiments are two powerful methods to test for local adaptation (Sakai *et al.* 2001). Reciprocal transplantation experiments allow comparing fitness of populations in their 'home' environment with fitness of 'foreign' populations (Kawecki & Ebert 2004) but for woody perennials, results are often constricted to short periods of the life-cycle (Ross *et al.* 2008, in press). We therefore applied an alternative approach and correlated results from a common garden experiment with environmental variables from the home site of each population (e.g. Anderson *et al.* 1996, Weber & Schmid 1998). We used progeny of 20 invasive *B. davidii* populations sampled across Western and Central Europe, and quantified frost resistance of leaves rather than plant fitness of this perennial species, which was beyond the time extent covered by this article. In case of local adaptation to winter temperatures, we expected a geographic cline in frost resistance: populations from oceanic regions should be more susceptible to frost than populations from sub-continental regions with low winter temperatures, in particular if resistance traits incurred a fitness cost (cf. Agrawal *et al.* 2004).

Macroecological range analyses can be regarded as additional approach to detect evolutionary changes: macroclimatic niche properties are intrinsic characteristics and thus important preconditions enabling a species to spread into new environments. If evolutionary changes resulted in niche expansions, this should become evident in differences of the macroecological niches between the native and the invaded range. Thus, we analyzed the native macroclimatic niche of *B. davidii* and compared it with the climatic properties of the currently invaded range. We performed ecological niche models based on native distribution data, and statistical comparisons of climatic data between native range and invaded range. Ecological niche models are commonly used to predict the geographic range of a taxon from presence/absence data and environmental variables (Pulliam 2000, Austin 2002). Regarding *B. davidii*, we expected indications of niche expansion in the invaded range, and furthermore, that the supposed frost-susceptibility (Krivanek & Pyšek 2006) was detectable in the limitation of the native macroclimatic niche, and that this limit is at least approached in the invaded range.

Materials and Methods

Study species

Buddleja davidii Franch. is a deciduous or semi-evergreen (Owen & Whiteway 1980) shrub of 2 to 5 m height (Wu & Raven 1996) and reaches an age of up to 30 years (Smale 1990). The species is native to the Chinese provinces Gansu, Guangdong, Guangxi, Guizhou, Hubei, Hunan, Jiangsu, Jiangxi, Shaanxi, Sichuan, Xizang, Yunnan, and Zhejiang, where it occurs as thickets on mountain slopes and along draws in mountains at elevations from 800 - 3000 m (Wu & Raven 1996). Since its introduction to Europe as an ornamental shrub at the end of 19th century, about 70 *B. davidii* cultivars have been bred by gardeners (Dirr 1997). In Europe and North America, *B. davidii* invades in particular naturally or anthropogenically disturbed habitats such as riverbanks, railway embankments, and brown fields due to tolerance of drought as well as flood events (Kunick 1970, Randall & Marinelli 1996).

The growth rate of *B. davidii* plants is at least 0.5 m per year. Although flowering typically occurs in the second year after germination, it can already happen in the first year. The plant flowers from July to late September. The terminal inflorescences are thyrsoid panicles, appearing at current-year stems or branches (Wu & Raven 1996). *B. davidii* has an enormous seed production of up to 3 million seeds per plant (Kreh 1952, Campbell 1984). Due to their low weight, seeds are well dispersed by wind and water (Campbell 1984). Until now, a detailed distribution dataset for the native and invaded ranges of the species has not been available. While numerous records of naturalized populations can be found documenting the occurrence in western European countries and regions after World War II (see Kreh 1952), they are missing for east European regions. In Europe, naturalized *B. davidii* populations grow predominantly in oceanic regions of temperate and Mediterranean zones (Rothmaler 2002), and their current eastern distribution border runs approximately along the 15° E meridian. Krivanek & Pyšek (2006) assume that the further spread of *B. davidii* into Eastern Europe is limited by a lack of frost tolerance. However, the Association of German Nurseries evaluated 21 currently traded *B. davidii* cultivars, and assessed four of them as highly frost

resistant and eight as indifferent (Albrecht 2004), indicating heritable variation in frost resistance.

Common garden experiment and measurement of frost resistance

In winter 2005/2006 we sampled seeds from 20 invasive populations of *B. davidii*, trying to cover the invaded range in Europe as much as possible (Figure 1, Table 1). This enabled us to analyze our experimental data in relation to the present climatic gradients. In April 2005, seeds of five individuals per populations were germinated in potting soil in a greenhouse.

Figure 1.   Map of the location of the 20 sampled invasive populations in Europe.

Table 1.   List of 20 sampled populations of *Buddleja davidii* in Europe.

| Population | coordinates | | Altitude |
	latitude	longitude	a.s.l. [m]
France / St. Malo	48.6122 N	2.0525 W	40
Germany / Cologne	50.9627 N	6.9647 E	46
Germany / Darmstadt	49.8894 N	8.6380 E	127
Germany / Duisburg	51.4827 N	6.7856 E	30
Germany / Essen	51.4652 N	7.0266 E	37
Germany / Halle	51.4811 N	11.9683 E	99
Germany / Oldenburg	53.1438 N	8.2138 E	68
Germany / Seligenstadt	50.0402 N	8.9681 E	114
Germany / Sulzbach	48.8630 N	8.3711 E	470
Germany / Tettnang	47.6300 N	9.5847 E	447
Italy / Merano	46.6666 N	11.1666 E	402
Spain / Leioa	43.3277 N	2.9869 W	77
Switzerland / Basel	47.5472 N	7.5892 E	280
Switzerland / Geneve	46.2083 N	6.1428 E	387
UK / Eastleigh	50.9666 N	1.3506 W	15
UK / Egham	51.4305 N	0.5467 W	17
UK / Manchester	53.4777 N	2.2456 W	50
UK / Reading	51.4527 N	0.9631 W	42
UK / Wallingford	51.6000 N	1.1258 W	48
UK / Yarmouth	50.7027 N	1.4967 W	1

In June 2005, two individuals of each population were planted in one of five blocks (10 plants per population overall) in a common garden in Halle (Germany, lat. 51.489664°, long. 11.961178°) characterized by sub-continental climate (for the period 1961-1990: mean annual temperature = 9.1°C, mean annual temperature amplitude = 18°C, mean annual precipitation = 459 mm, mean minimum temperature of the coldest month = -2.3°C, mean annual number of frost days: 72).

We used two methods to investigate frost resistance of leaves: First, we quantified frost damage by estimating the percentage of damaged area (brown spots or patches) of 20 randomly chosen leaves per plant visually in January 2007. We carefully chose this point in time when the plants had experienced first heavy frost periods (below -4°C in December) but still retained their leaves, which enabled us to get reliable estimates of frost damage. Second, we randomly sampled five leaves that were not damaged from each of five individuals per population to quantify frost hardiness using the electrolyte leakage method following Murray (1989). This method is based on the release of electrolytes through damaged membranes. The resulting electrolyte concentration in solution can then be measured as conductivity. The rate of electrolyte leakage is a first order process which is defined by a rate constant that varies directly with the extent of tissue damage (Murray *et al.* 1989). Leaves were exposed to control temperature at 4°C and to freezing temperatures at four levels (0°C, -4°C, -8°C, -16°C) in a climate test chamber (Sanyo MTH-4400). Each temperature level lasted for half an hour with a cooling rate of +4°C per hour between treatments. At the end of each temperature level, one leaf of each of the five individuals per population was removed from the freeze chamber and brought into a refrigerator at +4°C.

One day after frost treatment one disc of 10 mm diameter per leaf was cut from each leaf and placed in tubes with 3% iso-propanol. There were five replicates per population and per frost treatment, resulting in a total of 500 samples. The electrical conductivity of the solution in each test tube was measured at 4°C using an automated sample processor with conductivity meter (Metrohm) at the beginning (C_0), after 4, 24, 48, 72 hours (C_1 - C_4), each time after vortex mixing. The reference value was measured as the conductivity after the leaf disc in the solution was boiled for 20 minutes (C_{boiled}). The relative conductivity (RC) of each time series was fitted to a logistic regression (Murray *et al.* 1989; statistical software SAS 8.2):

$$RC = \frac{C_t - C_0}{C_{boiled} - C_0} = 1 - e^{-k*t}$$

The parameter k increases with the rate for electrolyte leakage and can therefore be used as an inverse measure of frost hardiness.

Statistical analysis of frost resistance

We applied a mixed-model ANOVA to test for differences between populations in terms of frost damage of leaves in the common garden (procedure MIXED, REML method, SAS version 9.1). Another mixed-model ANOVA was applied to the parameter k, thereby using the population x temperature interaction to test for differences in frost hardiness between populations. Population, block and population x temperature were considered as random effects. To investigate at which freezing temperature plants responded differently we carried out a post-hoc Dunnett test against the control at +4°C. To evaluate whether invasive populations from colder climates show increased frost resistance, we did a stepwise backward multiple regression of k-values of -16°C and -8°C against geographic coordinates (longitude and latitude) and several climatic variables from the origin of population using climate data from http://www.worldclim.org (Hijmans *et al.* 2005): annual mean temperature, mean minimum temperature of the coldest month, and mean annual precipitation. Akaike information criterion (AIC) was used as a criterion to evaluate the model (Akaike 1978). We considered a significant correlation as an indication for local adaptation to winter temperatures. Furthermore, we correlated data of frost damage estimation and k values of -16°C and -8°C (Pearson correlation). Estimated percentage of leaf damage was arcsine-transformed to normalize the values prior to analysis.

Distribution data

We were able to use new native range distribution data of reasonable resolution that covers the species' complete area of occurrence in China. The dataset was based on Wu & Ding (1999), a Chinese database on seed plants. Additional data were available from herbarium specimen information from CVH (2007). It provides access to data associated with plant specimens maintained in Chinese herbaria. The dataset was critically assessed and completed through the work of Zhiheng Wang and Zhi-Yao Tang from Department of Ecology, College of Environmental Sciences, Beijing University.

Distribution data for naturalized occurrences were compiled from a wide array of data sources. Since *B. davidii* has been naturalized rather recently in many regions, the geographical coverage in the floristic literature is uneven and depends on publishing date. For North America, data were compiled from Plants Database (Mehrhoff *et al.*2003, Klinkenberg2007, USDA2007, Weedmapper2007, OSU2007). Additional data sources used are GBIF (2007), AVH (2007) and BioCat (Font 2007).

Analysis of distribution data

We carried out ecological niche modelling to test for differences between native and invaded range, especially with respect to minimum temperatures. The native ecological niche dimensions of *B. davidii* in China (rectangular model area from 95° to 122° E, and 22° to 40 N) were modelled using the Genetic Algorithm for Rule-set Prediction (GARP, Stockwell & Noble 1992). GARP compares the ecological features of sites where a species is present with random sites in the region (i.e. with 'pseudo-absences'). The process tries and evaluates a selection of environmental rules, finally adding or rejecting them from the model. The final combination of rules constitutes the ecological niche model. This particular approach to modelling species' ecological niches is described in detail in Peterson *et al.* (2002).

We used point-occurrence data and digital layers of six climatic variables for the monitoring period 1961-1990 with a spatial resolution of 10 arc minutes (New *et al.* 2002): mean annual number of frost days, mean annual precipitation, mean annual temperature minimum, mean annual temperature maximum, and mean annual number of wet days. The native range occurrence data set contained 460 georeferenced point records. Models were trained on random subsets by half the points and cross-validated using the other half, i.e. the independent data points. The simplest type of GARP-parameterization was chosen: all rule types used, without combinations, best subset selection disabled, and no environmental layer combinations. We developed 100 replicate model runs with a maximum of 1.000 iterations and a convergence limit of 0.1, and summarized the resulting model grids to produce a final model. The 95%

model coincidence (area of raster cells where > 94 of the 100 models predicted presence of the species) was arbitrarily chosen as threshold between presence and absence.

Model quality for native range predictions was then tested in three ways. First, binomial tests (based on the proportional area predicted as present and the number of independent test points successfully predicted) were used to compare observed predictive success with that expected under random (null) models of no association between predictions and test points. Second, Cohens Kappa was computed to distinguish the prediction success from chance agreement between model and native distribution. Since Kappa is influenced by trait prevalence (distribution) and base-rates (Feinstein & Cicchetti 1990), we decided to use a weighted Kappa k_w computed as proportion of maximum possible unweighted kappa, given the observed marginal frequencies. Third, the coincidence between modelled distribution and the species range was assessed using the Jaccard-Index of similarity. In general, all quality measures are, at least partly, related to the ratio of specificity (percentage of true negatives correctly predicted) and sensitivity (percentage of true positives correctly predicted). Because our data include only presence data, sensitivity is more relevant to evaluate the models' quality.

To minimize sampling effects and other sources of spatial bias, we converted both the native range and the non-native range data points, and the modelled area into a grid with a cell size of 0.25°. This was necessary because we incorporated distribution data sets characterized by very different sampling effort and botanical collecting densities (see also Loiselle *et al.* 2008).

The rule sets of the native-range models were projected onto the remaining terrestrial regions (global land surface from -60° S to 80° N and -180° W to 180° E, excluding the rectangular model area of the native range analysis) for comparison with known occurrences in these regions. The models predictive ability for non-native range occurrences was tested using Cohens Kappa, specificity (percentage of true negatives correctly predicted) and sensitivity (percentage of true positives correctly predicted). It has to be noted here, that for non-indigenous species' data, ratios for absence

predictions are only relevant in terms of observations in the database, rather than in reality, because it cannot be assumed that the process of invasive spread is complete.

To visualize the potential impact of minimum temperatures on the native range, we selected from the original 10 arc minutes climate data layer 'mean annual temperature minimum' all grid cells with values between -7 and -9°C (according to the results of the leaf data analysis, see below) and mapped them as the putative margin of a 'frost damage zone'. Finally we extracted all grid cells of the same layer (long-term means of annual temperature minima) that are hit by (1) the model point data, or (2) the native range point data, or (3) the non-native range point data for statistical comparison of cold hardiness with Student's t-test.

Results

Frost resistance

Leaf damage of 20 European populations, measured in the common garden was on average 25.6% and varied among the populations from 14 to 37%. These differences were not significant (Table 2). We found only a weak relationship between frost damage and the minimum temperature of the coldest month at the home site of each population: with decreasing temperature, frost damage decreased slightly (Pearson's $r = 0.359$, $p = 0.119$).

Our test for frost hardiness using the electrolyte leakage method revealed, as expected, a strong effect of temperature on the leakage rate k (Figure 2, Table 2). Leakage rate was, on average, significantly larger at -16°C ($p < 0.001$) and -8°C ($p = 0.048$) compared to the control at 4°C, whereas leakage rate at -4°C and 0°C was similar to the control. Hence, destruction of leaf tissue of *B. davidii* becomes evident at temperatures of -8°C and lower.

Table 2. Summary of a mixed-model ANOVA for the effects of temperature and population on electrolyte leakage of leaf tissue. A significant population x temperature interaction indicates differences between invasive populations of *Buddleja davidii* in frost hardiness.

	df	log likelihood ratio	P-value
Leaf damage			
block	1	1.50	0.221
population	1	0.40	0.527
Frost hardiness			
random effects			
block	1	10.60	0.0011
population	1	1.70	0.1923
population x temperature	1	8.90	0.0029
	df	F	P-value
fixed effect			
temperature	4,76	332.83	<0.0001

Therefore, we used temperature values between -7 and -9°C to map putative 'frost damage zones' in our ecological niche model (see below). Furthermore, there was a highly significant population x temperature interaction (Table 2) indicating a different response of populations to freezing temperatures, i.e. a strong between-population variation in frost hardiness. Some populations showed a strong increase in electrolyte leakage rate already at the -8°C treatment while others responded first at the -16°C treatment.

Using stepwise multiple regressions, we neither found a relationship between k values of -8°C nor of -16°C and geographic coordinates of the origin of each population. There was also no correlation between k values and any climatic variables of the different provenances. Moreover, frost damage estimated in the experimental garden was not correlated with frost hardiness as measured by the electrolyte leakage method.
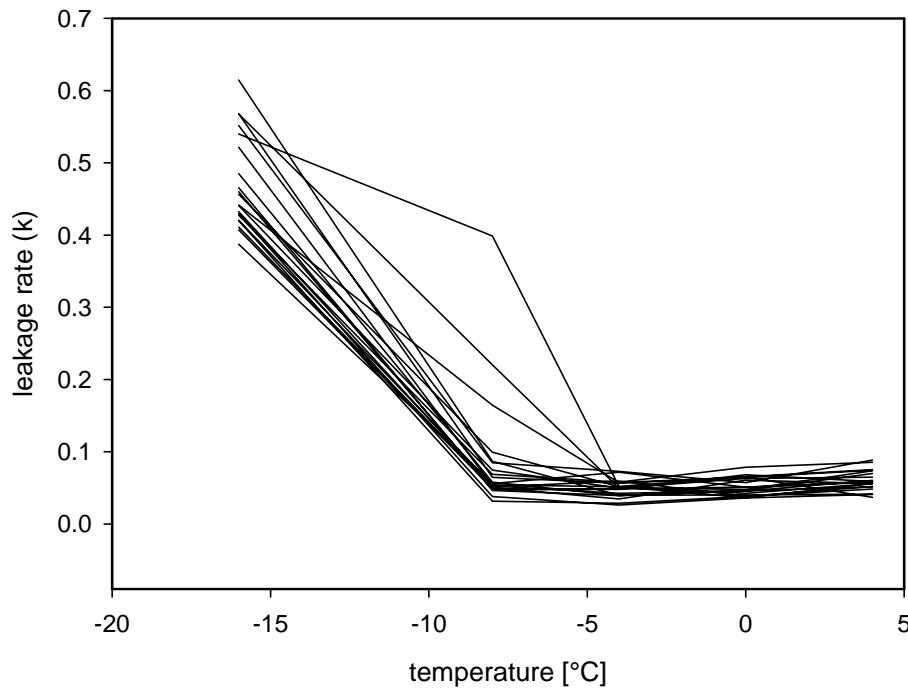
Figure 2. Leakage rate (k) for the 20 invasive populations of *B. davidii* at five temperature levels (- 16°C, - 8°C, - 4°C, 0°C and + 4°C).

Distribution data analysis

The 95% coincidence model for the native range (Figure 3a) showed a reasonable predictive ability when compared with native occurrence data. The agreement between test occurrence points and model predictions was significantly better than random (binomial tests, all $p < 0.05$). The value for weighted Kappa was $k_w = 0.85$, which can be interpreted as excellent agreement and better than by chance (Monserud & Leemans 1992). Sensitivity of the model was 0.95, and specificity was 0.43. The Jaccard-similarity between native range and model was 0.28 and points to constraints of the 'presence only' nature of the occurrence datasets, as well as to limitations of the climate data based model.

These latter limitations are easily observable in the prediction map for the native range (Figure 3a). While the western and south-western range margins are very well described by the model, this is obviously not the case for the eastern range margins. In

this region large areas are modelled as suitable while actual recorded occurrences are lacking, which leads to a relatively high rate of falsely predicted presences.

Independent native-range model projections to the remaining terrestrial regions were well supported by known non-native occurrence records (Figure 3b-c). The coincidence of our model predictions with actual invasion patterns was considerable: 83% of all grid cells with non-native occurrences were correctly predicted by the climatic envelope model. Weighted Kappa for model prediction was $k_w = 0.81$ (excellent, better than chance), sensitivity = 0.83, and specificity = 0.91. Falsely predicted non-native occurrences are mostly located in cool oceanic, boreo-atlantic regions of north-western Ireland and Scotland, and in the forelands of the Alps in Austria and Switzerland. Furthermore, Figure 3b-c indicates that there are vast potential distribution areas in southern Europe and eastern North America with 100% coincidence of the projected model that are currently uninhabited by *B. davidii*.

Statistical comparison of the climatic niche along the minimum temperature axis revealed only minor differences between the native range data and the native range model. The t-test for independent samples and unequal variance showed that the difference in means is just 0.35°C (Bonferroni adjusted p-value: 0.022). The difference in means between native range data and non-native range data was five times larger: 1.73°C (Bonferroni adjusted p-value: 0.000). For the 'cold tail' of the data distribution, the differences are even larger: At the 5th percentile there is a difference of 2.12°C between model and native range, whereas the difference between native and non-native minimum temperatures is 9.42°C. The values for the 10th percentile are 0.52°C *versus* 6.62°C in the same order. Hence, there are native *Buddleja davidii* populations that are thriving in Chinese regions with minimum temperatures 5 to 10°C colder than the hitherto established non-native populations in the coldest parts of the invaded range.
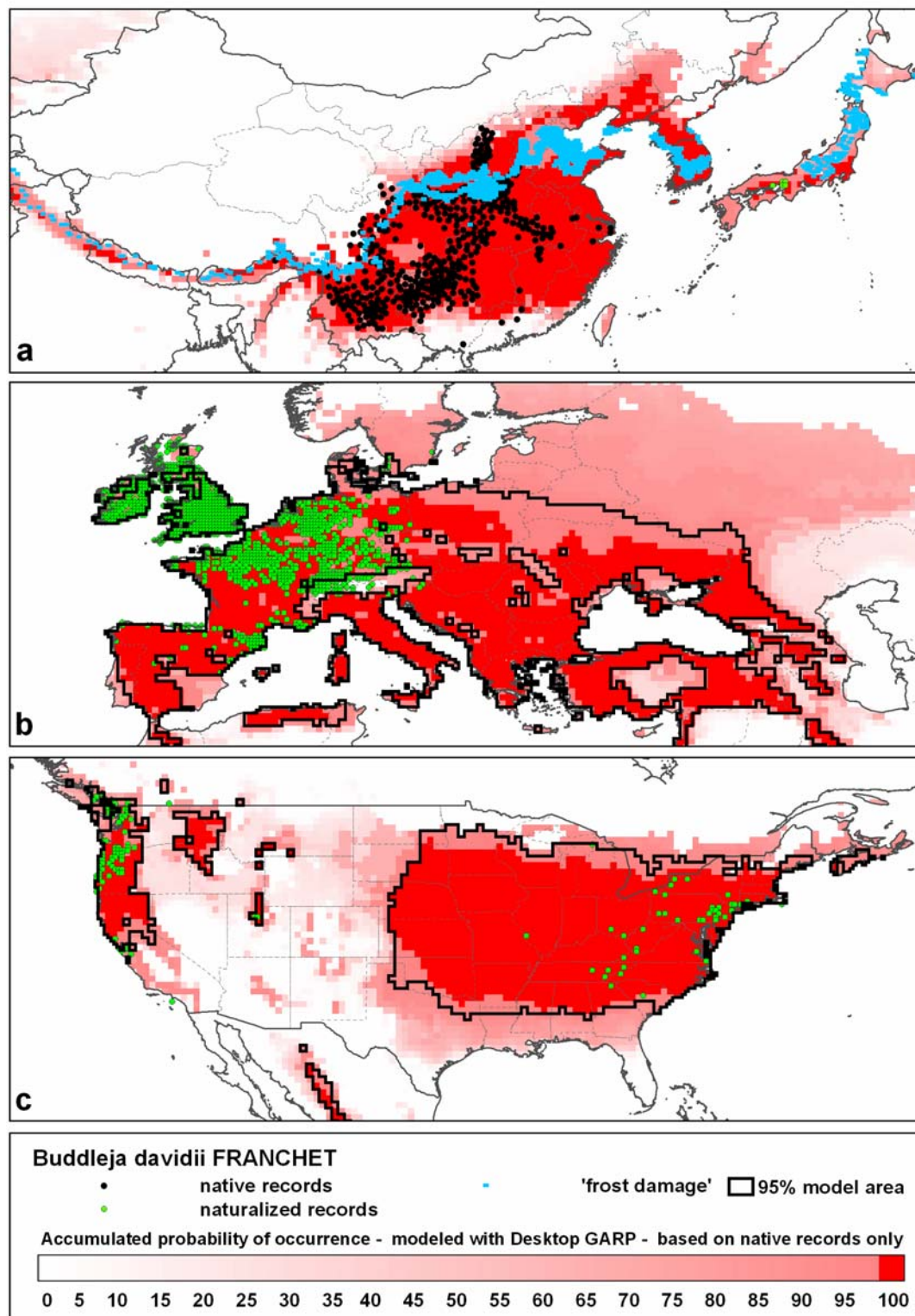
Figure 3. Ecological niche model for *Buddleja davidii* based on native distribution data (black dots) and climate layers indicating potential distribution in a) Asia, b) Europe, c) North America. The current non-native distribution is indicated by light green dots and the blue area marks grid cells with annual minimum temperatures between -7 and -9°C (see Figure 2).

Discussion

We can summarize our results as follows:

(1)   Frost hardiness of leaves varied among the invasive *B. davidii* populations but was not related to any climatic variable of their home site, indicating a lack of local adaptation to winter temperatures.

(2)   The ecological niche model indicates that the macroclimatic niche of *B. davidii* has not changed. Consequently, the potential invasive range of *B. davidii* is still predictable based on climatic data of the species' native distribution.

(3)   Our experiment revealed that frost damage in invasive *B. davidii* populations occurs at -8°C and lower temperatures, and the ecological niche model showed that the species has not yet filled out the potential invasive area. Thus, we can conclude from both approaches that the current distributional range of *B. davidii* across Europe is not limited by frost.

In the temperate zone frost acts an important environmental filter limiting the distribution of plant species (Franklin 1995). In non-indigenous horticultural plants, the probability of escape from gardens depends on their tolerance to low temperatures (Dehnen-Schmutz *et al.* 2007). However, adaptive evolution in response to novel environmental conditions can lead to an expansion of the distributional range (Bridle & Vines 2007). For instance, studies on various native plant species revealed that frost resistance of populations correlated with local climatic conditions (Scheumann & Schönbach 1968, Sakai & Wardle 1978, Bannister & Polwart 2001). Local adaptation to climatic conditions has also been shown for several invasive plant species within their new range, e.g. for *Impatiens glandulifera* (Kollmann & Banuelos 2004), *Solidago altissima* and *S. gigantea* (Weber & Schmid 1998), *Tamarix ramosissima* (Sexton *et al.* 2002), and *Datura stramonium (Weaver et al. 1984)*. In our study on 20 invasive populations of *Buddleja davidii* from different regions in Europe, we found strong between-population variation in frost hardiness measured by the electrolyte leakage method: All populations responded in a similar way at temperatures above -8°C, but at this temperature, some of them suffered much more from freezing conditions than others.

In contrast, the populations did not differ in visible frost damage of leaves in the common garden. The inconsistent results of the two resistance measures may derive from a lack of extreme temperatures at our study site. In December 2006, minimum temperatures were at least -4°C. Thus, although plants experienced frost, temperatures did not reach the threshold point of -8°C at which we detected population differentiation using the electrolyte leakage method. We assume that frost damage of leaves will affect plant fitness because less resistant individuals have to produce new leaves in spring, while individuals of higher resistance will retain their leaves. Frost damage should thus result in loss of assimilates and nutrients stored in leaf tissue, and in delay of photosynthesis. Therefore, we used leaves as model organs to study the impact of frost, but we clearly need more information about frost effects on other plant tissues, e.g. buds and roots, and how such damage actually translates into plant fitness. Furthermore, future studies on frost resistance of *B. davidii* should use a finer resolution of temperature treatments between -8°C and -20°C to reveal population differentiation more precisely. In our study, however, this was not possible without any a-priori knowledge of the species' response to frost.

Although our experimental results indicate genetic variation in frost resistance among invasive *B. davidii* populations, there was no relationship between frost resistance and geographic location, or climatic conditions of the home site of each population. Hence, our expectation that invasive populations from oceanic climates should be more susceptible to frost than populations from sub-continental climates was not confirmed. The obvious lack of local adaptation of invasive *B. davidii* populations to minimum temperatures might be due to several reasons. First, sufficient genetic variation in frost resistance is a prerequisite for local adaptation to minimum temperatures, and has been revealed in a number of native plant species (e.g. Agrawal *et al.* 2004, Savolainen *et al.* 2004). Species invasions, however, are supposed to be accompanied with genetic bottlenecks that should impair adaptive evolution (Allendorf & Lundquist 2003). *B. davidii* was imported to Europe from populations in the western provinces of China about 120 years ago (Lauener 1996). Thus, cultivated *B. davidii* are likely to originate from a small part of the native distribution only, and

might therefore be adapted to a narrower range of minimum temperatures. However, our experimental results are contradicting this speculation because we found large variation in frost hardiness among populations.

Second, it is a reasonable assumption that locally planted cultivars rather than unmodified genotypes from China are ancestors of the invasive *B. davidii* populations. Invasive cultivars are also known from other ornamental plants, e.g. *Ardisia crenata* (Kitajima *et al.* 2006), *Pyrus calleryana* (Culley & Hardiman 2007), and *Mahonia aquifolium* (Ross *et al.* 2008). For *B. davidii*, about 70 cultivars are known (Dirr 1997) which differ in various traits, among others also in frost hardiness (Albrecht 2004). We suppose that horticultural trade has lead to a mixing of *B. davidii* genotypes across Europe. Thus, variation in frost resistance among invasive populations may simply reflect the traits of cultivars haphazardly planted at each location and escaped from cultivation. Moreover, the large-scale mixing of cultivars may also have prevented local adaptation owing to gene-flow into invasive populations. This effect may be similar to genetic swamping which is supposed to hinder adaptation at range margin (Bridle & Vines 2007). Accordingly, Ross *et al.* (2008, in press) assumed that a possible reason for the lack of local adaptation in invasive *Mahonia aquifolium* populations is gene-flow from planted cultivars.

Third, the lack of local adaptation in *B. davidii* may be related to its invasion history in Europe, including the short time period since the first spread, the time of cultivation and the intensity of propagule pressure (cf. Dehnen-Schmutz *et al.* 2007). The naturalization of *B. davidii* was promoted by destruction of urbanized regions in Western Europe during the Second World War, where enormous amounts of building site rubble provided suitable habitats (Kreh 1952, Kunick 1970, Owen & Whiteway 1980). In parts of Eastern Europe, e.g. Poland, the cultivation of *B. davidii* started only in the 1970's (Adamowski *et al.* 2002). Although it has been demonstrated that plants can evolve in response to recent environmental changes within few generations (e.g. Bone & Farres 2001), we suppose that the short time span since cultivation (Eastern Europe) or first spread (Western Europe) combined with founder effects, may have prevented local adaptation so far. Finally, apart from frost resistance, an alternative

plant strategy to cope with frost is tolerance, i.e. the ability to compensate for frost damage (Agrawal *et al.* 2004). Aboveground susceptibility of *B. davidii* to frost does not necessarily lead to death of the whole plant because of a high re-sprouting ability in the following year (Albrecht 2004). In future studies, it will therefore be neccessary to consider also variation in frost tolerance to assess possible local adaptations of invasive *B. davidii* populations more comprehensively.

Our experimental data, indicating a lack of adaptive evolution in *B. davidii* in response to minimum temperatures, are in line with the results of our ecological niche model. The analyses of the climatic properties of the invaded and the native range showed that the distribution in North America and Europe of *B. davidii* corresponds to its climatic envelope in China. Hence, the species' realized macroclimatic niche appears to be conserved (cf. Wiens & Graham 2005), and unaffected by evolutionary changes including plant breeding. This finding is in contrast to recent studies providing evidence for niche shifts in the invasive knapweed *Centaurea maculosa (Broennimann et al. 2007)* in North America, and the invasive toad *Chaunus marinus* in Australia (Urban *et al.* 2007).

There is, however, a mismatch between the modelled and the known native distribution of *B. davidii* in Eastern China. Contrary to the situation in Europe and North America, temperate oceanic to sub-oceanic lowland regions are underrepresented in the known native range of *B. davidii*, where the species is confined to mountainous regions. A possible explanation may relate to our observation that the species prefers stony slopes with well drained, partly bare soils. The fit of the native range model could then be increased by including topography related variables like 'slope' or 'topographical index'. In addition, the occurrence of invasive populations in disturbed habitats of urbanized regions (such as railway tracks or brown fields) indicates a preference towards soil conditions similar to those of mountainous areas rather than to mountain climates. Overall, we found no evidence for an ecogeographical pattern that may result from evolutionary changes or ecological release in the invasive range. Moreover, we have to point out that in case of *B. davidii*, factors like rapid evolutionary adjustment and artificial selection do not impair the

prediction of the potential range. However, predictions by climate based ecological niche models produce just rough spatial estimates for possible future naturalizations, provided that other conditions, often acting on finer spatial scales, are favourable for the species.

Based on our experiments and the analysis of the geographical distribution, we cannot confirm the assumption of Krivanek & Pyšek (2006) that the distribution of *B. davidii* in Europe is limited by a lack of frost resistance. Both, the ecological niche model and the statistical comparison of climate, show that in its native range, the species is already adapted to colder temperatures than those present in the currently invaded range. So far, we cannot exclude that the eastern border of the current distribution in Europe is reflecting the history of introduction (e.g. the late introduction to Eastern Europe, Adamowski *et al.* 2002) and subsequent invasion rather than being caused by climatic factors. Thus, we suggest that historical factors, such as urbanisation, war damage, and fashion trends in gardening, may mask the relationship between the invasion dynamics of *B. davidii* and ecological factors, and that the coincidence between its current range margins and climatic factors is just produced by chance. We may therefore conclude that large parts of the potential range of *B. davidii* in Europe and North America are still unoccupied, and that the invasion process will continue. Thus, monitoring further spread as well as additional experiments testing for local adaptation, e.g. reciprocal transplantation experiments, are next steps to prove this hypothesis. In summary, it can be stated that the combination of experimental tests for frost resistance and ecological niche analyses based on native range distribution data enabled a better understanding, and thus prediction of the invasion patterns of *B. davidii* than separate analyses would have allowed.

Chapter 5

Herbicide tolerance in invasive Butterfly Bush (*Buddleja davidii)* populations in Europe

with Harald Auge

Abstract

In Europe, many invasive plant species grow in disturbed habitats like roadsides and railway tracks, where they have to be removed for safety reasons. Spraying chemical herbicides is the most commonly used technique for weed management along railway tracks. As a consequence of recurrent herbicide use plants can evolve resistance. This may in turn provoke either a higher frequency or an increased rate of herbicide application, causing additional environmental and economic costs. In Europe, invasive populations of introduced Butterfly Bush (*Buddleja davidii*) are spreading along railways although the herbicide glyphosate is used for weed management along railway tracks in most countries of Europe. Therefore, we asked to what extent *B. davidii* is susceptible to glyphosate, and whether populations invading railway tracks have evolved increased resistance compared with populations from other habitats. In order to investigate this, we carried out a greenhouse experiment using offspring of 12 *B. davidii* populations from three different habitat types: railway embankments, ruderal sites, and semi-natural habitats. After applying distilled water or four different concentrations of the herbicide glyphosate, we monitored plant survival. In contrast to our expectation, we found no evidence for glyphosate resistance in *B. davidii* because the recommended application rate of 30 mL/L resulted in 100% mortality. Furthermore, comparison of dose-response curves as well as LD_{50} and LD_{90} values revealed that populations from railway embankments have not evolved increased resistance to glyphosate so far.

Introduction

The application of herbicides is a very common method to manage invasive plant species (Culliney 2005). However, an important problem regarding the use of herbicides is the evolution of herbicide resistance in plants (Heap 1997). In this context, it is alarming that plants may evolve such resistance within few generations (Bone & Farres 2001). If plants become resistant to herbicides, probably more costly methods for controlling invasive plants must be applied. So far, most of the studies documenting resistance to herbicides refer to agricultural weeds: By the mid 1990ies, herbicide resistance has been demonstrated for more than 120 weed species, and their number is increasing continuously (Heap 1997). The evolution of herbicide resistance in the context of exotic plant invasions, however, has been marginalized in the past. This is in remarkable contrast to the general opinion that the ability for adaptive evolution is a key feature of successful invaders (Caroll & Dingle 1996, Sakai *et al.* 2001, Bossdorf *et al.* 2005). Indeed, rapid evolutionary adjustments to novel environments have been shown for several invasive plant species, e.g. by Bradshaw *et al.* (2004), Galloway & Fenster (2000), Maron *et al.* (2004) and Kollmann & Banuelos (2004).

Many introduced plant species occur along railway tracks, which represent not only structures fragmenting natural habitats but are also dispersal corridors for plants of disturbed habitats. Railway tracks can facilitate the spread of non-indigenous plants by providing suitable substrates, favorable light conditions and reduced competition (Brandes 1983, Theoharides & Dukes 2007). For instance, Brandes (1993) found that more than 20% of plant species growing in railway stations in Germany are neophytes. The occurrence of plant species along railways generally causes loss of elasticity and drainage properties of the railway bed (Schweinsberg *et al.* 1999). To ensure safety and comfort of the line, it is essential to remove plants from the railway tracks (Schweinsberg *et al.* 1999, Ramwell *et al.* 2004, Torstensson *et al.* 2005). Since root stocks are mostly not removed by manual eradication, weeds usually continue growing. Application of chemical herbicides seems therefore to be the only economically efficient method to control plants on railways (Torstensson *et al.* 2005), and contributes

to the increasing economic costs to mitigate the impact of plant invasions (Pimentel *et al.* 2000). Economic losses caused by herbicide application to manage invasive plants are enormous: annual estimates range from 1 billion $ in New Zealand (Culliney 2005) to 39 billion $ in India (Williams & Timmens 2002). At present, the most often used herbicide on railway embankments in Sweden (Torstensson *et al.* 2005), Germany (Schweinsberg *et al.* 1999) and in the UK (Ramwell *et al.* 2004) is the non-selective glyphosate, frequently sold under the brand name RoundUp®. It is also the most common herbicide in agriculture, landscape management and in weed control programs (Zelaya *et al.* 2004, Perez *et al.* 2004).

Recent studies on herbicides rarely integrated evolutionary and population biology, which would promote the understanding of those forces that support evolution of herbicide resistance (Neve 2007). We think that comparing resistance among populations or habitats with different history of herbicide application may be a first step in this direction. We therefore conducted a study on the pattern of glyphosate resistance among different invasive populations of the Butterfly Bush, *Buddleja davidii*. This perennial shrub is native in China and invasive in several man-made and semi-natural habitats in western and central Europe (Ebeling *et al.* 2008b). Invasive *B. davidii* populations occur in enormous numbers along railway tracks and are still spreading although glyphosate is used for weed control on railways in Germany since 1996. We therefore hypothesized that populations from railway tracks, which are regularly treated with glyphosate, have evolved herbicide resistance and are, thus, less susceptible than conspecific populations from ruderal or semi-natural habitats without a history of herbicide use.

Materials and Methods

Study species

The perennial shrub *Buddleja davidii* Franch. (Scrophulariaceae) is native to China and was introduced for ornamental reasons to Europe around 1890. The species is naturalized in central and west Europe (Kreh 1952, Kunick 1970, Anisko & Im 2001), and is still spreading across the continent. The shrub occurs at forest edges, roadsides, rural dumps, stream and river banks and other disturbed habitats. It is a deciduous or semi-evergreen shrub, 2-4 m tall, with an annual growth rate of at least 0.5 m. The plant typically flowers in the second year after germination but flowers can also occur in the first year. The lilac flowers, standing in terminal panicles at stem apex, are pollinated by butterflies. *Buddleja* produces large quantities of seeds (about 3 Mill. per plant), which are tailed for an efficient wind or water dispersal. It can regenerate by producing adventitious shoots and roots on buried stems.

Herbicide glyphosate

Glyphosate herbicides, N-(posphonomethyl)-glycine are the most commonly used herbicides in the world (Cox 2004). It inhibits the enzymatic reaction for the biosynthesis of the amino acids phenylalanine and tyrosine. Therefore it is a broad-spectrum, non-selective systemic herbicide used for control of annual and perennial plants including grasses, sedges, broad-leaved weeds and woody plants.

Nevertheless, resistance have been revealed in *Conyza canadensis* (VanGessel 2001, Koger *et al.* 2004, Main *et al.* 2004), *Lolium rigidum* (Heap2007) and *Lolium multiflorum* (Perez & Kogan 2003), *Eleusine indica* (Lee & Ngim 2000), *Conyza bonariensis* and *Plantago lanceolata* (Heap2007).

Experimental design

In Europe, we collected seeds from four invasive *B. davidii* populations within each of three habitat types in which this species generally occurs: (1) railway embankments, (2) ruderal sites, e.g. brownfields, gaps between buildings, roadsides, and (3) semi-natural sites such as forest edges, abandoned gravel-pits and riverbanks (Table 1).

Table 1. Habitats and sampling locations of the twelve invasive *Buddleja davidii* populations used for the experiment.

population	coordinates		Altitude
	latitude	longitude	a.s.l. [m]
1. railway			
Switzerland / Basel	47.5472 N	7.5892 E	280
Germany / Essen	51.4652 N	7.0266 E	37
Germany / Oldenburg	51.4694 N	6.9014 E	68
Germany / Seligenstadt	50.0402 N	8.9681 E	114
2. ruderal			
Germany / Darmstadt	49.8894 N	8.6380 E	127
Germany / Duisburg	51.4827 N	6.7856 E	30
Germany / Halle	51.4811 N	11.9683 E	99
Germany / Cologne	50.9627 N	6.9647 E	46
3. semi-natural			
Switzerland / Geneve	46.2083 N	6.1428 E	387
Italy / Merano	44.6666 N	11.1666 E	272
Germany / Sulzbach	48.8630 N	8.3711 E	470
Germany / Tettnang	47.6300 N	9.5847 E	447

The seeds were sampled from 5 individuals in each of the twelve populations in winter 2004/2005. In May 2005, seeds were sown in seedling trays filled with a 1:1 mixture of sand and potting soil. They were placed in a greenhouse with a 12:12 h light/dark cycle at 21/18°C. In July 2005, 25 plants from each population were repotted into 750 ml pots with the same substrate mixture.

The pots were then placed in an unheated greenhouse and watered as needed. In August 2005, plants were randomly assigned to five groups, with five plants of each population in each group. The herbicide was applied in different concentrations of the solution to each group: the recommended application rate (30 mL/L), ¼ of this dose (7.5 mL/L), 1/16 (1.88 mL/L) and 1/64 (0.47 mL/L), and distilled water. We sprayed leaves of the plants until all of them were moistened with the respective solution. After this treatment, plants were completely randomized on a greenhouse table, maintaining day/night temperatures as described above. Five weeks after sprinkling with herbicide or distilled water, we determined the number of survived plants in each treatment.

Analysis

The response of plants to the different glyphosate concentrations was recorded as dead or alive resulting in a binomial distribution of data. The statistical analysis of mortality rates was not possible when the effect of populations (nested within habitats) was included because the model did not converge (neither with proc glimmix nor with proc genmod; SAS version 9.1). We therefore calculated a reduced model without the population effect, being aware that this model did not exactly reflect our experimental design. The reduced model included habitat as factor, $\log(x + 0.1)$ of glyphosate concentration as continuous variable, and the habitat x glyphosate interaction which compares the slope of the dose-response curves between the three habitats (proc genmod, logit link, binomial error distribution).

To characterize the lethal doses of glyphosate we performed logistic regression analyses of mortality on glyphosate concentration for each of the twelve populations separately, and used the intercepts and slopes to calculate LD_{50} and LD_{90}. We performed an analysis of variance (ANOVA, proc glm) on these LD_{50} and LD_{90} values followed by Tukey's post hoc test to compare the three habitats.

Results

Mortality increased significantly with increasing concentration of glyphosate among *B. davidii* plants from all three habitats (Figure 1, Table 2). Mortality rate was only marginally affected at a concentration of 1/64 of the recommended dose (i.e. 0.47 mL/L) but increased considerably at 1/16 (1.88 mL/L). Already ¼ of the recommended dose (7.5 mL/L) caused virtually 100% mortality among the plants. We found no general effect of habitat on plant mortality (Table 2), and dose-response curve of plants from the three habitats were not significantly different (habitat x treatment interaction, Table 2).
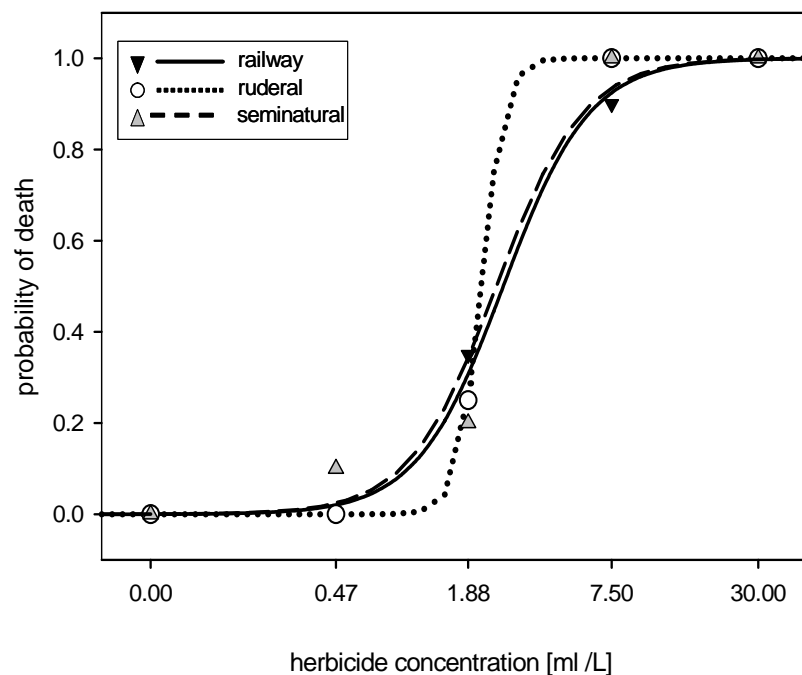
Figure 1. Probability of death of *Buddleja davidii* populations from three different habitats (railway tracks, ruderal sites and semi-natural sites) in response to glyphosate treatment at five different concentrations. The logistic regression lines are not significantly different between the three habitats.

Table 2. Analysis of deviance for the effects of habitat type and glyphosate concentration on plant mortality in invasive populations of *Buddleja davidii* (generalized linear model, logit link, binomial error, glyphosate concentration logarithmically transformed).

	Devianz	d.f.	X^2	p
dose	106.9088	1	306.36	< 0.001
habitat	106.7871	2	0.12	0.941
dose x habitat	103.2637	2	3.52	0.172

The lethal doses LD_{50} and LD_{90}, i.e. the two concentrations resulting in 50% and 90% mortality, were on average 3.24 ± 1.20 mL/L and 4.74 ± 2.19 mL/L, respectively (mean ± s.e). It is remarkable that populations from ruderal sites had slightly lower LD_{50} (2.73 ± 0.56 mL/L, mean ± s.e.) and LD_{90} (3.37 ± 0.65 mL/L) compared with populations from the other two habitats (railway embankments: $LD_{50} = 3.73 \pm 0.53$ mL/L, $LD_{90} = 6.09 \pm 1.17$ mL/L; semi-natural habitats:

$LD_{50} = 3.26 \pm 0.53$ mL/L, $LD_{90} = 4.75 \pm 1.49$ mL/L). However, these differences were not significant ($F_{2,9} = 0.06$, $p = 0.993$, and $F_{2,9} = 1.64$, $p = 0.246$, for LD_{50} and LD_{90}, respectively).

Discussion and Conclusion

In our study, we compared invasive *Buddleja davidii* populations from Europe with respect to their response to different glyphosate concentrations. We investigated whether *B. davidii* is generally susceptible to glyphosate, and whether populations from railway tracks with a history of glyphosate application have evolved some level of herbicide resistance compared to populations from other habitat types (seminatural and ruderal habitats).

In contrast to our expectation, we did not find significant differences in glyphosate resistance between populations from the three habitats. Thus, we can not confirm our hypothesis that plants from railways are less susceptible than plants from other habitats. Population differentiation in glyphosate resistance or tolerance, respectively, has been shown for wild populations of *Brassica juncea* in China (Huangfu *et al.* 2007), and *Ipomoea purpurea* as well as *I. hederacea* in North America (Baucom & Mauricio 2008). Furthermore, Vila-Aiub *et al.* (2007) found differences in resistance between *Sorghum halepense* populations from glyphosate-treated and glyphosate-free sites in Argentina. It should be noted that the latter is also an invasive non-indigenous plant in South America.

One explanation for our result might be a general lack of genetic variation for herbicide resistance in this species, or at least a lack of such variation in the invasive populations. Sufficient genetic variation is an important prerequisite for the response of a population to selection (Falconer & MacKay 1996). The lack of genetic variation in traits conferring herbicide resistance might be related to the source of the populations: *B. davidii* was introduced as an ornamental shrub and thus plant breeders manipulated native genotypes of the species and bred new cultivars (Albrecht 2004). Therefore, it is likely that individuals of naturalized populations originated from cultivars planted in the surrounding area. Even though we do not know the amount of cultivars and genetic composition with respect to our populations, it is a reasonable assumption that locally planted cultivars rather than non manipulated genotypes from the native range induced the spread of *B. davidii*. Depending on the popularity, probably only few cultivars have escaped from cultivation in a particular region (Dehnen-Schmutz *et al.* 2007) resulting in low genetic variation, thus impeding the invasive populations' response to selection.

A second reason for the lack of glyphosate resistance in railway populations of *B. davidii* may reside in the properties of the herbicide itself. Glyphosate is the most widely used herbicide and considered as a low-risk herbicide, because of its way of action, chemical structure and the relatively small disappearance time (Heap 1997). Non-selectively inhibiting enzymatic reactions, this broad-spectrum herbicide may

effectively aggravate resistance evolvements in plants. This seems convincing regarding to the small number of plant species that have been revealed to be resistant to glyphosate (Heap 2007). Until now, only two mechanisms, both inherited as single gene, nuclear traits, are known, but it is likely that other resistance mechanisms will become evident (Powles & Preston 2006). This may even include the evolution of quantitative resistance which has been shown for other herbicides (Neve & Powles 2005). Evolution of herbicide resistance depends in general on the frequency, the duration and the area of use (Heap 1997, Perez *et al.* 2004). Thus, it remains possible that resistance may arise with the increasing reliance of glyphosate (Powles & Preston 2006) and resistant populations than will act as a source of resistant alleles via gene flow through pollen or seeds. Moreover, it should be noted that our study does not consider glyphosate tolerance, i.e. the ability of plants to regrow after damage, which is an alternative to resistance known from a few other plant species (e.g. Baucom & Mauricio 2008).

Nevertheless, our study shows that glyphosate is still a useful herbicide to control stands of *Buddleja davidii* in railway embankments in Germany. Our study reveals that even a quarter of the recommended dose is sufficient to control *B. davidii*. To avoid evolution of herbicide resistance for the long term and considering ecological risks of herbicides penetrating to the groundwater (Torstensson *et al.* 2005), we hold the view, that the application rate should be as low as possible. On the other hand, application rate should be still high enough to ensure high mortality thus preventing the evolution of quantitative resistance traits (Neve & Powles 2005). Future studies on herbicide response of invasive *B. davidii* populations should include the compensatory potential after damage, i.e. tolerance (Baucom & Mauricio 2008), and should cover the whole life cycle using both, greenhouse trials and field investigations (Neve 2007).

Synthesis

Human activity is one of the world's greatest evolutionary forces (Palumbi 2001) with impact on ecosystems in various ways: climatic change, alteration in land use, degradation, isolation and fragmentation of habitats (Vitousek 1990), and changes in biodiversity by deliberately or accidently introducing non-indigenous species (Mooney & Cleland 2001, Lee 2002). Although a majority of the species that are introduced fail to survive, others thrive and become invasive (Williamson & Fitter 1996, Pyšek *et al.* 2004b). Such biological invasions are considered to be a major cause of extinction of native species throughout the world (Sala *et al.* 2000). Therefore, scientists focus on the prevention, eradication and control of invasive organisms. The understanding of biological invasions and the mechanisms associated with their success is an essential step in the development of meaningful control measures.

In plants the most conspicuous observation is the different performance of introduced plant species in the native compared to the invasive range, where they often grow more vigorously (e.g. Buckley *et al.* 2003, Prati & Bossdorf 2004, Jakobs *et al.* 2004). The increased vigour might be due to the absence of their natural enemies (Maron & Vila 2001, Keane & Crawley 2002). Alternatively, it might be possible that genetically fixed traits mirror the adaptations to the new environmental factors (Galloway & Fenster 2000). Such evolutionary changes can be rapid and may explain why some introduced species become invasive after a lag phase while others do not. The debate about adaptive evolution of invasive plant species as a possible explanation for their success has emerged during the last years. Evolution after introduction maybe caused either by genetic drift or by natural selection exerted by the novel biotic and abiotic environment (Mack *et al.* 2000, Leger & Rice 2003, Parker *et al.* 2003). Hence, differences in the performance between native and invasive range might be a result of rapid evolution expressed as increased competitive ability (EICA hypothesis, Blossey & Nötzold 1995). Several studies have already revealed local adaptation in invasive plant species that are accounting for there invasion success, for example, in *Solidago canadensis* (Weber 1997) and *S. gigantea* (Weber & Schmid 1998) or *Impatiens glandulifera* (Kollmann & Banuelos 2004). In this thesis I studied the genetic differentiation and local adaptation of a perennial shrub, *Buddleja davidii* (butterfly bush) that is native to

China yet invasive in West Europe, New Zealand and North America. Introduced to Europe as an ornamental in the late 1800's, it has been subject to artificial selection in its invasive range. Therefore comparisons between native and invasive populations of *B. davidii* offer excellent opportunities for examining invasion processes.

In the first study (chapter 2) it has turned out that the performance of *B. davidii* populations is enhanced in the new range, similar to observations on other species. Through a comparative approach we confirmed our hypothesis of a better growth and reproduction of *B. davidii* in the invasive range. Stems and inflorescences in European populations were 79% and 23% larger, respectively, in comparison with Chinese populations. The three subsequent experimental approaches (chapter 3-5) were conducted to determine reasons for the differences between the naturalized European (henceforth referred to as the invasive populations) and Chinese (henceforth referred to as the native populations) populations. On one hand leaf herbivory was substantially reduced in the invasive populations, which suggests that the escape from natural enemies may be responsible for increased vigour. On the other hand a more benign abiotic environment may positively influence the performance in the new range.

Climate is a primary driver of natural selection in plants (Kawecki & Ebert 2004), often explaining the distributional ranges of plant species (Woodward & Williams 1987). Adaptive divergence in relation to climatic conditions in the invaded range have been revealed in several plant species (e.g. Weber & Schmid 1998, Kollmann & Banuelos 2004, Jakobs *et al.* 2004). Climatic limitation of distributional ranges and its overcoming by genetic adaptation may be of particular importance for plant invasions. To study genetic differentiation with respect to climatic conditions among 20 invasive populations of *B. davidii* a common garden experiment was carried out (chapter 3). This approach was replicated in three different climatic regions within the invasive range (Basel/Switzerland, Halle/Germany, Oldenburg/Germany). Although rarely implemented, replication across climatic regions studies reveal conclusions more accurately than a garden study conducted at a single location. We found high genetic variation in growth and reproduction traits among invasive populations of *B. davidii*,

which may be either due to neutral forces such as founder effects or drift, or natural selection leading to local adaptation. Differentiation in plant traits did not correlate with geographic distances among populations, indicating that between-population variation is not likely due to isolation by distance. Plant fitness did not decrease with increasing geographic distance of the common garden to the respective home site of a population, and traits of growth and reproduction did not correlate with climatic characteristics of the populations' home site. These two latter results suggest that *B. davidii* populations are not locally adapted to their home environments. In addition to such correlative studies, local adaptation can also be investigated by reciprocal transplant experiments. Thus, a reciprocal transplantation was integrated into the common garden experiment: populations originating from the three cities harbouring the common gardens were included into the population subset. In contrast to our hypothesis, we found no significant genotype x environment interaction, again indicating a lack of local adaptation.

A second study dealing with genetic differentiation and local adaptation focused on frost as an important climatic factor limiting the distribution of plant species in the temperate zone (Woodward & Williams 1987). If *B. davidii* populations are locally adapted to minimum temperatures, one would expect that populations in sub-continental climate are less susceptible to frost than populations in oceanic climate. To test this hypothesis, we investigated frost resistance of plants growing in the common garden located in the sub-continental region (chapter 4). The study consisted of both, an experimental approach to local adaptation to minimum temperatures in the common garden and an ecological niche model to compare distribution of native and invasive populations of *B. davidii*. After all, we found significant differences in frost resistance among populations (population x frost interaction), but these differences were not related to any climatic variable of their home site, indicating that populations are not genetically adapted to climatic conditions of their home site within the invasive range. However, the ecological niche model revealed that invasive *B. davidii* populations have not saturated their potential range suggesting that climatic conditions are not currently limiting the further spread of the species.

Neither the experiment nor the model confirmed Krivanek and Pyšek´s (2006) assumption of that the current distribution border of *B. davidii* in Europe, running between the oceanic and sub-continental climate, is limited by the lack of frost tolerance.

Already established invasive plants must be managed to minimize their impact on the ecosystem. The most frequently used method to control plant populations is the chemical control by herbicides. However, recurrent herbicide application may lead to evolution of resistance in plants (Powles & Preston 2006). In Europe, *Buddleja davidii* often occurs along railways, where it is removed for safety reasons. Since the herbicide glyphosate is commonly used for this purpose, the question arises to what extent *B. davidii* may have evolved resistance to this herbicide. Specifically, we asked, if populations that invaded railway tracks show an increased resistance compared to populations from other habitats (chapter 5). We examined twelve *B. davidii* populations from three different habitat types: railway embankments, ruderal sites, and semi-natural habitats. Since the recommended application rate of 30 mL/L resulted in 100% mortality, it could be concluded that there is no evidence for glyphosate resistance in *B. davidii*. Furthermore, comparison of dose-response curves and LD_{50} and LD_{90} values revealed that populations from railway embankments have not evolved increased resistance to glyphosate so far. Therefore, glyphosate can be considered as an effective herbicide to be used to control the species.

A major conclusion that can be drawn from all three experimental studies of this thesis (chapter 3-5) is that we found no evidence for local adaptation in invasive *B. davidii* populations. This is in contrast to our expectation and may be due to a lack of genetic variation which is an important prerequisite for the response of a population to selection (Falconer & MacKay 1996). We speculate that the pattern of genetic differentiation among *B. davidii* populations may rather reflect founder effects. Being a popular garden plant, the genetic variation of cultivated *B. davidii* may have been increased by plant breeding resulting in more than 90 cultivars (Stuart 2006). It is likely that the cultivars planted in the surrounding area were the seed source for the naturalized populations included in our studies.

Furthermore, trade and transport of cultivars across Europe may have lead to a mixing of *B. davidii* genotypes and thus, have prevented local adaptation owing to gene-flow into invasive populations so far. Molecular studies are needed to determine the amount of genetic variation in invasive *B. davidii* populations, and the sources (i.e. which cultivars) that have particularly contributed to the gene pool of these populations.

The absence of adaptations may also be attributed to the relatively short period of time that has passed since the invasion of *B. davidii* in Europe began. Although it has been demonstrated that evolution in response to recent environmental conditions can occur within few generations (Davison & Reiling 1995, Thompson 1998, Bone & Farres 2001), the recentness of *B. davidii* invasion combined with founder effects and associated bottlenecks at each location has prevented the evolution of local adaptation thus far. However, the absence of evidence for local adaptation does not exclude rapid evolution in *B. davidii* in future. Furthermore, there remains the possible evolution of glyphosate resistance with the increasing use of herbicides (Powles & Preston 2006). Resistant populations will than serve as the sources of resistant alleles via gene flow through pollen or seeds.

Nevertheless, we could clearly demonstrate that the investigated populations of *B. davidii* that were included in this research show high phenotypic plasticity that enables the plants to grow and reproduce under different environmental conditions. Since other studies found a higher degree of plasticity in invasive than in native populations (see Bossdorf *et al.* 2005), the results support the suggestions, that high phenotypic plasticity is an important feature of successful invaders (Rice & Mack 1991a, Sexton *et al.* 2002).

In conclusion, *Buddleja davidii* is a highly invasive species that may cause serious problems in the invaded range due to its high growth rates, early maturity, high reproductive output of easily distributed seeds, and tolerance of a broad range of environmental conditions. The importance of plant breeding for invasion success of introduced plant species should be watched carefully, and should be considered in future studies on *B. davidii* in particular and on cultivated plants in general.

Overall, the next step should be a prospective monitoring of this plant species and to explore the effects of artificial selection on *B. davidii* invasiveness, realizing that, as the ecological niche model demonstrated, the invasion process is still on-going because the species has not saturated its potential distribution range.

Chapter 7

Zusammenfassung

Menschliche Aktivitäten haben einen enormen Einfluss auf ökologische und evolutionäre Prozesse. Diese Einflüsse vollziehen sich innerhalb und zwischen den Ökosystemen auf verschiedenen Ebenen und führen zu Änderungen des Klimas und der Landnutzung, zu Degradation und Fragmentierung von Habitaten und zu einer Veränderung der Biodiversität. Ein wichtiger Teilprozess der Änderung der biologischen Vielfalt ist die absichtliche Einführung oder unbeabsichtigte Einschleppung nicht-einheimischer Arten. Obwohl die meisten eingeführten Arten sich nicht etablieren, sind doch einige in der Lage im neuen Gebiet zu überleben und sich verstärkt auszubreiten, also invasiv zu werden. Solche biologischen Invasionen gelten weltweit als eine Hauptursache für das Aussterben einheimischer Arten. Deshalb liegt ein Schwerpunkt der Invasionsbiologie in der Erforschung gebietsfremder Arten, der Risikoabschätzung ihres Einflusses und der Verhinderung des Einschleppens bzw. der Beseitigung und der Kontrolle solcher Organismen. Zudem ist es erforderlich, biologische Invasionen und die damit verbundenen Mechanismen zu verstehen, um daraus sinnvolle Managementmaßnahmen ableiten zu können.

Eine auffällige Beobachtung bei pflanzlichen Invasionen ist das unterschiedliche Verhalten der Individuen im neuen Areal verglichen mit denen des Heimatareals. Im neuen Areal scheinen sie oft vitaler zu sein, schneller zu wachsen und eine höhere Reproduktionsrate aufzuweisen. Dies wird oft auf das Fehlen ihrer natürlichen Feinde – der Herbivoren und Pathogene, die häufig nicht mit in das neue Areal verschleppt werden – zurückgeführt (Enemy Release Hypothese). Andererseits ist es möglich, dass genetisch fixierte Merkmale eine Anpassung an die neuen Umweltbedingungen widerspiegeln. Solche evolutionären Veränderungen können rasch erfolgen und erklären, warum manche eingeführten Arten erst nach einer so genannten lag-Phase invasiv werden und eine erhöhte Fitness aufweisen. Generell können evolutionäre Veränderungen bei invasiven Arten durch genetische Drift oder durch natürliche Selektion hervorgerufen werden. Im Verlauf der letzten Jahre wurde zunehmend über die adaptive Evolution als Ursache für den Invasionserfolg von Pflanzenarten diskutiert. So kann der Unterschied in der Pflanzenfitness zwischen dem neuen und den ursprünglichen Verbreitungsgebiet das Ergebnis einer solcher schnellen

evolutionären Veränderung sein, z.B. in Form der Evolution erhöhter Konkurrenzfähigkeit als Antwort auf das Fehlen der natürlichen Feinde (EICA-Hypothese). Verschiedene Studien haben bereits nachgewiesen, dass bei invasiven Pflanzenarten wie zum Beispiel *Solidago canadensis* (Weber 1997), *S. gigantea* (Weber & Schmid 1998) oder *Impatiens glandulifera* (Kollmann & Banuelos 2004), lokale Anpassung an die neue Umwelt zum Invasionserfolg beigetragen hat.

In meiner Dissertation habe ich die genetische Differenzierung und lokale Anpassung einer invasiven Art am Beispiel des Sommerflieders, *Buddleja davidii*, untersucht. Dieser aus China stammende Strauch ist in Westeuropa, Neuseeland und Nordamerika als invasive Art weit verbreitet. Er wurde als Kulturpflanze eingeführt und unterliegt seitdem züchterischen Veränderungen. Diese Art ist deshalb besonders geeignet, um Invasionsprozesse „in Aktion" zu untersuchen.

Die erste, vergleichende Studie (Kapitel 2) zeigte, dass Populationen von *B. davidii* im neuen Areal (Deutschland) im Vergleich zu den Populationen im Heimatareal (China) vitaler sind und bestätigt somit dieses Phänomen auch für den Sommerflieder. Besonders bemerkenswert ist, dass in den europäischen Populationen die Sprosse um 79% und die Infloreszenzen um 23% länger waren als bei den chinesischen Populationen.

Die folgenden drei experimentellen Untersuchungen (Kapitel 3-5) beschäftigten sich mit möglichen Gründen dieser Unterschiede zwischen den beiden Kontinenten. Auf der einen Seite war die Blattherbivorie in den invasiven Populationen deutlich reduziert. Dieses Ergebnis deutet – in Übereinstimmung mit der Enemy-Release-Hypothese – darauf hin, dass der Verlust an natürlichen Feinden im neuen Areal für die erhöhte Vitalität der Art im neuen Areal verantwortlich ist. Auf der anderen Seite können günstigere abiotische Umweltbedingungen das verbesserte Wachstum im neuen Areal verursacht haben. Das Klima ist eine der Haupttriebkräfte natürlicher Selektion und begrenzt häufig die Verbreitungsgebiete von Pflanzenarten. Adaptive Divergenz in Bezug auf die klimatischen Bedingungen im invasiven Areal wurden bereits bei verschiedenen Pflanzenarten nachgewiesen. Das Überwinden klimatischer

Grenzen mit Hilfe genetischer Anpassung ist vermutlich von großer Bedeutung für Pflanzeninvasionen.

Um zu überprüfen inwieweit dieser Aspekt auf invasive Populationen von *B. davidii* in Europa zutrifft, wurde ein so genanntes Common-Garden-Experiment durchgeführt (Kapitel 3). Dieser Ansatz wurde in drei klimatisch verschiedenen Regionen innerhalb des invasiven Areals wiederholt (Halle und Oldenburg in Deutschland und Basel in der Schweiz). Bisher wurde diese Form des Experimentes nur sehr selten durchgeführt, obwohl sie eine viel genauere Schlussfolgerung erlaubt als ein einzelnes Common-Garden-Experiment. Es stellte sich heraus, dass invasive Populationen von *B. davidii* genetisch variabel sind, was entweder auf neutrale Prozesse (wie Gründereffekte und Drift) oder auf natürliche Selektion zurückzuführen ist. Die Populationsunterschiede korrelierten nicht mit der geographischen Entfernung zwischen den Herkunftsorten der Populationen. Daraus lässt sich schlussfolgern, dass die Populationsdifferenzierung nicht allein auf genetische Isolation infolge räumlicher Distanz zurückzuführen ist. Außerdem korrelierte die Pflanzenfitness nicht mit der Entfernung des Versuchsgartens zum entsprechenden Herkunftsort, und die gemessenen Wachstums- und Reproduktionsmerkmale korrelierten nicht mit den klimatischen Merkmalen des Herkunftsortes. Beides deutet darauf hin, dass sich die Differenzierung zwischen den *B. davidii*-Populationen auch nicht auf einer lokalen Anpassung an ihre jeweilige Umwelt beruht.

Außer durch solche korrelative Ansätze kann lokale Anpassung mit Hilfe reziproker Verpflanzungsexperimente untersucht werden. Deshalb integrierten wir ein solches Verpflanzungsexperiment in das Common-Garden-Experiment, indem Nachkommen einer Population aus jeder der Common-Garden-Städte in die Populationsauswahl einbezogen wurde. Obwohl genetische Variation zwischen den Populationen nachgewiesen wurde, gab es keine signifikante Genotyp x Umwelt-Interaktion, was als weiteres Indiz für das Fehlen lokaler Anpassung gewertet werden muss.

Eine zweite Studie untersuchte die genetische Differenzierung und die lokale Anpassung von *B. davidii* an Frost als einen wichtigen klimatischen Faktor, der die Verbreitung von Arten in der temperaten Klimazone beschränkt. Es galt zu klären, ob Populationen aus sub-kontinentalem Klima weniger frostempfindlich sind als Populationen aus ozeanischem Klima. Dazu wurde die Frostresistenz jener Pflanzen untersucht, die gemeinsam in dem im sub-kontinentalen Klima gelegenen Common-Garden (Halle) wuchsen (Kapitel 4). Diese Studie bestand zum einen aus einem experimentellen Ansatz, der lokale Anpassung an Minimumtemperaturen testete und zum anderen aus einem ökologischen Nischenmodell, um die Verbreitung von *B. davidii* im invasiven und nativen Areal zu vergleichen. Die Populationen wiesen zwar signifikante Unterschiede in ihrer Frostresistenz auf (Population x Frost Interaktion), diese korrelierten jedoch nicht mit den klimatischen Merkmalen des Herkunftsortes. Das weist darauf hin, dass die Populationen nicht genetisch an die klimatischen Bedingungen ihres Herkunftsgebietes innerhalb des invasiven Areals adaptiert sind. Jedoch zeigte das ökologische Nischenmodell, dass das potentielle Verbreitungsgebiet von *B. davidii* noch nicht vollständig ausgefüllt ist. Das legt die Vermutung nah, dass die zukünftige Verbreitung nicht durch die klimatischen Bedingungen begrenzt sein wird. Die Ergebnisse beider Untersuchungen konnten die Annahme von Krivanek and Pyšek (2006) nicht bestätigen: die gegenwärtige, zwischen dem ozeanischen und subkontinentalem Klima verlaufende Verbreitungsgrenze von *B. davidii* in Europa ist offensichtlich nicht auf mangelnde Frostresistenz zurückzuführen.

In vielen Fällen ist eine Bekämpfung bereits etablierter invasiver Arten oft notwendig, um ihre Einflüsse auf die Ökosysteme zu minimieren. In Europa wächst *B. davidii* hauptsächlich an Bahnstrecken, von denen sie aus Sicherheitsgründen entfernt werden muss. Am häufigsten werden dazu Herbizide, vor allem Glyphosat verwendet. Der Einsatz von Herbiziden kann allerdings zur Evolution von Resistenz führen. Aufgrund dieser Tatsache ergibt sich die Frage, ob sich bei *B. davidii* bereits eine Herbizidresistenz entwickelt hat. Insbesondere galt es herausfinden, ob Individuen von *B. davidii*, die entlang von Bahngleisen wachsen, weniger empfindlicher auf das Herbizid reagieren als Individuen aus anderen Habitaten

(Kapitel 5). Zu diesem Zweck wurden zwölf Populationen aus drei verschiedenen Habitattypen (Bahnanlagen, Ruderalstellen und halbnatürliche Habitate) in einem Gewächshausexperiment auf ihre Glyphosat-Resistenz untersucht. Eine 100%ige Mortalität bei der Anwendung der empfohlenen Dosis von 30 mL/L verdeutlichte das Fehlen der erwarteten Glyphosat-Resistenz. Des Weiteren zeigte ein Vergleich der Dosis-Antwort-Kurven und der LD_{50}- sowie der LD_{90}-Werte (d.h., der letalen Dosis, bei der 50% bzw. 90% der Pflanzen sterben), dass Populationen der Bahnanlagen keine erhöhte Resistenz entwickelt haben. Deshalb kann Glyphosat weiterhin als effektives Herbizid für die Kontrolle dieser Pflanzenart betrachtet werden.

Aus diesen drei experimentellen Untersuchungen kann zusammenfassend geschlussfolgert werden, dass es entgegen der ursprünglichen Erwartungen keine lokale Anpassung in invasiven Populationen von *B. davidii* gibt. Unzureichende genetische Variation, als grundlegende Voraussetzung für evolutionäre Veränderungen, könnte eine Ursache dafür sein. Es ist anzunehmen, dass das Muster genetischer Differenzierung zwischen den Populationen von *B. davidii* vielmehr Gründereffekte widerspiegelt.

Die Beliebtheit als Zierpflanze führte dazu, dass heute 70 verschiedene Kultivare von *B. davidii* im Handel sind. Es ist daher sehr wahrscheinlich, dass die Individuen der invasiven Populationen von denjenigen Kultivaren abstammen, die in der unmittelbaren Umgebung angepflanzt wurden. Ferner führten Handel und Transport dieser Kultivare zu einer Durchmischung der Genotypen von *B. davidii* quer durch Europa. Durch Genfluss von diesen kultivierten Beständen in die invasiven Populationen könnte eine Anpassung an lokale Bedingungen bisher verhindert worden sein. Zukünftige molekulare Studien sollten die genetische Variation in invasiven Populationen quantifizieren, und herausfinden, welche Kultivare besonders zum Genpool dieser Populationen beigetragen haben. Des Weiteren kann der kurze Zeitraum seit der ersten erfolgreichen Ausbreitung im neuen Areal das Fehlen von Adaptationen begründen. Bei anderen Arten konnte bereits gezeigt werden, dass Anpassungen an bestehende Umweltbedingungen innerhalb weniger Generationen erfolgen können. Jedoch scheint der kurze Zeitraum im Zusammenspiel mit

Gründereffekten und dem damit verbundenen genetischen Flaschenhals an jedem Standort eine lokale Anpassung bei *B. davidii* verhindert zu haben. Dennoch schließt die gegenwärtige Abwesenheit von lokalen Anpassungen, eine schnelle adaptive Evolution in Zukunft nicht aus. Außerdem besteht weiterhin das Risiko, dass *B. davidii* mit dem weiteren einseitigen Gebrauch von Glyphosat letztendlich doch Resistenzen entwickelt. Solche Populationen dienen dann als Quelle resistenter Gene, die sich durch Pollen oder Samen weiter verbreiten können.

Die im Rahmen meiner Dissertation durchgeführten Untersuchungen verdeutlichen, dass die invasiven Populationen von *B. davidii* eine hohe phänotypische Plastizität aufweisen. Dadurch wird es den Pflanzen offenbar ermöglicht, unter verschiedenen Umweltbedingungen zu wachsen und sich zu reproduzieren. Auch andere Studien belegten bereits ein erhöhtes Maß an phänotypischer Plastizität in invasiven Populationen im Vergleich zu nativen Populationen. Die Ergebnisse der vorliegenden Untersuchungen bestätigen, dass phänotypische Plastizität eine wichtige Eigenschaft eines erfolgreichen Eindringlings wie *B. davidii* ist.

Buddleja davidii bleibt aufgrund ihrer hohen Wachstumsrate, ihrer schnellen Entwicklung zur Fruchtreife, der hohen Samenproduktion und den geringen Ansprüchen an ihre Umweltbedingungen, eine Pflanzenart mit hohem Invasionspotenzial. Damit verbunden besteht auch weiterhin die Gefahr, einheimische Arten zu verdrängen bzw. Interaktionen zwischen den verschiedenen trophischen Stufen zu beeinflussen. Die Bedeutung der Pflanzenzucht für den Invasionserfolg eingeführter Pflanzenarten sollte nicht unterschätzt werden, sondern sorgfältig beobachtet und in zukünftigen Untersuchungen von *B. davidii* bzw. anderer kultivierter Pflanzenarten unbedingt berücksichtigt werden. Ein vorausblickendes Monitoring dieser Pflanzenart und weitere Untersuchungen zum Einfluss züchterischer Selektion auf die Invasibilität sind weitere wichtige Schritte zum Verständnis des Invasionsprozesses des Sommerflieders. Da *B. davidii* ihr potentielles klimatisches Verbreitungsgebiet innerhalb des neuen Areals noch nicht vollständig ausgefüllt hat, ist anzunehmen, dass der Invasionsprozess zukünftig fortschreiten wird.

Bibliography

Adamowski, W., Dvorak, L. & Ramanjuk, I. (2002) Atlas of alien woody species of the Bialowieza primaeval forest. *Phytocoenosis*, **14**: 304.

Agrawal, A.A., Conner, J.K. & Stinchcombe, J.R. (2004) Evolution of plant resistance and tolerance to frost damage. *Ecology Letters*, **7**: 1199-1208.

Akaike, H. (1978) Bayesian-Analysis of minimum AIC procedure. *Annals of the Institute of Statistical Mathematics*, **30**: 9-14.

Albrecht, H.-J. (2004) Ergebnis der Buddleja-Sichtung. *Gartenpraxis*, **1**: 30-35.

Allendorf, F.W. & Lundquist, L.L. (2003) Introduction: Population biology, evolution, and control of invasive species. *Conservation Biology*, **17**: 24-30.

Alpert, P., Bone, E. & Holzapfel, C. (2000) Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. *Perspectives in Plant Ecology, Evolution and Systematics*, **3**: 52-66.

Anderson, J.E., Williams, J., Kriedemann, P.E., Austin, M.P. & Farquhar, G.D. (1996) Correlations between carbon isotope discrimination and climate of native habitats for diverse eucalypt taxa growing in a common garden. *Australian Journal of Plant Physiology*, **23**: 320.

Angiosperm Phylogeny Group II (2003) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society*, **141**: 399-436.

Anisko, T. & Im, U. (2001) Beware of Butterfly Bush. *American Nurseryman*, **194**: 46-49.

Armitage, A.M. & Dirr, M.A. (1995) Influence of spacing on yield of *Buddleia* and *Salix* grown as cut flowers and stems. *Journal of Environmental Horticulture*, **13**: 176-177.

Auge, H. & Brandl, R. (1997) Seedling recruitment in the invasive clonal shrub, *Mahonia aquifolium*. *Oecologia*, **110**: 205-211.

Austin, M.P. (2002) Spatial prediction of species distribution: an interface between ecological theory and statistical modelling. *Ecological Modelling*, **157**: 101-118.

AVH (2007) Australia's Virtual Herbarium. - <http://www.cpbr.gov.au/cgi-bin/avh.cgi>.

Baker, H.G. (1974) The evolution of weeds. *Annual Review of Ecology and Systematics*, **5**: 1-24.

Bannister, P. & Polwart, A. (2001) The frost resistance of ericoid heath plants in the British Isles in relation to their biogeography. *Journal of Biogeography*, **28**: 589-596.

Barrett, S.C.H. (1992) Genetics of weed invasions. In: *Applied population biology*, (ed. by Jain, S.K. & Botsford, L.W.), Kluwer, Dordrecht, The Netherlands.

Baucom, R.S. & Mauricio, R. (2008) The evolution of novel herbicide tolerance in a noxious weed: the geographic mosaic of selection. *Evolutionary Ecology*, **22**: 85-101.

Becker, U., Colling, G., Dostal, P., Jakobsson, A. & Matthies, D. (2006) Local adaptation in the monocarpic perennial *Carlina vulgaris* at different spatial scales across Europe. *Oecologia*, **150**: 506-518.

Bellingham, P.J., Peltzer, D.A. & Walker, L.R. (2005) Contrasting impacts of a native and an invasive exotic shrub on flood-plain succession. *Journal of Vegetation Science*, **16**: 135-142.

Blossey, B. & Nötzold, R. (1995) Evolution of increased competitive ability in invasive nonindigenous plants: A hypothesis. *Journal of Ecology*, **83**: 887-889.

BMU (1992) Konvention über die Biologische Vielfalt (deutsche Fassung). In: *Dokumente zur Konferenz der Vereinten Nationen für Umwelt und Entwicklung im Juni 1992 in Rio de Janeiro,* (ed. by BMU- Bundesumweltministerium), Bonn.

BMU (2003) Ökonomische Folgen der Ausbreitung von gebietsfremden Organismen in Deutschland. In: *Forschungsbericht 201 86 211 des Umweltbundesamtes. Umweltforschungsplan des Bundesministeriums für Umwelt, Naturschutz und Reaktorsicherheit.,* (ed. by BMU-Bundesumweltministerium).

Bone, E. & Farres, A. (2001) Trends and rates of microevolution in plants. *Genetica*, **112**: 165-182.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W., Siemann, E. & Prati, D. (2005) Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, **144**: 1-11.

Bradshaw, W.E., Zani, P.A. & Holzapfel, C.M. (2004) Adaptation to temperate climates. *Evolution*, **58**: 1748-1762.

Brandes, D. (1983) Flora und Vegetation der Bahnhöfe Mitteleuropas. *Phytocoenologia*, **11**: 31-115.

Brandes, D. (1993) Eisenbahnanlagen als Untersuchungsgegenstand der Geobotanik. *Tuexenia*, **13**: 415-444.

Brandl, R., Klotz, S., Stadler, J. & Auge, H. (2001) Nischen, Lebensgemeinschaften und biologische Invasionen. In: *Rundgespräche der Kommission für Ökologie,* (ed. by Reichholf, J.H.), pp. 81-98. Bayerische Akademie der Wissenschaften 22, München.

Bridle, J.R. & Vines, T.H. (2007) Limits to evolution at range margins: when and why does adaptation fail? *Trends in Ecology & Evolution*, **22**: 140-147.

Brockerhoff, E.G., Withers, T.M., Kay, M. & Faulds, W. (1999) Impact of the defoliator *Cleopus japonicus* (Coleoptera: Curculionidae) on *Buddleja davidii* in the laboratory. *Proceedings of the N.Z.Plant Protection Conference*, **52**: 113-118.

Broennimann, O., Treier, U.A., Muller-Scharer, H., Thuiller, W., Peterson, A.T. & Guisan, A. (2007) Evidence of climatic niche shift during biological invasion. *Ecology Letters*, **10**: 701-709.

Brown, K. (1990) The weed status and ecology of *Buddleja davidii* in the Orongorongo Valley (Tararua Ecological District). Bachelor Thesis. Victoria University of Wellington.

Bruelheide, H. (2002) Climatic factors controlling the eastern and altitudinal distribution boundary of *Digitalis purpurea* L. in Germany. *Flora*, **197**: 475-490.

Buckley, Y.M., Downey, P., Fowler, S.V., Hill, R., Memmot, J., Norambuena, H., Pitcairn, M., Shaw, R., Sheppard, A.W., Winks, C., Wittenberg, R. & Rees, M. (2003) Are invasives bigger? A global study of seed size variation in two invasive shrubs. *Ecology*, **84**: 1434-1440.

Cabin, R.J. & Mitchell, R.J. (2000) To Bonferroni or not to Bonferroni: When and how are the questions. *Bulletin of the Ecological Society of America*, **81**: 246-248.

Callaway, R.M. & Aschehough, E.T. (2000) Invasive plants versus their new and old neighbors: a mechanism for exotic invasions. *Science*, **290**: 521-523.

Campbell, D.J. (1984) The vascular flora of the DSIR study area, lower Orongorongo Valley, Wellington, New Zealand. *New Zealand Journal of Botany*, **22**: 223-270.

Cano, L., Escarre, J., Fleck, I., Blanco-Moreno, J.M. & Sans, F.X. (2008) Increased fitness and plasticity of an invasive species in its introduced range: a study using *Senecio pterophorus*. *Journal of Ecology*, **96**: 468-476.

Caroll, S.P. & Dingle, H. (1996) The biology of postinvasion events. *Biological Conservation*, **78**: 207-214.

CBD (2007) The convention on biological diversity. - <http://www.cbd.int/convention/convention.shtml>.

Chapin, F.S. & McNaughton, S.J. (1989) Lack of compensatory growth under phosphorus deficiency in grazing-adapted grasses from the Serengeti Plains. *Oecologia*, **79**: 551-557.

Chittka, L. & Schurkens, S. (2001) Successful invasion of a floral market. *Nature*, **411**: 653.

Clausen, J., Keck, D.D. & Hiesey, W. (1940) Experimental studies on the nature of species. I. Effects of varied environments on western North American plants . *Carnegie Institute of Washington*, **520**: 1-452.

Cooke, F., Taylor, P.D., Francis, C.M. & Rockwell, R.F. (1990) Directional selection and clutch size in birds. *American Naturalist*, **136**: 261-267.

Cox, C. (2004) Glyphosate. *Journal of Pesticide Reform*, **24**: 10-15.

Crawley, M.J. (1986) The population biology of invaders. *Philosophical Transactions of the Royal Society of London.Series B*, **314**: 711-731.

Crawley, M.J. (1987) What makes a community invasible. In: *Colonization, Succession and Stability,* (ed. by Gray, A.J., Crawley, M.J. & Edwards, P.J.), pp. 429-453. Blackwell Scientific Publications, Oxford.

Csurshes, S. & Edwards, R. (1998) *Potential environmental weeds in Australia: Candidate species for preventive control.* Biodiversity Group, Environment Australia, Canberra.

Culley, T.M. & Hardiman, N.A. (2007) The beginning of a new invasive plant: A history of the ornamental Callery pear in the United States. *Bioscience*, **57**: 956-964.

Culliney, T.W. (2005) Benefits of classical biological control for managing invasive plants. *Critical Reviews in Plant Sciences*, **24**: 131-150.

CVH (2007) Chinese Virtual Herbarium. - <www.cvh.org.cn>.

Davison, A.W. & Reiling, K. (1995) A rapid change in ozone resistance of *Plantago major a*fter summers with high ozone concentrations. *New Phytologist*, **131**: 337-344.

Dehnen-Schmutz, K., Touza, J., Perrings, C. & Williamson, M. (2007) The horticultural trade and ornamental plant invasions in Britain. *Conservation Biology*, **21**: 224-231.

Dewalt, S.J., Denslow, J.S. & Ickes, K. (2004) Natural-enemy release facilitates habitat expansion of the invasive tropical shrub *Clidemia hirta*. *Ecology*, **85**: 471-483.

Dietz, H., Fischer, M. & Schmid, B. (1999) Demographic and genetic invasion history of a 9-year-old roadside population of *Bunias orientalis* L. (Brassicaceae). *Oecologia*, **120**: 225-234.

Dirr, M.A. (1997) *Dirr's Hardy Trees and Shrubs. An Illustrated Encyclopedia.* Timber Press, Portland, Oregon.

Drake, J.A., Mooney, H.J., Di Castri, F., Grovers, R.H., Kruger, F.J., Reijmanek, M., & Williamson, M. (1989) *Biological invasions: a global perspective.* Wiley, New York.

Durka, W. (2000) Autogame Reproduktion: die Bedeutung der populationsgenetischen Struktur für den Artenschutz am Beispiel der Stromtalart *Corrigiola litoralis* L. *Schriftenreihe für Vegetationskunde*, **32**: 61-71.

Dybdahl, M.F. & Kane, S.L. (2005) Adaptation vs. phenotypic plasticity in the success of a clonal invader. *Ecology*, **86**: 1592-1601.

Ebeling, S.K., Welk, E., Auge, H. & Bruelheide, H. (2008a) Predicting the spread of an invasive plant: Combining experiments and ecological niche models. *Ecography*, -accepted.

Ebeling, S.K., Hensen, I. & Auge, H. (2008b) The invasive shrub *Buddleja davidii* performs better in its introduced range. *Diversity and Distributions*, **14**: 225-233.

Ellstrand, N.C. & Schierenbeck, K.A. (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences of the United States of America*, **97**: 7043-7050.

Elton, C.S. (1958) *The ecology of invasions by animals and plants.* Methuen, London.

Esler, A.E. (1988a) The naturalisation of plants in urban Auckland, New Zealand. 5. Success of the alien species. *New Zealand Journal of Botany*, **26**: 565-584.

Esler, A.E. (1988b) The naturalisation of plants in urban Auckland, New Zealand. 4. The nature of the naturalised species. *New Zealand Journal of Botany*, **26**: 345-385.

Falconer, D.S. & MacKay, T.F.C. (1996) *Introduction to Quantitative Genetics.* Longman, Essex.

Feinstein, A.R. & Cicchetti, D.V. (1990) High agreement but low Kappa. 1. The problems of 2 paradoxes. *Journal of Clinical Epidemiology*, **43**: 543-549.

Fischer, M., Husi, R., Prati, D., Peintinger, M., van Kleunen, M. & Schmid, B. (2000) RAPD variation among and within small and large populations of the rare clonal plant *Ranunculus reptans* (Ranunculaceae). *American Journal of Botany*, **87**: 1128-1137.

Font, X. (2007) *Mòdul flora i vegetació. Banc de dades de biodiversitat de Catalunya.* Generalitat de Catalunya i Universitat de Barcelona, Barcelona.

Franklin, J. (1995) Predictive vegetation mapping: Geographic modelling of biospatial patterns in relation to environmental gradients. *Progress in Physical Geography*, **19**: 474-499.

Frazer, A. (1965) Colonization and genetic drift. In: *The genetics of colonizing species*, (ed. by Baker, H.G. & Stebbins, G.L.), pp. 117-125. Academic Press Inc., New York.

Frenzel, M. & Brandl, R. (2003) Diversity and abundance patterns of phytophagous insect communities on alien and native host plants in the Brassicaceae. *Ecography*, **26**: 723-730.

Galloway, L.F. & Fenster, C.B. (2000) Population differentiation in an annual legume: Local adaptation. *Evolution*, **54**: 1173-1181.

GBIF (2007) Data Portal of the Global Biodiversity Information Facility. -<http://data.gbif.org/welcome.htm>.

Gordon, D.R. (1998) Effects of invasive, non-indigenous plant species on ecosystem processes: Lessons from Florida. *Ecological Applications*, **8**: 975-989.

Harper, J.L. (1977) *Population Biology of Plants.* Academic Press, London.

Heap, I.M. (2007) The international survey of herbicide resistent weeds. -<www.weedscience.com>.

Heap, I.M. (1997) The occurrence of herbicide-resistant weeds worldwide. *Pesticide Science*, **51**: 235-243.

Hierro, J.L., Maron, J.L. & Callaway, R.M. (2005) A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. *Journal of Ecology*, **93**: 5-15.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**: 1965-1978.

Hochwender, C.G., Marquis, R.J. & Stowe, K.A. (2000) The potential for and constraints on the evolution of compensatory ability in *Asclepias syriaca*. *Oecologia*, **122**: 361-370.

Huangfu, C.H., Song, X.L., Qiang, S. & Zhang, H.J. (2007) Response of wild *Brassica juncea* populations to glyphosate. *Pest Management Science*, **63**: 1133-1140.

Humphries, R.N., Jordan, M.A. & Guarino, L. (1982) The effect of water stress on the mortality of *Betula pendula* Roth. and *Buddleia davidii* Franch. seedlings. *Plant and Soil*, **64**: 273-276.

Humphries, R.N. & Guarino, L. (1987) Soil nitrogen and the growth of birch and buddleia in abandoned chalk quarries. *Reclamation and Revegetation Research*, **6**: 55-61.

Jakobs, G., Weber, E. & Edwards, P.J. (2004) Introduced plants of the invasive *Solidago gigantea* (Asteraceae) are larger and grow denser than conspecifics in the native range. *Diversity and Distributions*, **10**: 11-19.

Joshi, J., Schmid, B., Caldeira, M.C., Dimitrakopoulos, P.G., Good, J., Harris, R., Hector, A., Huss-Danell, K., Jumpponen, A., Minns, A., Mulder, C.P.H., Pereira, J.S., Prinz, A., Scherer-Lorenzen, M., Siamantziouras, A.-S.D., Terry, A.C., Troumbis, A.Y. & Lawton, J.H. (2001) Local adaptation enhances performance of common plant species. *Ecology Letters*, **4**: 536-544.

Joshi, J. & Vrieling, K. (2005) The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores. *Ecology Letters*, **8**: 704-714.

Kawecki, T.J. & Ebert, D. (2004) Conceptual issues in local adaptation. *Ecology Letters*, **7**: 1225-1241.

Kay, M. & Smale, M.C. (1990) The potential for biological control of *Buddleja davidii* Franchet in New Zealand. *FRI Bulletin - New Zealand Ministry of Forestry, Forest Research Institute*, **155**: 29-33.

Kay, N. (2002) Variety in Buddleia Biocontrol. *Biocontrol News and Information*, **23.**

Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, **17**: 164-170.

Kitajima, K., Fox, A.M., Sato, T. & Nagamatsu, D. (2006) Cultivar selection prior to introduction may increase invasiveness: Evidence from *Ardisia crenata*. *Biological Invasions*, **8**: 1471-1482.

Klinkenberg, B. (2007) E-Flora BC: Electronic Atlas of the Plants of British Columbia. - <http://www.eflora.bc.ca/>.

Koger, C.H., Poston, D.H., Hayes, R.M. & Montgomery, R.E. (2004) Glyphosate-resistant horseweed (*Conyza canadensis*) in Mississippi. *Weed Technology*, **18**: 820-825.

Kollmann, J. & Banuelos, M.J. (2004) Latitudinal trends in growth and phenology of the invasive alien plant *Impatiens glandulifera* (Balsaminaceae). *Diversity and Distributions*, **10**: 377-385.

Kowarik, I. (1995) Time lags in biological invasions with regard to the success and failure of alien species. In: *Plant Invasions - General Aspects and Special Problems,* (ed. by Pyšek, P., Prach, K., Rejmánek, M. & Wade, M.), pp. 15-38. Academic Publishing, Amsterdam.

Kowarik, I. (2003) *Biologische Invasionen – Neophyten und Neozoen in Mitteleuropa.* Ulmer, Stuttgart.

Kreh, W. (1952) Der Fliederspeer (*Buddleja variabilis*) als Jüngsteinwanderer unserer Flora. *Aus der Heimat: naturwissenschaftliche Monatszeitschrift*, **60**: 20-25.

Krivanek, M., Pyšek, P. & Jarosik, V. (2006) Planting history and propagule pressure as predictors of invasion by woodysSpecies in a temperate region. *Conservation Biology*, **20**: 1487-1498.

Krivanek, M. & Pyšek, P. (2006) Predicting invasions by woody species in a temperate zone: a test of three risk assessment schemes in the Czech Republic (Central Europe). *Diversity and Distributions*, **12**: 319-327.

Krüssmann, G. (1976) *Handbuch der Laubgehölze.* Parey, Berlin & Hamburg.

Kunick, W. (1970) Der Schmetterlingsstrauch (*Buddleja davidii* Franch.) in Berlin. *Berliner Naturschutzblätter*, **14**: 407-410.

Lauener, L.A. (1996) *The introduction of Chinese plants into Europe*. Amsterdam, SPB Academic Publishing.

Lee, C.E. (2002) Evolutionary genetics of invasive species. *Trends in Ecology & Evolution*, **17**: 386-391.

Lee, L.J. & Ngim, J. (2000) A first report of glyphosate-resistant goosegrass (*Eleusine indica* (L) Gaertn) in Malaysia. *Pest Management Science*, **56**: 336-339.

Leeuwenberg, A.J.M. (1979) *The Loganiaceae of Africa XVIII, Buddleja L. II*. Revision of the African and Asiatic species. Wageningen, Veenman, H. & Zonen, B.V.

Leger, E.A. & Rice, K.J. (2003) Invasive California poppies (*Eschscholzia californica* Cham.) grow larger than native individuals under reduced competition. *Ecology Letters*, **6**: 257-264.

Lennartsson, T., Tuomi, J. & Nilsson, P. (1997) Evidence for an evolutionary history of overcompensation in the grassland biennial *Gentianella campestris* (Gentianaceae). *American Naturalist*, **149**: 1147-1155.

Lennartsson, T., Nilsson, P. & Tuomi, J. (1998) Induction of overcompensation in the field gentian, *Gentianella campestris*. *Ecology*, **79**: 1061-1072.

Levine, J.M., Vilá, M., D'Antonio, C.M., Dukes, J.S., Grigulis, K. & Lavorel, S. (2003) Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society B: Biological Sciences*, **270**: 775-781.

Linhart, Y.B. & Grant, M.C. (1996) Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics*, **27**: 237-277.

Littell, R.C., Milliken, G.A., Stroup, W.W., & Wolfinger, R.D. (1996) *SAS System for Mixed Models.* SAS Institute Inc., Cary.

Lodge, D.M. (1993) Biological Invasions - Lessons for Ecology. *Trends in Ecology & Evolution*, **8**: 133-137.

Loiselle, B.A., Jorgensen, P.M., Consiglio, T., Jimenez, I., Blake, J.G., Lohmann, L.G. & Montiel, O.M. (2008) Predicting species distributions from herbarium collections: does climate bias in collection sampling influence model outcomes? *Journal of Biogeography*, **35**: 105-116.

Luijten, S.H., Dierick, A., Oostermeijer, J.G.B., Raijmann, L.E.L. & Den Nijs, H.C.M. (2000) Population size, genetic variation, and reproductive success in a rapidly declining, self-incompatible perennial (*Arnica montana*) in The Netherlands. *Conservation Biology*, **14**: 1776-1787.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzaz, F.A. (2000) Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications*, **10**: 689-710.

Main, C.L., Mueller, T.C., Hayes, R.M. & Wilkerson, J.B. (2004) Response of selected horseweed (*Conyza canadensis* (L.) cronq.) Populations to glyphosate. *Journal of Agricultural and Food Chemistry*, **52**: 879-883.

Maron, J.L. & Vila, M. (2001) When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos*, **95**: 361-373.

Maron, J.L., Vila, M., Bommarco, R., Elmendorf, S. & Beardsley, P. (2004) Rapid evolution of an invasive plant. *Ecological Monographs*, **74**: 261-280.

Matthies, D. (2000) The genetic and demographic consequences of habitat fragmentation for plants. Examples from declining grassland species. *Schriftenreihe Vegetationskunde*, **32**: 129-140.

Mehrhoff, L.J., Silander, J.A.Jr., Leicht, S.A., Mosher, E.S., & Tabak, N.M. (2003) IPANE: Invasive Plant Atlas of New England, Department of Ecology & Evolutionary Biology, University of Connecticut, Storrs, CT, USA. - <http://nbii-nin.ciesin.columbia.edu/ipane/>.

Memmott, J., Fowler, S.V., Paynter, Q., Sheppard, A.W. & Syrett, P. (2000) The invertebrate fauna on broom, *Cytisus scoparius*, in two native and two exotic habitats. *Acta Oecologica*, **21**: 213-222.

Miller, A. (1984) The distribution and ecology of *Buddleja davidii* Franch. in Britain, with particular reference to conditions supporting germinationand the establishment of seedlings. PhD thesis. Department of Biology, Oxford Polytechnic.

Monserud, R.A. & Leemans, R. (1992) Comparing global vegetation maps with the Kappa-statistic. *Ecological Modelling*, **62**: 275-293.

Mooney, H.A. & Cleland, E.E. (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences of the United States of America*, **98**: 5446-5451.

Moran, M.D. (2003) Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos*, **100**: 403-405.

Müller-Schärer, H., Schaffner, U. & Steinger, T. (2004) Evolution in invasive plants: implications for biological control. *Trends in Ecology & Evolution*, **19**: 417-422.

Müller-Schärer, H. & Steinger, T. (2004) Predicting evolutionary change in invasive, exotic plants and its consequences for plant-herbivore interactions. In: *Genetics, Evolution and Biological Control*, (ed. by Ehler L.E., Sforza R. & Mateille T.), pp. 137-162. CABI Publishing, Wallingford, UK.

Murray, M.B., Cape, J.N. & Fowler, D. (1989) Quantification of frost damage in plant-tissues by rates of electrolyte leakage. *New Phytologist*, **113**: 307-311.

Neve, P. & Powles, S. (2005) High survival frequencies at low herbicide use rates in populations of *Lolium rigidum* result in rapid evolution of herbicide resistance. *Heredity*, **95**: 485-492.

Neve, P. (2007) Challenges for herbicide resistance evolution and management: 50 years after Harper. *Weed Research*, **47**: 365-369.

New, M., Lister, D., Hulme, M. & Makin, I. (2002) A high-resolution data set of surface climate over global land areas. *Climate Research*, **21**: 1-25.

Novak, S.J., Mack, R.N. & Soltis, D.E. (1991) Genetic-variation in *Bromus tectorum* (Poaceae) - Population differentiation in its North-American range. *American Journal of Botany*, **78**: 1150-1161.

Novak, S.J. & Mack, R.N. (1993) Genetic-variation in *Bromus tectorum* (Poaceae) - Comparison between native and introduced populations. *Heredity*, **71**: 167-176.

OSU (2007) Oregon Flora Project. - <http://www.oregonflora.org/>.

Owen, D.F. & Whiteway, W.R. (1980) *Buddleia davidii* in Britain: History and development of an associated fauna. *Biological Conservation*, **17**: 149-155.

Paige, K.N. & Whitham, T.G. (1987) Overcompensation in response to mammalian herbivory - the advantage of being eaten. *American Naturalist*, **129**: 407-416.

Palumbi, S.R. (2001) Evolution - Humans as the world's greatest evolutionary force. *Science*, **293**: 1786-1790.

Parker, I.M., Rodriguez, J. & Loik, M.E. (2003) An evolutionary approach to understanding the biology of invasions: Local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. *Conservation Biology*, **17**: 59-72.

Perez, A. & Kogan, M. (2003) Glyphosate-resistant *Lolium multiflorum* in Chilean orchards. *Weed Research*, **43**: 12-19.

Perez, A., Alister, C. & Kogan, M. (2004) Absorption, translocation and allocation of glyphosate in resistant and susceptible Chilean biotypes of *Lolium multiflorum*. *Weed Biology and Management*, **4**: 56-58.

Pergl, J., Perglova, I., Pyšek, P. & Dietz, H. (2006) Population age structure and reproductive behavior of the monocarpic perennial *Heracleum mantegazzianum* (Apiaceae) in its native and invaded distribution ranges. *American Journal of Botany*, **93**: 1018-1028.

Peterson, A.T., Ball, L.G. & Cohoon, K.P. (2002) Predicting distributions of Mexican birds using ecological niche modelling methods. *Ibis*, **144**: E27-E32.

Pfitzner, G. (1983) Der Stellenwert eines Buddleja-Beobachtungsnetzes für die Erfassung von Tagfalterbeständen. *Öko L.*, **5**: 10-16.

Pimentel, D., Lach, L., Zuniga, R. & Morrison, D. (2000) Environmental and economic costs of nonindigeniuos species in the United States. *Bioscience*, **50**: 53-63.

Powles, S.B. & Preston, C. (2006) Evolved glyphosate resistance in plants: Biochemical and genetic basis of resistance. *Weed Technology*, **20**: 282-289.

Prati, D. & Bossdorf, O. (2004) A comparison of native and introduced populations of the South African Ragwort *Senecio inaequidens* DC. in the field. *2.Symposium der A.F.W.Schimper-Stiftung*, pp.1-7, Hohenheim.

Pulliam, H.R. (2000) On the relationship between niche and distribution. *Ecology Letters*, **3**: 349-361.

Pyšek, P. (1995) Recent studies on plant invasions (1974-1993). In: *Plant invasions. General aspects and special problems,* (ed. by Pyšek, P., Prach, K., Rejmanek, M. & Wade, M.), SPB Academic Publ, Amsterdam.

Pyšek, P. (1998) Alien and native species in Central European urban floras: a quantitative comparison. *Journal of Biogeography*, **25**: 155-163.

Pyšek, P., Richardson, D.M., Rejmánek, M., Webster, G.L., Williamson, M. & Kirschner, J. (2004a) Alien plants in checklists and floras: Towards better communication between taxonomists and ecologists. *Taxon*, **53**: 131-143.

Pyšek, P., Richardson, D.M. & Williamson, M. (2004b) Predicting and explaining plant invasions through analysis of source area floras: some critical considerations. *Diversity and Distributions*, **10**: 179-187.

Pyšek, P. & Richardson, D. (2007) Traits associated with invasiveness in alien plants: Where do we stand? In: *Biological invasions, Ecological Studies 193,* (ed. by Nentwig, W.), pp. 97-125. Springer, Berlin & Heidelberg.

Ramwell, C.T., Heather, A.I.J. & Shepherd, A.J. (2004) Herbicide loss following application to a railway. *Pest Management Science*, **60**: 556-564.

Randall, J.M. & Marinelli, J. (1996) *Invasive plants: weeds of the global garden.* Brooklyn Botanic Garden, New York.

Ream, J. (2006) Production and invasion of Butterfly Bush (*Buddleja davidii*) in Oregon. Oregon State University,University of Honors College and Bioresource Research.

Reichard, S.H. & Hamilton, C.W. (1997) Predicting invasions of woody plants introduced into North America. *Conservation Biology*, **11**: 193-203.

Reinhart, K.O., Royo, A.A., Van der Putten, W.H. & Clay, K. (2005) Soil feedback and pathogen activity in *Prunus serotina* throughout its native range. *Journal of Ecology*, **93**: 890-898.

Rice, K.J. & Mack, R.N. (1991a) Ecological genetics of *Bromus tectorum*. 2. Intraspecific variation in phenotypic plasticity. *Oecologia*, **88**: 84-90.

Rice, K.J. & Mack, R.N. (1991b) Ecological Genetics of Bromus-Tectorum .3. the Demography of Reciprocally Sown Populations. *Oecologia*, **88**: 91-101.

Richards, C.L., Bossdorf, O., Muth, N.Z., Gurevitch, J. & Pigliucci, M. (21-8-2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters*, **9**: 981-993.

Richardson, B., Kimberley, M.O., Ray, J.W. & Coker, G.W. (1999) Indices of interspecific plant competition for *Pinus radiata* in the central north island of New Zealand. *Canadian Journal of Forest Research-Revue Canadienne de Recherche Forestiere*, **29**: 898-905.

Richardson, D.M., Wilgen, B.W., Higgins, S.I., Trinder-Smith, T.H., Cowling, R.M. & McKell, D.H. (1996) Current and future threats to plant biodiversity on the Cape Peninsula, South Africa. *Biodiversity and Conservation*, **5**: 607-647.

Richardson, D.M., Allsopp, N., D'Antonio, C.M., Milton, S.J. & Rejmanek, M. (2000a) Plant invasions - the role of mutualisms. *Biological Reviews*, **75**: 65-93.

Richardson, D.M., Pyšek, P., Rejmanek, M., Barbour, M.G., Panetta, F.D. & West, C.J. (2000b) Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions*, **6**: 93-107.

Richardson, D.M., Rouget, M. & Rejmánek, M. (2004) Using natural experiments in the study of alien tree invasions: opportunities and limitations. In: *Experimental approaches to conservation biology,* (ed. by Gordon.M.S. & Bartol, S.M.), pp. 180-201. University of California Press, Berkeley.

Richardson, D.M. & Pyšek, P. (2006) Plant invasions: merging the concepts of species invasiveness and community invasibility. *Progress in Physical Geography*, **30**: 409-431.

Roach, D.A. & Wulff, R.D. (1987) Maternal effects in plants. *Annual Review of Ecology and Systematics*, **18**: 209-235.

Rosenthal, J.P. & Kotanen, P.M. (1994) Terrestrial plant tolerance to herbivory. *Trends in Ecology & Evolution*, **9**: 145-148.

Ross, C. & Auge, H. (2008) Invasive Mahonia plants outgrow their native relatives. *Plant Ecology*, DOI 10.1007/s11258-008-9408-z.

Ross, C., Faust, D. & Auge, H. (2008) Mahonia invasions in different habitats: local adaptation or general-purpose genotypes? *Biological Invasions*, DOI 10.1007/s10530-008-9261-y.

Rothmaler, W. (2002) *Exkursionsflora von Deutschland*. Gefäßpflanzen: Grundband. Jena, Gustav Fischer.

Sakai, A. & Wardle, P. (1978) Freezing resistance of New Zealand trees and shrubs. *New Zealand Journal of Ecology*, **1**: 51-61.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N. & Weller, S.G. (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**: 305-332.

Sala, O.E., Chapin, F.S., Armesto, J.J., Berlow, E., Bloomfield, J., Dirzo, R., Huber-Sanwald, E., Huenneke, L.F., Jackson, R.B., Kinzig, A., Leemans, R., Lodge, D.M., Mooney, H.A., Oesterheld, M., Poff, N.L., Sykes, M.T., Walker, B.H., Walker, M. & Wall, D.H. (2000) Biodiversity - Global biodiversity scenarios for the year 2100. *Science*, **287**: 1770-1774.

Savolainen, O., Bokma, F., Garcia-Gil, R., Komulainen, P. & Repo, T. (2004) Genetic variation in cessation of growth and frost hardiness and consequences for adaptation of *Pinus sylvestris* to climatic changes. *Forest Ecology and Management*, **197**: 79-89.

Scheumann, W. & Schönbach, H. (1968) Die Prüfung der Frostresistenz von 25 *Larix leptolepis* - Herkünften eines internationalen Provenienzversuches mit Hilfe von Labor-Prüfverfahren. *Archiv für Forstwesen*, **17**: 597-611.

Schlichting, C.D. (1986) The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics*, **17**: 667-693.

Schmitz, J. (1991) Vorkommen und Soziologie neophytischer Sträucher im Raum Aachen. *Decheniana*, **144**: 22-38.

Schweinsberg, F., Abke, W., Rieth, K., Rohmann, U. & Zullei-Seibert, N. (1999) Herbicide use on railway tracks for safety reasons in Germany? *Toxicology Letters*, **107**: 201-205.

Segal, S. (1969) *Ecological notes on wall vegetation.* Junk, The Hague.

Settele, J., Hammen, V., Hulme, P., Karlson, U., Klotz, S., Kotarac, M., Kunin, W., Marion, G., O'Connor, M., Petanidou, T., Peterson, K., Potts, S., Pritchard, H., Pyšek, P., Rounsevell, M., Spangenberg, J., Steffan-Dewenter, I., Sykes, M., Vighi, M., Zobel, M. & Kühn, I. (2005) ALARM -- Assessing LArge-scale environmental Risks for biodiversity with tested Methods. *GAIA*, **14**: 69-72.

Sexton, J.P., Mckay, J.K. & Sala, A. (2002) Plasticity and genetic diversity may allow saltcedar to invade cold climates in North America. *Ecological Applications*, **12**: 1652-1660.

Shaw, R.G. & Mitchell-Olds, T. (1993) ANOVA for unbalanced data: an overview. *Ecology*, **74**: 1638-1645.

Siemann, E. & Rogers, W.E. (2001) Genetic differences in growth of an invasive tree species. *Ecology Letters*, **4**: 514-518.

Simberloff, D., Parker, I.M. & Windle, P.N. (2005) Introduced species policy, management, and future research needs. *Frontiers in Ecology and the Environment*, **3**: 12-20.

SKEW (2008) Schweizerische Kommission für die Erhaltung von Wildpflanzen. - <www.cps-skew.ch>.

Smale, M.C. (1990) Ecological role of Buddleia (*Buddleja davidii*) in streambeds in Te Urewera National Park. *New Zealand Journal of Ecology*, **14**: 1-6.

Soldaat, L.L. & Auge, H. (1998) Interactions between an invasive plant, *Mahonia aquifolium*, and a native phytophagous insect, *Rhagoletis meigenii*. In: *Plant Invasions,* (ed. by Starfinger U., Edwards K., Kowarik I. & Williamson M.), pp. 347-360. Backhuys Publishers, Leiden.

Starr, F., Starr, K., & Loope, L. (2003) *Buddleia davidii*. - <http://www.hear.org/starr/hiplants/reports/html/buddleia_davidii.htm>.

Stockwell, D.R.B. & Noble, I.R. (1992) Induction of sets of rules from animal distribution data - a robust and informative method of data-analysis. *Mathematics and Computers in Simulation*, **33**: 385-390.

Storz, J.F. (2002) Contrasting patterns of divergence in quantitative traits and neutral DNA markers: analysis of clinal variation. *Molecular Ecology*, **11**: 2537-2551.

Strauss, S.Y. & Agrawal, A.A. (1999) The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology & Evolution*, **14**: 179-185.

Stuart, D.D. (2006) *Plant collector guide: Buddlejas.* Timber Press Royal Horticultural Society, Portland, Oregon, USA.

Thebaud, C. & Simberloff, D. (2001) Are plants really larger in their introduced ranges? *American Naturalist*, **157**: 231-236.

Theoharides, K.A. & Dukes, J.S. (2007) Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. *New Phytologist*, **176**: 256-273.

Thompson, J.N. (1998) Rapid evolution as an ecological process. *Trends in Ecology & Evolution*, **13**: 329-332.

Torstensson, L., Borjesson, E. & Stenstrom, J. (2005) Efficacy and fate of glyphosate on Swedish railway embankments. *Pest Management Science*, **61**: 881-886.

Trumble, J.T., Kolodnyhirsch, D.M. & Ting, I.P. (1993) Plant compensation for arthropod herbivory. *Annual Review of Entomology*, **38**: 93-119.

Tutin, T.G. (1972) *Flora Europaea*. 3. Diapensiaceae to Myoporaceae. Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M., and Webb, D. A. (eds.). Cambridge, Cambridge Univ. Press.

Urban, M.C., Phillips, B.L., Skelly, D.K. & Shine, R. (2007) The cane toad's (*Chaunus [Bufo] marinus*) increasing ability to invade Australia is revealed by a dynamically updated range model. *Proceedings of the Royal Society B: Biological Sciences*, **274**: 1413-1419.

USDA (2007) The PLANTS Database. - <http://plants.usda.gov/>.

VanGessel, M.J. (2001) Glyphosate-resistant horseweed from Delaware. *Weed Science,* **49**: 703-705.

Verhoeven, K.J.F., Simonsen, K.L. & McIntyre, L.M. (2005) Implementing false discovery rate control: increasing your power. *Oikos*, **108**: 643-647.

Vermeij, G.J. (1991) When biotas meet - Understanding biotic interchange. *Science*, **253**: 1099-1104.

Vila, M., Weber, E. & D' Antonio, C.M. (2000) Conservation implications of invasion by plant hybridization. *Biological Invasions*, **2**: 207-217.

Vilà, M., Maron, J.L. & Marco, L. (2005) Evidence for the enemy release hypothesis in *Hypericum perforatum*. *Oecologia*, **142**: 474-479.

Vila-Aiub, M.M., Balbi, M.C., Gundel, P.E., Ghersa, C.M. & Powles, S.B. (2007) Evolution of glyphosate-resistant Johnsongrass (*Sorghum halepense*) in glyphosate-resistant soybean. *Weed Science*, **55**: 566-571.

Vitousek, P.M. (1990) Biological invasions and ecosystem processes - Towards an integration of population biology and ecosystem studies. *Oikos*, **57**: 7-13.

Vitousek, P.M., D'Antonio, C.M., Loope, L., Rejmanek, M. & Westbrooks, R. (1997) Introduced species: A significant component of human-caused global change. *New Zealand Journal of Ecology*, **21**: 1-16.

Weaver, S.E., Dirks, V. & Warwick, S.I. (1984) Variation and climatic adaptation in northern populations of *Datura stramonium*. *Canadian Journal of Botany*, **63**: 1303-1308.

Webb, C.J., Sykes, W.R., and Garnock-Jones, P.J. (1988) *Flora of New Zealand Volume IV*. Naturalised pteridophytes, gymnospermes, dicotyledons. Christchurch, Botany Division, Department of Scientific and Industrial Research.

Weber, E. (1997) Morphological variation of the introduced perennial *Solidago canadensis* L. sensu lato (Asteraceae) in Europe. *Botanical Journal of the Linnean Society*, **123**: 197-210.

Weber, E. (2003) *Invasive plant species of the world*. CAB International.

Weber, E. & Schmid, B. (1998) Latitudinal population differentiation in two species of *Solidago* (Asteraceae) introduced into Europe. *American Journal of Botany*, **85**: 1110-1121.

Weedmapper (2007) <http://www.weedmapper.org/>.

Welk, E., Schubert, K. & Hoffmann, M.H. (2002) Present and potential distribution of invasive garlic mustard (*Alliaria petiolata*) in North America. *Diversity and Distributions*, **8**: 219-233.

Wiens, J.J. & Graham, C.H. (2005) Niche conservatism: Integrating evolution, ecology, and conservation biology. *Annual Review of Ecology Evolution and Systematics*, **36**: 519-539.

Williams, J.L., Auge, H. & Maron, J.L. (2008) Different gardens, different results: Native and introduced populations exhibit contrasting phenotypes across common gardens. *Oecologia*, - accepted.

Williams, P.A. & Timmens, S. (2002) Economic impacts of weeds in New Zealand: some examples. In: *Biological invasions: economic and environmental costs of alien plant, animal, and microbe species,* (ed. by Pimentel, D.), pp. 175-184. CRS Press, Boca Raton.

Williamson, M. (1993) Invaders, weeds and the risk from genetically manipulated organisms. *Experientia*, **49**: 219-224.

Williamson, M.H. & Fitter, A. (1996) The characters of successful invaders. *Biological Conservation*, **78**: 163-170.

Willis, A.J. & Blossey, B. (1999) Benign environments do not explain the increased vigour of non-indigenous plants: A cross-continental transplant experiment. *Biocontrol Science and Technology*, **9**: 567-577.

Willis, S.G. & Hulme, P.E. (2002) Does temperature limit the invasion of *Impatiens glandulifera* and *Heracleum mantegazzianum* in the UK? *Functional Ecology*, **16**: 530-539.

Wilson, S.B., Thetford, M., Mecca, L.K. & Raymer, J.S. (2004a) Evaluation of 14 butterfly bush taxa grown in western and southern Florida: I. Visual quality, growth, and development. *Horttechnology*, **14**: 605-612.

Wilson, S.B., Thetford, M., Mecca, L.K., Raymer, J.S. & Gersony, J.A. (2004b) Evaluation of 14 butterfly bush taxa grown in western and southern Florida: II. Seed production and germination. *Horttechnology*, **14**: 612-618.

Woitke, M. & Dietz, H. (2002) Shifts in dominance of native and invasive plants in experimental patches of vegetation. *Perspectives in Plant Ecology Evolution and Systematics*, **5**: 165-184.

Wolfe, L.M. (2002) Why alien invaders succeed: Support for the escape-from-enemy hypothesis. *The American Naturalist*, **160**: 705-711.

Woodward, F.I. & Williams, B.G. (1987) Climate and plant-distribution at global and local scales. *Vegetatio*, **69**: 189-197.

Wu, Z.Y. & Ding, T.Y. (1999) *China Seed Plant Database.* Yunnan Science and Technology Press, Kunming, Yunnan.

Zelaya, I.A., Owen, M.D.K. & VanGessel, M.J. (2004) Inheritance of evolved glyphosate resistance in *Conyza canadensis* (L.) Cronq. *Theoretical and Applied Genetics*, **110**: 58-70.

Wu, Z.Y. and Raven, P.H. (1996) *Flora of China, Myrsinaceae through Loganiaceae*. Beijing, Science Press.

Supplement

Acknowledgement / Danksagung

Curriculum vitae

Contact information

Name: Susan Ebeling

Address: Geseniusstraße 8, 06110 Halle, Germany

Phone: 0049-(0)345 -120 79 63

e-mail: susan.ebeling@web.de

Personal Details

Date of birth: 19.11.1977,
City / Country: Halle, Germany
Languages: German, English, Russian

Education

1984 - 1991   Polytechnische Oberschule „Friedrich Schiller", Halle
1991 - 1996   Christian-Thomasius-Gymnasium, Halle
1996 - 2002   Martin-Luther-University Halle-Wittenberg, Diploma in
                 Biology, with a major in animal ecology and minors in plant ecology,
                 biochemistry and geography
2004 - 2008   PhD-thesis at the Helmholtz Centre for Environmental Research
                 (UFZ), Department for Community Ecology, supported by the
                 scholarship programme of the German Federal environmental
                 foundation (DBU)
2008   Field assistance for the DFG-project "Biodiversity Exploratories"

Diploma thesis: "Einfluss der Populationsgröße auf Besucherfrequenz,
                 Samenansatz und Keimung am Beispiel von *Pulsatilla vulgaris
                 ssp. vulgaris*, MILLER (1768) in der Porphyrkuppenlandschaft
                 bei Halle."

                 Supervision: Prof. Isabell Hensen, Dr. Monika Partzsch

PhD thesis	" Local Adapations as a reason for the success of plant invasions: A Case study on *Buddleja davidii"*
	Supervision: Prof. Isabell Hensen, Dr. Harald Auge

Work Experience

1999	assistance in the national park Harz
1999	internship at the biosphere reserve Spreewald
2001	student assistance at the University Halle, Institute of Botany
2003	"Praktikum für die Umwelt" (Environmental Education) in the Naturpark Altmühltal, Centre for Tourism and Environment in Eichstätt
2003-2008	internship and freelancing at the Zoo Halle, Department Environmental Education
2005	research stay in China, province Yunnan

Achievements

Publications: Ebeling, Hensen & Auge (2008) *Buddleja davidii* Franch. performs better in the introduced area. Diversity & Distribution, 14 (2): 225-233.

Feng, Auge & Ebeling (2008) More nitrogen allocation to the photosynthetic apparatus in *Buddleia davidii*, a noxious invasive shrub, than in five native woody species. Oecologia, 153 (3): 501-510.

Ebeling, Welk, Auge & Bruelheide: Predicting the spread of an invasive plant: Combining experiments and ecological niche models. Ecography, accepted

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Scholarship for participation in EMAPI Conference in Perth/Australia 2007 by the German Research Foundation (DFG)

Scholarship for participation in GfÖ Conference in Marburg / Germany 2007 by the Prof. Dr. Zerweck-/Casella-Stiftung

Scholarship for participation in EMAPI Conference in Katowice/ Poland 2005 by the sponsorship for women of the Martin-Luther-University in Halle

Volunteering

since 1997	group leader at the Red Cross Youth in Halle (JRK)
2000	child care at the Expo 2000 Hannover, Red Cross Youth Germany
since 2003	commissary for nature conservation for the German Red Cross Water Rescue Organisation (Wasserwacht) in Saxony-Anhalt
since 2006	assistance in the German Red Cross Youth working group „ contest"

Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit mit dem Titel " Does local adaptation facilitate the success of plant invasions? A case study on *Buddleja davidii*" bisher weder der Mathematisch-Naturwissenschaftlich-Technischen Fakultät der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt wurde.

Ferner erkläre ich, dass ich die vorliegende Arbeit selbständig und ohne fremde Hilfe verfasst sowie keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen wurden als solche von mir kenntlich gemacht.

Ich erkläre weiterhin, dass ich mich bisher noch nie um einen Doktorgrad beworben habe.

Halle, 10. Juni 2008