Resistance of Plant Communities to Invasive Species - Disentangling Invasiveness from Invasibility

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La mer est un espace de rigueur et de liberté
Victor Hugo

La recherche aussi ...

Summary

Invasive species are nowadays considered as one of the most important threat to biodiversity. By displacing native species, modifying ecosystem functioning and causing substantial losses to agricultural production, they represent a menace to natural and managed ecosystems.

Although ecology of invasions has become an important research topic since the last decades, the mechanisms that determine why a given species may invade a given ecosystem and why some biomes are less resistant to invasion are still not clarified. Ecology of invasions is divided into 2 main topics: invasiveness and invasibility. While invasiveness refers to species ability to invade a community, invasibility focuses on the resistance of a community to invasion. Invasiveness may be the result of ecological processes, such as release from biotic constraints or human alteration of the environment (disturbance, stress…) or the consequence of evolutionary processes, such as hybridization or polyploidization that may increase genetic variation and therefore, enhance niche breadth. Invasibility has been said to be influenced by disturbance and biotic factors such as community diversity, dominant species identity, biotic interactions or community compositional stability. The invasion success is the consequence of the interaction between species invasiveness and community invasibility. Most studies in ecological invasions have focused on either invasiveness or invasibility, but hardly both together. By working at the same time and in the same conditions with native and introduced genotypes and by comparing their ecological performances, this thesis aims at a better understanding of both invasiveness and invasibility mechanisms.

Two worldwide invasive species, *Centaurea maculosa* and *Senecio inaequidens*, were used in several experiments (pot, microcosm, field) to disentangle the importance of invasiveness and community invasibility in their invasion success. Both species encountered polyploidisation in their native range, leading to the presence of diploid and tetraploid populations, whereas only tetraploid populations have been found in the introduced range. Using native diploid, native tetraploid and introduced tetraploid genotypes of the two model-species, allows assessing the effects of genetic variation (diploid vs. tetraploid genotypes) and environmental variation (genotype from native vs. introduced range) on species phenotypic traits variations and consequently on fitness variation and invasiveness. In the community context, studying response of different genotypes to experimental factors and community change gives information on the interaction between invasiveness and invasibility.

Plants were grown in pot, in field or in artificially built communities where (i) the management treatment, (ii) the community diversity and (iii) the spatial organisation of resident species were manipulated. In addition, (iv) community species composition, (v) community competitive ability and (vi) compositional and functional stability of the community were monitored. According to the experiments, data were gathered on survival, morphological traits (vegetative height, lateral spread, shoot and root biomass), leaf traits (specific leaf area, leaf dry matter content) and reproductive traits (probability of flowering, capitulum production) of the genotypes (native diploid, native tetraploid and introduced tetraploid) of both model species. Through the use of statistical models and multivariate approaches, the effects of management and biotic factors on survival, growth and reproduction of native and introduced genotypes of these two worldwide invasive species were assessed.

Invasion strategy of the two model species was investigated through a growth experiment in optimal conditions in a pot experiment. High investment in seed production could explain invasive success of *S. inaequidens* through high propagule pressure, whereas *C. maculosa's* strategy seemed to be oriented towards interactions with belowground communities, as shown by the shift in rhizosphere bacterial communities between genotypes. For both species, polyploidisation in the native range could be linked to a specialisation towards higher competitive ability, which could have allowed the first step of invasion. Introduction in the new range could be related to a loss of specialisation through selection of traits allowing coping with various or changing environments, improving successful spread.

Survival of both model species was highly affected by community spatial pattern, management and neighbouring competition. Responses of growth and reproductive output of native and introduced genotypes to management, community spatial pattern and community diversity were species-specific. Growth and reproductive output of both genotypes of *S. inaequidens* were affected by experimental factors whereas introduced genotypes of *C. maculosa* were less affected than native ones. Comparison of response of native and introduced genotypes to experimental factors allowed defining two strategies of invasion based on phenotypic plasticity. *Centaurea maculosa* was able to maintain fitness in all kinds of environments, either favourable or stressful (“Jack-of-all-trades” invader). *Senecio inaequidens* was able to deal with all kinds of environments and was also able to increase its fitness in favourable conditions (“Jack-and-Master” invader).

The combination of the effects of management, community spatial pattern and community diversity on species genotypes allowed defining, for both invasive species, two invasion phases which were impacted by different factors. The introduction phase corresponds to the survival of seedlings and their ability to deal with neighbouring competition. If seedlings manage to survive despite neighbouring competition, they grow and reproduced in order to spread, which corresponds to the establishment phase. In terms of management perspectives, regular mowing or use of highly covering species could limit invasive success of *S. inaequidens* and *C. maculosa* respectively.

The synthesis of all the experiments conducted in this thesis with *C. maculosa* and *S. inaequidens* highlights (1) the importance of polyploidisation in the invasion process as well as (2) the species-specific invasion strategies and consequently (3) the species-specific response of invasive species to abiotic and biotic factors. It also emphasizes on (4) the temporal evolution of the interaction between invasiveness and invasibility since the community factors that affected invasive species fitness changed according to the invasion stage (introduction vs. establishment phase) of the invader. The provided insights into the importance of the interaction between species invasiveness and community invasibility will contribute to improve knowledge in ecology of invasions, in addition to supply some clues for management efficiency.

Keywords: *Centaurea maculosa*, community diversity, community dynamics, competitive ability, invasibility, invasiveness, invasive plants, Jack-of-all-Trades, Jack-and-Master, phenotypic plasticity, plant-soil interactions, polyploidisation, *Senecio inaequidens*

Résumé

Les plantes invasives sont aujourd'hui considérées comme une des menaces les plus importantes pour la biodiversité. En éliminant des espèces natives, en modifiant les processus écosystémiques et en causant des dommages importants à la production agricole, elles représentent un danger pour les écosystèmes agricoles et naturels.

Bien que l'écologie des invasions soit devenue un axe majeur de recherche ces dernières décennies, les mécanismes permettant de comprendre pourquoi une espèce envahit un écosystème et pourquoi certains milieux sont plus résistants que d'autres ne sont toujours pas élucidés. L'écologie des invasions est actuellement divisée en deux domaines: l'étude du potentiel invasif de l'espèce et celle de la résistance de la communauté végétale, *i.e.* l'invasibilité. Le potentiel invasif est la conséquence i) de processus environnementaux, tels que la levée de contraintes biotiques ou l'altération anthropique de l'environnement (perturbation, stress…) et ii) de processus évolutifs, tels que l'hybridation ou la polyploïdisation, qui tendent à augmenter la variation génétique de l'espèce et donc à élargir la niche potentielle de la plante invasive. L'invasibilité peut résulter de perturbations ou de facteurs biotiques tels que la diversité végétale, les caractéristiques des espèces dominantes, les interactions biotiques ou encore la stabilité de la communauté en terme de composition spécifique ou fonctionnelle. La plupart des études porte sur l'un ou l'autre de ces deux domaines, bien que le succès d'une invasion biologique soit reconnu comme étant le résultat de l'interaction entre le potentiel invasif de l'espèce et l'invasibilité. Cette thèse a pour but de mieux comprendre les mécanismes de l'invasion en abordant conjointement l'étude du potentiel invasif de l'espèce et celle de l'invasibilité. Elle s'appuie sur plusieurs approches expérimentales (pot, microcosme ou plein champ) et l'analyse des performances écologiques des génotypes natifs et invasifs d'espèces modèles *Centaurea maculosa* et *Senecio inaequidens* dans différentes conditions environnementales

Suite à une évolution génétique (polyploïdisation), *C. maculosa* et *S. inaequidens*, présentent des individus diploïdes et tétraploïdes dans leur aire d'origine, tandis que seuls des individus tétraploïdes colonisent l'aire d'introduction. Travailler avec des individus génétiquement et d'origine géographique différents permet d'étudier les conséquences de la variation génétique et des changements environnementaux sur les traits phénotypiques et donc sur les performances écologiques à l'origine du potentiel invasif. Au sein de la communauté végétale, l'étude de la réponse des différents génotypes aux facteurs expérimentaux ou à l'évolution des caractéristiques de la communauté végétale permet d'aborder l'interaction entre le potentiel invasif de l'espèce et l'invasibilité de la communauté.

Les génotypes natifs (diploïdes et tétraploïdes) et invasifs (tétraploïdes) ont été transplantés dans des communautés artificielles (microcosmes) ou naturelle (plein champ) soumises à différentes conditions expérimentales : (i) type de gestion (fauche *vs.* pâturage), (ii) diversité de la communauté et (iii) organisation spatiale des espèces résidentes. Nous avons également suivi (iv) la composition floristique, (v) le potentiel compétiteur de la communauté végétale et (vi) la stabilité de la communauté végétale en terme de composition spécifique et fonctionnelle. La survie, la croissance et le potentiel reproducteur des plantes invasives ont été mesurés via les traits morphologiques (hauteur végétative, étalement latéral de la canopée, biomasses racinaire et aérienne), foliaires (surface spécifique foliaire, contenu des feuilles en matière sèche), ou reproducteurs (taux de floraison, nombre de capitules produits). L'utilisation de modèles statistiques et d'approches multivariées a permis d'estimer les effets des facteurs expérimentaux sur la survie, la croissance et la reproduction des différents génotypes des deux espèces invasives.

La stratégie invasive des deux espèces a été étudiée lors d'une expérience de croissance en conditions optimales, en pot. L'invasion de *S. inaequidens* s'expliquerait par un investissement important des ressources dans la production de capitules conduisant à une forte pression de propagules sur le milieu environnant. La stratégie de *C. maculosa* serait liée aux interactions avec les micro-organismes du sol, comme le suggère l'évolution de la communauté bactérienne en fonction du génotype. Pour les deux espèces, la polyploïdisation dans l'aire native serait associée à une spécialisation vers un meilleur potentiel compétiteur favorisant la première étape de l'invasion. Ensuite, la sélection de traits plus généraux contribuerait à l'adaptation de l'espèce à des environnements variés et changeants, étape nécessaire à l'expansion dans l'aire d'introduction.

La survie des plantules des deux espèces est relativement faible dans une communauté fauchée, à faible diversité spécifique ou avec un assemblage aléatoire des espèces résidentes. En termes de croissance et de reproduction, les réponses des génotypes natifs et invasifs aux différents facteurs varient selon l'espèce modèle considérée. Alors que le type de gestion, la diversité spécifique et les patrons d'agrégation spatiaux de la communauté influencent la croissance et la reproduction des génotypes natifs et invasifs de *S. inaequidens*, les génotypes invasifs de *C. maculosa* sont moins affectés que les génotypes natifs. Deux stratégies d'invasion basées sur la plasticité phénotypique des espèces ont été proposées. Les génotypes invasifs de *C. maculosa* ont une forte capacité à maintenir leur performance écologique dans les milieux défavorables (« Jack-of-all-Trades invader ») alors que les génotypes invasifs de *S. inaequidens* présentent une aptitude à maintenir de bonnes performances dans des milieux défavorables, associée à une capacité à améliorer leurs performances dans un milieu favorable (« Jack-and-Master invader »).

L'intégration des effets des différents facteurs expérimentaux sur les génotypes natifs et invasifs des deux espèces modèles permet de définir deux phases d'invasion, influencées par différents facteurs. La phase d'introduction, *i.e.* l'arrivée des individus dans un nouveau milieu, dépend de leur capacité de survie et leur aptitude à gérer les interactions compétitives avec leurs voisins. Si les individus survivent à cette première sélection, leur croissance et leur reproduction conduisent à terme, à former une population auto-suffisante. Cette deuxième phase correspond à la phase d'établissement. En terme de gestion, une fauche régulière ou le semis d'espèces à forte couverture végétale pourrait limiter le succès invasif de *S. inaequidens* et *C. maculosa* respectivement.

La synthèse des expériences conduites sur les deux espèces modèle *C. maculosa* et *S. inaequidens* permet de mettre en valeur (1) l'importance de la polyploïdisation dans le processus d'invasion ainsi que (2) la spécificité de la stratégie d'invasion à l'échelle de l'espèce et, par conséquent, (3) la spécificité des réponses des espèces aux facteurs biotiques et abiotiques. Elle met également l'accent sur (4) l'évolution de l'interaction entre le potentiel invasif de l'espèce et la résistance de la communauté végétale puisque les facteurs influençant les performances de l'espèce invasive changent au cours du processus d'invasion (phase d'introduction *vs.* phase d'établissement). Cette nouvelle approche basée sur l'interaction entre le potentiel invasif de l'espèce et l'invasibilité renforce les connaissances relatives aux invasions biologiques, et contribue au développement d'une gestion efficace.

Mots clés : *Centaurea maculosa*, diversité spécifique, dynamique temporelle, interactions biotiques, interactions plantes-sol, Jack-of-all-Trades, Jack-and-Master, plantes invasives, plasticité phénotypique, potentiel compétitif, potentiel invasif, polyploidisation, résistance de la communauté, *Senecio inaequidens*

Institutional context

This PhD was integrated in the larger project “Evolution and spread of potentially invasive species”, funded by the Swiss National Centre of Competence in Research (SNF-NCCR) “Plant Survival” of the University of Neuchâtel (Switzerland). The research group was originally composed by people from the universities of Lausanne, Fribourg and EPFL as well as of the WSL Research Institute of Birmensdorf. To improve understanding of the invasion process, the four teams worked on a common model species, *Centaurea maculosa,* to find the ecological and evolutionary factors that explain the success of invasive plants.

The four research groups addressed the question from various angles: historical evolution of its geographical distribution, genetic comparisons between introduced and native populations, mechanisms that allow the expansion of the weed (for example, a favourable landscape structure), modelling of potential spread in favourable ecological niches, and factors that allow certain natural environments to resist invasion. The approach intended to be multi-disciplinary and included collection of samples in the field in the native and the introduced ranges, physiological, ecological and genetic analyses, experimental approach as well as statistical modelling integrating the various observations.

The team based at the University of Lausanne (Antoine Guisan and Olivier Brönnimann) worked on niche modelling. Their aim was to test niche differentiation between ranges and ploidy levels. At the University of Fribourg, Heinz Müller-Schärer and his team (Gillianne Bowman, Thomas Steinger and Urs Treier) explored the inter-relationship between the life-cycle habit, ploidy level and breeding system, and their associations with the invasion success. At the WSL Research Institute of Birmensdorf, Helene Wagner and Jacqueline Bolli focused on dispersal modelling of invasive plants. They left the group 2 years after the beginning of the project, H. Wagner having been appointed as a professor in Toronto. In 2007, Markus Fischer and his team from the University of Bern (Melanie Glättli and Mark van Kleunen) joined the project to experimentally assess the determinants of invasiveness by using pairs of invasive and non-invasive naturalized plant species in their native range. Lastly, at EPFL Lausanne, Alexandre Buttler and his team (Aurélie Thébault and François Gillet) focused on the factors involved in community resistance.

In the context of technology transfer, I also wanted to work on a species considered as invasive in Switzerland. I decided to carry out all the experiments with *Senecio inaequidens,* a species registered on the Black List of invasive plants in Switzerland (Swiss Commission for Wild Plant Conservation CPS/SKEW). Using two worldwide invasive species also allows discussing on generalization of processes involved in community invasibility.

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Chapter 1

General Introduction

1.1. Biological invasions: definitions and concepts

Exotic plant invasions represent a threat to natural and managed ecosystems (Prieur-Richard and Lavorel 2000). They are considered as one of the most important hazard to biodiversity (Clavero and Garcia-Berthou 2005; Rejmanek 1996). Major impacts are displacement of native species (Charles and Dukes 2007; Levine *et al*. 2003; Reinhart and Callaway 2006; Walker and Vitousek 1991), modification of ecosystem functioning or community dynamics (Charles and Dukes 2007; D'Antonio and Vitousek 1992; Dukes and Mooney 2004; Mack and D'Antonio 1998; Vitousek *et al*. 1997), as well as substantial losses to plant and animal agricultural production (Pimentel *et al.* 2000). Understanding invasion processes and mechanisms is therefore of major concern.

Definition of invasion has been and is still a big issue. Indeed, the term of "invasive species" is often used in different ways, despite some attempts to unify terminology (Alpert *et al.* 2000; Colautti and MacIsaac 2004; Richardson *et al.* 2000). Most definitions integrate the two concepts of movement into a new place and negative effects in this new place. Thus an invasive species is usually defined as a species that both spreads in space (outside its natural biogeographical range) and has negative impacts on species already established in the area that it enters (Alpert *et al.* 2000). This definition will be the one used in this thesis.

Invasion ecology is organised around three main topics: invasiveness, invasibility and impacts (Alpert *et al.* 2000). While invasiveness refers to the intrinsic properties of a species that make it able to invade a community, invasibility focuses on the properties of a community that make it able to resist (or not) to invasion. A community is said invasible when an introduced species is able to establish and persist or expand (Burke and Grime 1996). The ecology of invasions has became an important subject of research over the nineties (Kolar and Lodge 2001; Lodge 1993; Rejmanek 1996; Richardson and Pysek 2006), however the underlying mechanisms of why a given species may invade a given ecosystem and why some biomes are less resistant to invasion, although thoroughly studied, are still heavily debated. My work aims at understanding invasion success by studying simultaneously traits responsible for invasiveness as well as factors affecting community invasibility.

Invasion is a multistage process that comprises four phases: initial dispersal, introduction, establishment and spread (Kolar and Lodge 2001; Williamson 1996). The initial dispersal is the phase during which an organism moves from its native habitat to a new one, outside its natural biogeographical range. The distance between native and new habitat can be important, even intercontinental. Once arrived in a new habitat, the new organism has to established itself, *i.e.* be self-sustaining within this new habitat. Lastly, the exotic species can spread within the community and become dominant and spread to nearby habitats. Each of these stages is considered as a major filter for the species, causing high mortality (Williamson 1996). Nevertheless, even if most invasions fail (Williamson 1996), exotic species that do manage to invade a new community are incredibly successful (Figure 1).

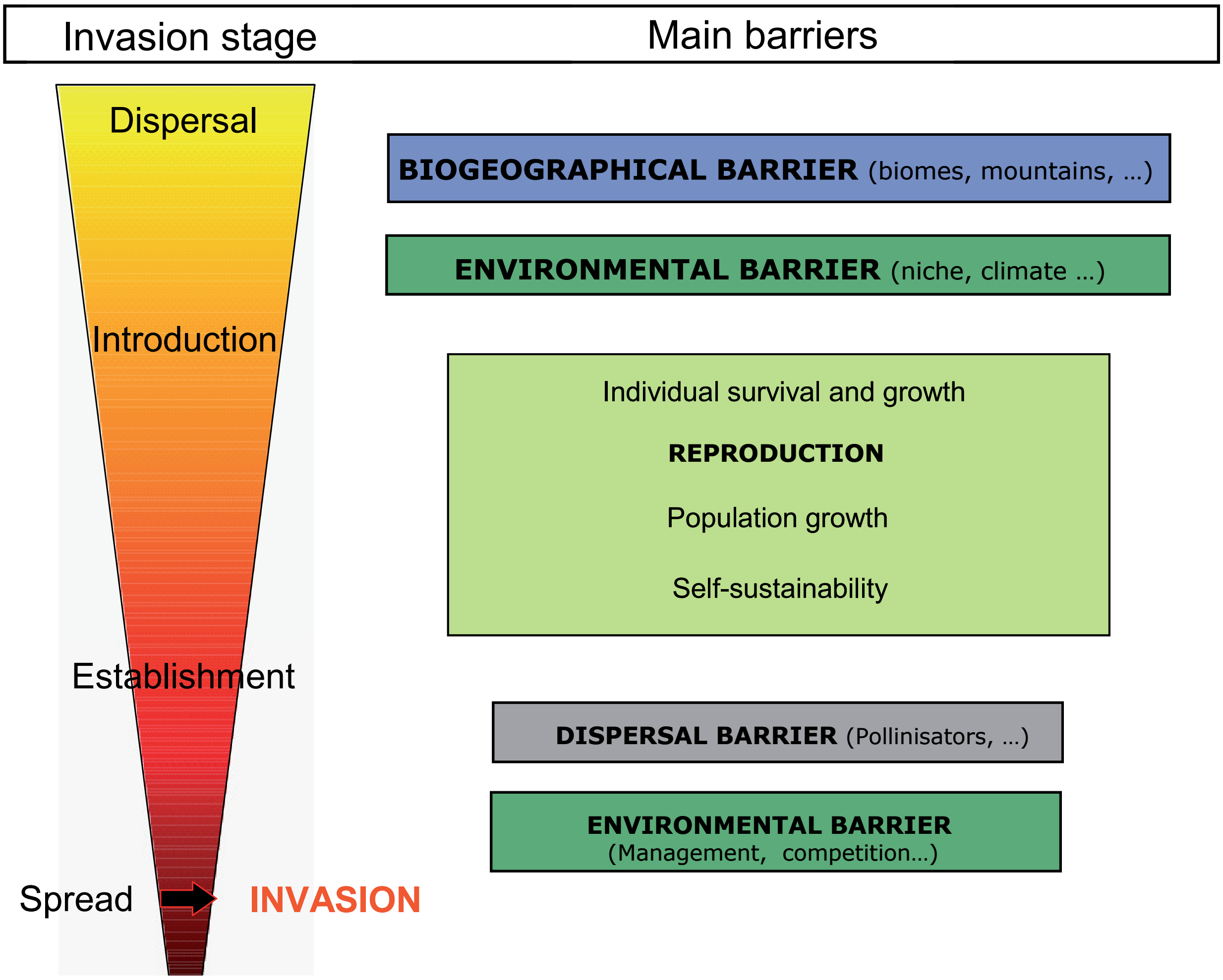

Figure 1: Description of invasion stages related to main barriers and alien species status. (*adapted from* Richardson *et al.* 2000 and Sol 2007).

Establishment success can be related to propagule pressure, which is a measure of the number of individual released in a region to which they are not native (Lockwood *et al.* 2005). It combines estimates of the absolute number of individuals involved in any one release event (propagule size) and the number of discrete release events (propagule number). Therefore, whenever propagule size or propagule number increases, propagule pressure also increases. Propagule pressure is considered as an event-level characteristic and can differ for each introduced population in a given community, or between different communities regarding one particular exotic species. Thus, the relation between propagule pressure and establishment success can be influenced both by species traits and the recipient environment characteristics.

1.2. Invasiveness of exotic species: a review of theories

During the last decades, few theories based on ecological or evolutionary processes have been suggested to explain invasive success of exotic species in new environments (Figure 2). In accordance with ecological processes, invasion success is the consequence of extrinsic changes in the new environment that favour the invading species, such as disturbance or creation of empty niches (Elton 1958; Hierro *et al*. 2005; Mack *et al*. 2000), release from competitively superior neighbours (Bossdorf *et al.* 2004) or natural enemies (Keane and Crawley 2002; Maron and Vila 2001), inhibition of resident species through the release of chemical compounds into the soil (Callaway and Aschehoug 2000) or alteration of the soil community (Eppinga *et al.* 2006). Evolutionary processes such as hybridization (Ellstrand and Schierenbeck 2000; Suarez and Tsutsui 2007), polyploidization (Pandit *et al.* 2006; Soltis and Soltis 2000; Verlaque *et al.* 2002) or trait selection have also been mentioned for explaining invader success. Indeed, they lead to intrinsic changes of the invasive species such as adaptive evolution of traits (Blossey and Notzold 1995; Bossdorf *et al*. 2004), which can lead to higher fitness (Hufbauer and Torchin 2007) or change in ecological breadth from the native range to the introduced one (Petit and Thompson 1999; Soltis and Soltis 2000; Weber and Schmid 1998). However, until recently, evolutionary processes have been hardly discussed in the framework of invasion ecology despite the fact that their study could give new insights into mechanisms of invasion (Blair and Wolfe 2004; Blumenthal and Hufbauer 2007; Dietz and Edwards 2006; Facon *et al.* 2006; Lee 2002; Maron *et al.* 2004; Müller-Schärer *et al*. 2004; Prentis *et al.* 2008; Suarez and Tsutsui 2007).

1.2.1. Ecological processes

Few theories suggest that invaders are simply inherently superior to native species in the community they invade. In this perspective, the ***empty niche hypothesis*** argues that exotic species are able either to use resources of the community not used by native ones or to use them more efficiently (Elton 1958; Hierro *et al*. 2005; Mack *et al*. 2000). From this point of view, invaders might be able to take advantage more easily of a ***stochastic event*** which changes the resources used by the community such as disturbance for example (Davis *et al.* 2000).

The ***enemy release hypothesis*** (Keane and Crawley 2002) is based on the assumption that when introduced in a new area, an exotic species is often released from its natural specialist enemies. According to this hypothesis, (i) specialist enemies of the new habitat hardly abandon their usual preys to shift to new ones and (ii) generalists often have greater impact on endemic species than exotic ones. Therefore, losses encountered by exotic species in the new environment are less damaging than in their native one, leading to faster spread.

Since few years, belowground processes have been integrated in ecology of invasions. So far, two theories involving soil biota have been suggested to explain invasion success. The ***novel weapon hypothesis*** (Callaway and Aschehoug 2000) argues that exotic species are able to invade plant communities through alteration of plant-soil interactions *via* release of inhibitory chemicals. These weapons act as powerful allelopathic agents or as mediator of new plant-soil microbial interactions. They may be highly inhibitory to newly encountered plants in recipient community which have not coevolved with these substances, by contrast to local neighbours in the native range. Exotic species can also develop species-specific soil communities by amplifying a particular subset of the soil community leading to ***accumulation of local pathogens*** (Eppinga *et al.* 2006). Indeed, even if the accumulation of local pathogens may limit the abundance of the exotic species, the inhibitory effect should even be higher on native plants, leading to a competitive advantage of exotic species.

1.2.2. Evolutionary processes

Beyond these ecological factors, evolutionary factors may be essential to explain invasive success (Lee 2002; Prentis *et al*. 2008). Indeed, ***hybridization***, either with another introduced population or with native populations could lead to increase of invasiveness through rapid evolution of genomes (Ellstrand and Schierenbeck 2000). ***Polyploidisation*** is known to play an important role in plant evolution since it can have profound consequences for gene expression (Osborn *et al.* 2003), and offer the potential for novel physiological (Soltis *et al*. 2004; Soltis and Soltis 2000) and ecological (Lumaret *et al.* 1997) behaviours in plant. Moreover, since introduced species face a strong selection pressure, they may evolve quickly in the introduced range through ***rapid evolution of traits***. As an example, once released from its natural enemies, an exotic plant may allocate less resources to herbivore defence and thus more resources to *increase competitive ability* (Blossey and Notzold 1995). However, since introduction in a new area may also release exotics from their competitive neighbours, there might be a selection of traits leading to *reduced competitive ability*, especially if competition involves traits that have fitness cost (Bossdorf *et al.* 2004).

So far, a lot of studies have focused on the characteristics of exotic species that allow them to invade an environment. A large set of biological attributes has been found to explain invasiveness (Kolar and Lodge 2001; Rejmanek 2000; Rejmanek and Richardson 1996) but unfortunately it is difficult to generalize and make predictions (Kuster *et al.* 2008). To be a successful invader, an individual must have a higher fitness (survival, growth and reproduction) than competitors present in the new area (Colautti *et al*. 2006; Daehler 2003; Pyšek and Richardson 2007) and/or a high competitive ability allowing them to better capture resources (Daehler 2003). Phenotypic plasticity, the property of a genotype to express different phenotypes in different environments (Bradshaw 1965), has recently been suggested to explain invasiveness (Richards *et al.* 2006), as a consequence of ecological or evolutionary processes (Figure 2).

Phenotypic plasticity could enhance niche breadth as plastic response may allow organisms to manage in a broader range of environments (Bradshaw 1965; Richards *et al*. 2008). In the case of invasive species, this could explain why species arriving in a new area are able to adapt very quickly and establish successfully.

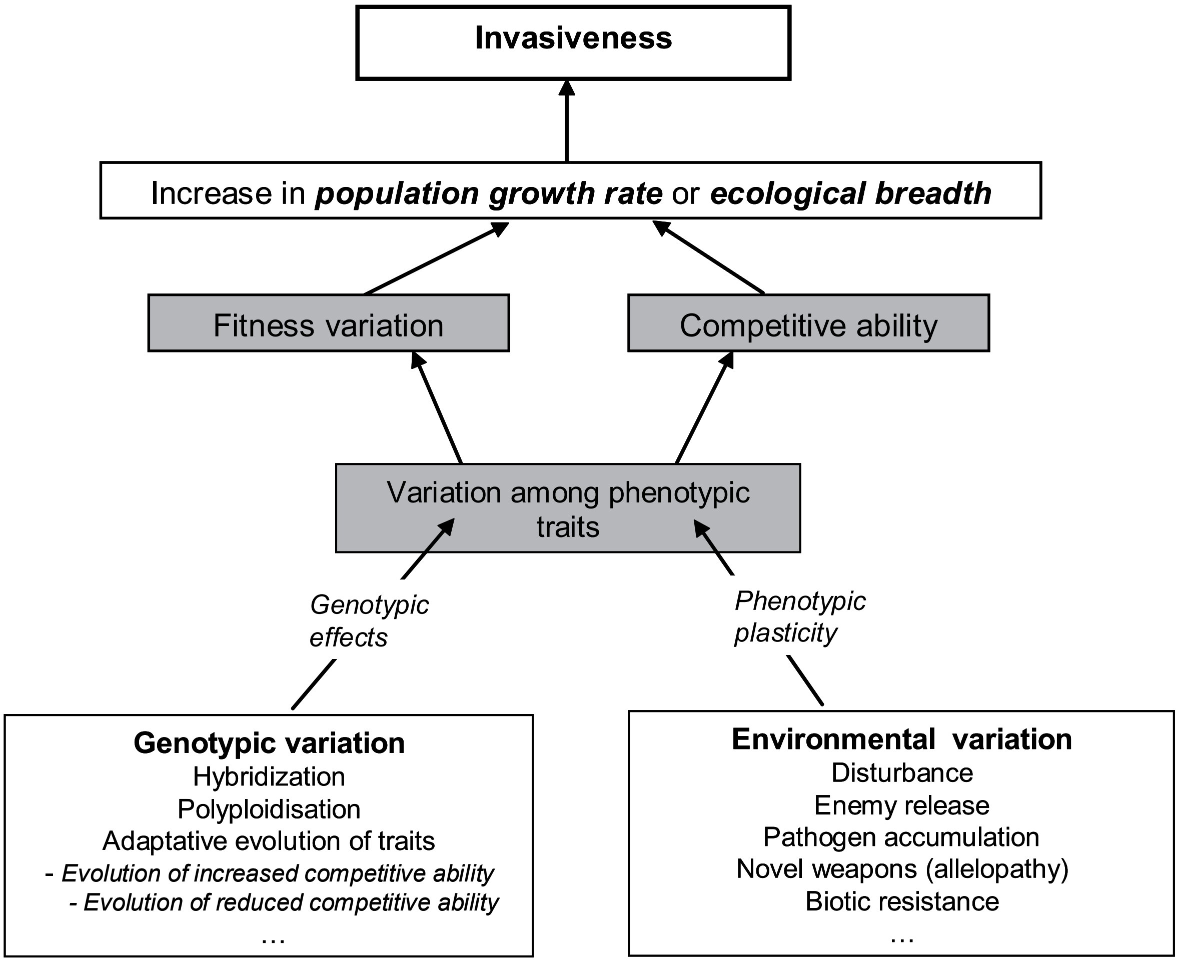

Figure 2: Ecological and evolutionary mechanisms involved in invasion success: overview of the relationships between environmental, genetic, phenotypic and fitness variation (*adapted from* Richards *et al*. 2006)

1.3. Invasibility of natural communities

Until the renewed debate on the relationship between community diversity and stability (Frank and McNaughton 1991; Tilman 1996; Tilman and Downing 1994), very few studies had addressed the question of invasion from the community viewpoint (Prieur-Richard and Lavorel 2000).

Community historical context strongly influences invasibility (Prieur-Richard and Lavorel 2000). Both ***history of disturbance*** and ***history of previous*** invasions need to be taken into account when studying plant community characteristics that might favour invasions (Alpert *et al*. 2000; Diaz *et al*. 1994). Habitats in which competition has been intense for a long time will contain species with a high competitive ability. Consequently, invasion by a new species will be more difficult since established species will be able to outcompete it (Alpert *et al*. 2000; Prieur-Richard and Lavorel 2000). European grasslands which present a long history of disturbance and colonization should be quite safe regarding to invasion risk. The recent establishment and spread of invasive species in European natural areas emphasizes that beyond the evolutionary history of communities, natural meadows have properties that allow exotic species to establish, spread and dominate the community. Therefore, ***competition*** with the resident vegetation, even if very important, is likely not the principal factor influencing invasibility (Kulmatiski *et al.* 2006).

Invasibility might not be an intrinsic, static, component of community (Davis *et al.* 2000). The ***fluctuating resource availability theory*** states that every community is susceptible to invasion whenever a pulse of resource supply coincides with the presence of invading propagules (Davis *et al.* 2000). The increase in resource supply can be due to a decline in its use by the established vegetation (*e.g.* a disturbance that damages or destroys resident vegetation such as herbivory or a widespread disease) or to an increase in resource availability (*e.g.* fertilisation). However, the effect of ***disturbance*** on invasion success is still debated. While some authors argue that disturbance promotes biological invasions (Burke and Grime 1996; Chabrerie *et al*. 2008; Frenot *et al*. 2001; Leishman and Thomson 2005), others claim that invasion can take place with a little or even without disturbance (Buckland *et al*. 2001; Diemer and Schmid 2001; Wiser et al. 1998).

Invasibility is an essential component of ecosystem stability which can be affected by ***species diversity*** (Chapin *et al*. 1997; Levine and D'Antonio 1999). A wide set of studies has focused on the relationship between invasibility and diversity since Elton (1958). Elton's hypothesis is based on the theory of competitive exclusion and niche displacement. It predicts that the most diverse communities are the least invaded, as empty niches are scarce and resources are more completely used. Controversial results highlight the fact that this relationship is not straightforward (Cleland *et al*. 2004; Emery and Gross 2005; Gilbert and Lechowicz 2005; Hector *et al.* 2001; Kennedy *et al.* 2002; Lanta and Leps 2008; Levine 2000; Levine and

D'Antonio 1999; Lonsdale 1999; Maron and Marler 2007; Naeem *et al.* 2000; Palmer and Maurer 1997; Planty-Tabacchi *et al*. 1996; Robinson *et al.* 1995; Stohlgren *et al*. 1999; Tilman 1997a). This relationship could be only a matter of scale (Byers and Noonburg 2003; Wardle et al. 2008), or due to a "sampling effect" since the more diverse a community is, the more probability it has to contain a dominant species with extreme traits such as invasion resistance (Levine and D'Antonio 1999; Palmer and Maurer 1997; Tilman 1997b). Therefore, ***species identity*** might be more important than species richness in resistance to invasion (Crawley *et al.* 1999; Emery 2007; Emery and Gross 2007). The removal of rare or subordinate species can surprisingly highly decrease invasion resistance considering their low abundance (Lyons and Schwartz 2001; Meiners *et al*. 2004; Smith *et al*. 2004). This could be explained by the fact that those rare native species exploited the same niche as the invader (Hector *et al*. 2001; Lyons and Schwartz 2001). Thus, ***functional diversity*** could explain invasibility better than floristic diversity since a species might have more difficulties to invade a community containing species of the same guild (Fargione *et al*. 2003; Xu *et al.* 2004). A possible explanation for such a result holds in the stochastic niche theory of Tilman (2004), based on resource partitioning. This theory predicts that once a species is established within a community, the probability that a similar species is successful to invade is low. Functional groups present in the community could also influence invasibility. Legumes for example, by fixing atmospheric dinitrogen, might be expected to increase levels of available resources over time and consequently facilitate invasion (Palmer and Maurer 1997).

The debate on diversity-invasibility relationship could profit from insights on ***temporal dynamics*** of the resident community, *i.e.* the local colonization and extinction of species over time (*turnover rate*). Bakker *et al.* (2003) found a positive relationship between species turnover rate and species richness in North American grasslands. Since species richness has often been thought to be linked to invasibility, species turnover rate might be involved in community resistance. To our knowledge, only few study tried to link species turnover rate at local scale with invasibility (Foster *et al.* 2002 Schoolmaster and Snyder 2007), although this component of community structure might affect invasibility, because of its stochasticity (Davis *et al.* 2000; Tilman 2004).

Lastly, ***spatial patterns*** of communities have important impacts on biotic interactions (Monzeglio and Stoll 2005; Murrell *et al*. 2001; Stoll and Prati 2001), and likely on resistance to invasion of natural communities. Experimental manipulations of plant aggregation in artificial communities showed that spatial distribution of plants deeply affects competitive interactions among species. Weaker competitors increase their fitness when surrounded by conspecifics whereas performance of stronger competitors decreases when grown in the neighbourhood of conspecifics (Stoll and Prati 2001). The decrease competitive ability of stronger invader might therefore decrease community resistance in aggregated communities.

1.4. Impacts of plant invasions

Despite the overwhelming evidence of the impacts of invasive species on ecosystem processes, studies are still needed to understand mechanisms leading to these impacts (Charles and Dukes 2007). Ecosystem services comprise all the benefits to human society by natural ecosystems. According to the Millenium Ecosystem Assessment (2005), ecosystem services can be separated into four main categories. *Provisioning services*, also named ecosystem goods, are products directly usable by humans (food, freshwater, fiber, fuel, genetic resources, biochemicals, pharmaceuticals, natural medicines and ornamental resources). *Regulating services* are those services that control ecosystem functioning (air quality regulation, climate regulation, water regulation and purification, disease regulation, natural pest control, pollination, erosion control, coastal protection). *Cultural services* are all non-material benefits such as aesthetic values, recreational and touristic values, spiritual, religious, education and scientific values. Lastly, *supporting services* are overarching and occur at larger scale to maintain other services. They include for example photosynthesis, primary production, nutrient and water cycling as well as soil formation, maintenance of fertility and atmospheric composition (Millennium Ecosystem Assessment 2005).

Production and maintenance of ecosystem goods and services is ensured by ecosystems' structure (composition and biological/physical organization) and functions (or processes). Invasive species may alter production, maintenance and quality of theses goods and services through diverse mechanisms. As pointed out by Charles and Dukes (2007), all the mechanisms are interrelated since they all affect some aspects of the ecosystems' structure and functions. However it is possible to group them into three main categories: mechanisms that mainly biotic factors, those that tend to disrupt natural cycles and mechanisms that alter other abiotic factors (Figure 3). Main mechanisms of impacts of invasive plant species on ecosystem structure and function and therefore, on ecosystem services are represented in Figure 3.

More studies are still needed to understand how invasive species alter ecosystems and how ecosystems resist to impacts of invasions (Charles and Dukes 2007; Dukes and Mooney 2004; Levine *et al*. 2003). Some studies have suggested that impacts of the invasion consequently favour spread of the invader (D'Antonio and Vitousek 1992). There is therefore an urgent need to test how invasion success is enhanced or retarded by the impact of the invader on the recipient community (Levine *et al.* 2003). Only a precise understanding of the links between invasive species, ecosystem structure and function, and provision of ecosystem goods and services will improve the ability to 1) recognize invasive species' impact on these goods and services and 2) better manage these impacts (Charles and Dukes 2007).

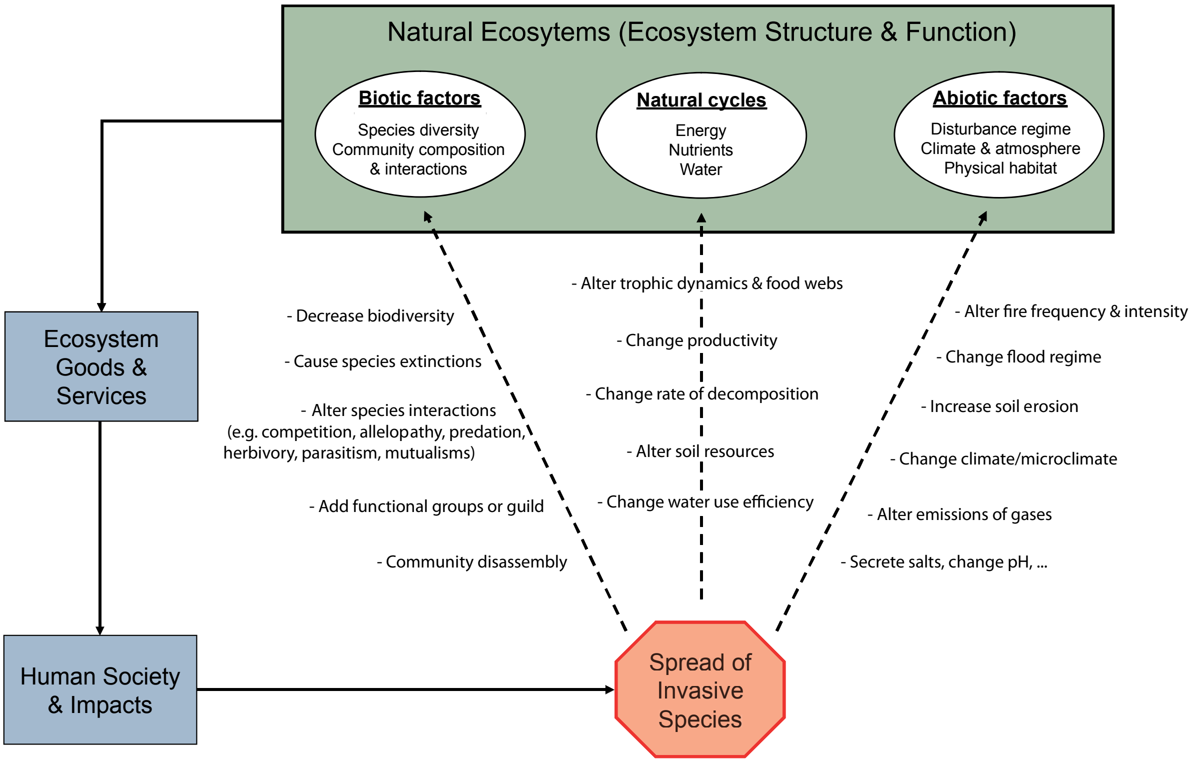

Figure 3: Mechanisms of ecosystem services alteration by invasive species (Charles and Dukes 2007)

1.5. Thesis objectives

This project aims at studying simultaneously invasiveness and invasibility aspects of plant biological invasions. Working with two invasive species, *Centaurea maculosa* and *Senecio inaequidens*, allows assessing species effect and emphasizing the importance of using more than one species before drawing conclusions about invasion success. Since these two species encountered polyploidisation in their native range, they are particularly good model species to assess effects of evolutionary *vs.* ecological processes. Indeed, using native diploid, native tetraploid and introduced tetraploid genotypes of these two mode species will allow to assess effects of genetic variation (diploid *vs.* tetraploid genotypes) and environmental variation (genotypes from native *vs.* introduced range) on species phenotypic traits variations (including plasticity) and consequently on fitness variation and invasiveness. In this manuscript, we define for convenience a geocytotype as a ploidy level in a given area (native or introduced range). Thus, both model species have three geocytotypes (native diploid, native tetraploid and introduced tetraploid).

By working at the same time and in the same conditions with native and introduced genotypes and by comparing their ecological performances, we will be able to assess effects of tested factors on invasiveness and thus the interaction between invasibility and invasiveness. Through experimental and observational studies in controlled and natural conditions, we will look at the effects of management, community small scale structure and community dynamics on invasive species establishment. Community structure will be studied through species diversity and composition in terms of functional traits, revealing competitive ability. Effects of small-scale temporal (resident species turnover) and spatial (resident species aggregation) patterns of aboveground vegetation and the consequences in terms of biotic interactions will also be assessed.

1.6. Chapters' description

The manuscript is divided into two main parts. While the first one deals with invasiveness aspect of biological invasions, the second part focuses on invasibility and the mechanisms involved in community resistance to invasive plant. Each of these two parts is further divided in different chapters addressing one specific question (Figure 4). Since the chapters correspond to scientific papers (submitted to or in preparation for peer-reviewed journals), we apologize for redundancies that could occur, especially in introductive or methodological parts. This manuscript ends with a synthesis of the results and some perspectives.

Part 1 deals with invasiveness aspects. In a first chapter (chapter 2), we present results of a nine month greenhouse experiment in which we grew individual plants in pots. We compare fitness and performance traits of geocytotypes of the two model species to assess whether intrinsic differences between geocytotypes could be responsible for invasion success. In a second experiment (Chapter 3), all the geocytotypes of the two species were grown during two years in a field experiment within a european plant community. By studying survival, growth and flowering of the individuals, we aim at assessing importance of competition and disturbance effects on geocytotypes establishment success. In the last chapter of this part (Chapter 4), we study belowground aspect of invasion success by comparing rhizosphere biota of the geocytotypes of both model-species.

Part 2 deals with invasibility aspects of invasion success. Through field and microcosms experiments, we aimed at assessing small scale effects of (i) community diversity, (ii) neighbouring competition and of (iii) temporal and (iv) spatial patterns of the recipient community. The first chapter of this part (Chapter 5) presents results of combined effects of spatial patterns of community assemblage and community diversity on native and introduced tetraploid genotypes' fitness. The second chapter (Chapter 6) focuses on effects of community diversity, competitive ability and dynamics on different geocytotypes' growth.

The last part of the manuscript (Chapter 7) gives a synthesis of all the results and places them in a larger context, highlighting the major issues and the new perspectives in ecology of invasions.

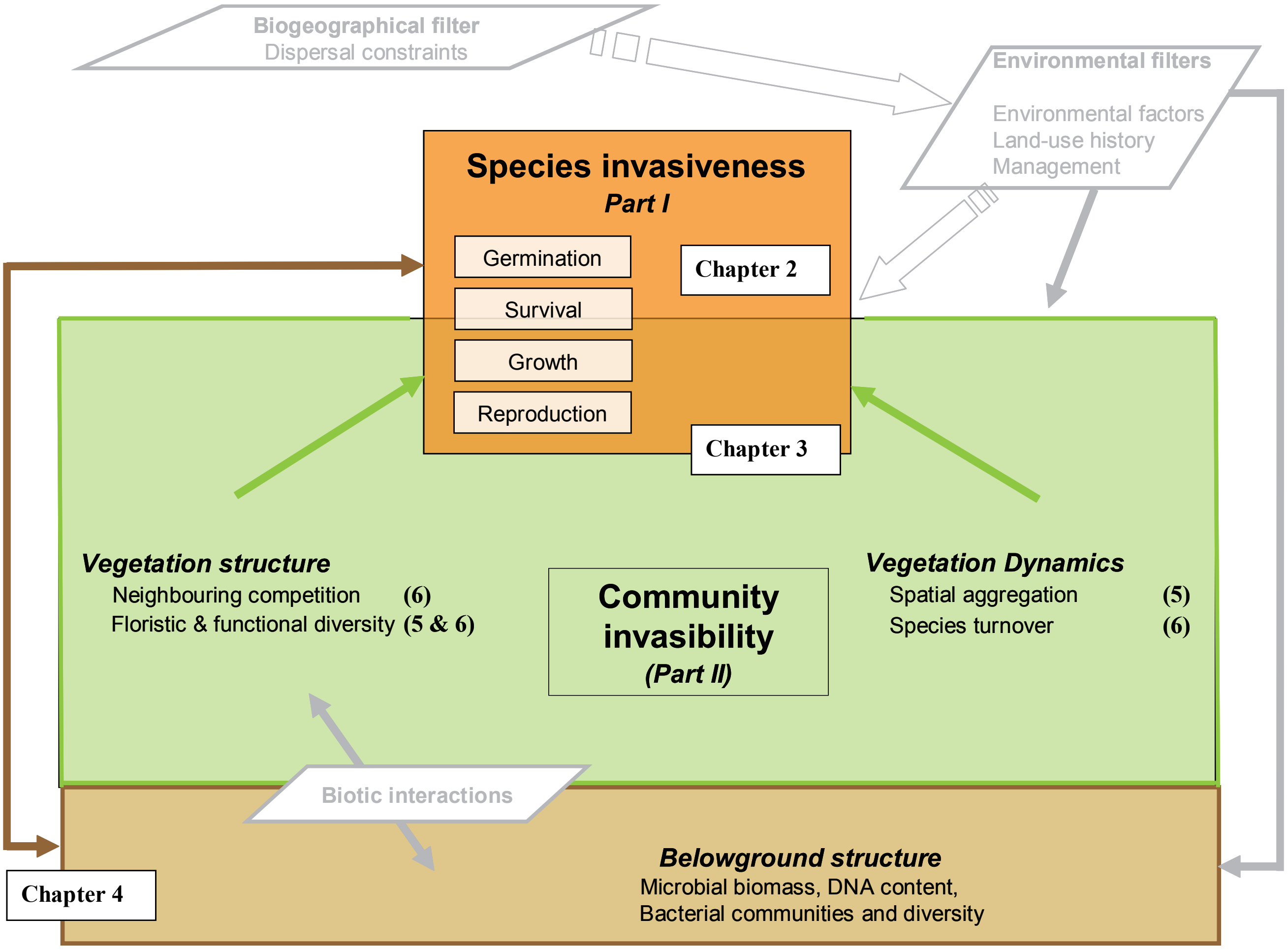

Figure 4: General scheme of the thesis structure and chapter objectives. Numbers in parentheses correspond to chapter numbers.

1.7. Model species

1.7.1. The spotted knapweed, Centaurea maculosa

Centaurea maculosa Lam. (1785) is a herbaceous biennial or short-lived perennial tap-rooted forb with a rosette of basal leaves and relatively thick flowering stems (Hook *et al.* 2004). It reproduces by seeds which are wind dispersed. Its native range spans from Western Asia to Western Europe. It is mainly found in continental xeric plant communities (Steinger and Müller Schärer 1992) but can also grow on sites ranging from semi-arid grasslands to open canopy forests, at low to mid elevation (Watson and Renney 1974), as well as on ruderal sites such as roadsides (Steinger and Müller Schärer 1992).

Centaurea maculosa was introduced in the Pacific Northwest of the United States in the late 1800s (Watson and Renney 1974) through Alfafa seeds imported from Asia Minor. It is also thought to have been introduced through ships' ballasts. Since its introduction, the species did not stop disseminating within North America and spread rapidly throughout north-western America and western Canada where it is now widespread in rangelands, pastures and on roadsides (Duncan 2001; Skinner *et al.* 2000). It causes substantial economic damage through its low nutritive value which reduces forage quality (Campobasso *et al.* 1994), and its invasion of agricultural land, displacing forage species (Watson and Renney 1974).

This species is known for its release of (±) catechine in the soil which may be allelopathic to other plants (Bais *et al*. 2002; Blair *et al*. 2006; Perry *et al*. 2005; Ridenour and Callaway 2001). Allelopathy is thought to be one of the main mechanisms involved in invasion success of *C. maculosa*. However, it has recently been argued that the release of catechine in the field was not important enough to be responsible for soil biota inhibition (Blair *et al.* 2006). Therefore, mechanisms of invasion of *C. maculosa* are still unclear and need further studies.

Centaurea maculosa belongs to the taxonomic complex named *C. stoebe* s.l. (*sensu lato*). Indeed, *C. maculosa* is very difficult, if not impossible, to differentiate from relatives such as *C. stoebe* or *C. bieberstinii* (Ochsmann 2001). Lastly, *C. maculosa* has encountered polyploidisation in its native range, resulting in presence of both diploid and tetraploid genotypes throughout Europe. Only tetraploid genotypes have been found in the introduced range, suggesting that either only tetraploid genotypes have been introduced, or that both genotypes have been introduced but only tetraploid ones managed to survive and spread.

1.7.2. The South-African ragwort, Senecio inaequidens

Senecio inaequidens DC. (1838) is an erect perennial dwarf shrub native from South Africa and Lesotho. Originally found in grasslands from 1400 m a.s.l. to 2800 m a.s.l., it is now widespread in South Africa. *Senecio inaequidens* reproduces mainly by wind dispersed seeds but vegetative reproduction may also occur.

It was accidentally introduced to Europe at the end of 1880s (Bornkamm 2002) where it is now widespread. Its presence was first recorded in Germany in 1889. It has then been found in Belgium in 1922, in Scotland 6 years later, in France and Italy respectively in 1935 and 1947. Although *S. inaequidens* only started to spread from these foci to the other European countries in the 1970's, it is considered as one of the most successful recent invading plant in Europe. It is also found nowadays in Australia and South America (see Ernst 1998 and Lafuma *et al.* 2003 for review). Due to its broad ecological range, it can be found in ruderal sites such as railroads and riverbeds (Garcia-Serrano 2004), as well as in disturbed pastures, wetlands and heathlands (Bornkamm 2002) and, more recently, also in undisturbed grassland ecosystems (Garcia-Serrano 2004). *Senecio inaequidens* contains pyrrolizidine alkaloids (Macel and Vrieling 2003) which are toxic to livestock (Noble *et al.* 1994). Therefore, when growing in pastures, *S. inaequidens* is avoided by cattle, which is expected to lead to reduction of forage quality and thus to cause economical losses.

S. inaequidens belongs to a taxonomical complex including *S. madagascariensis* and *S. harveianus* as the three species are impossible to differentiate morphologically (Lafuma *et al.* 2003). Lastly, *S. inaequidens* encountered polyploidisation in its native range, resulting in the presence of both diploid and tetraploid genotypes. However, contrary to *C. maculosa*, *S. inaequidens* presents a latitudinal differentiation of genotypes with the tetraploid ones being found at higher altitude than diploid ones (Lafuma *et al.* 2003). As for *C. maculosa*, only tetraploid genotypes have been found in the invasive range, arguing that either only tetraploids have been introduced into Europe, or that both genotypes have been introduced but only tetraploid ones managed to survive and disperse (Bossdorf *et al.* 2008).

The mechanisms leading to invasion of *Senecio inaequidens* are still unknown. Since the species is quite a recent invader, only few attempts have been undertaken to understand its invasion process.

To summarise, these two model species have been used in all experiments since they present ecological and genetic similarities (Table 1). Ecological similarities are (1) belonging to the same family (Asteraceae), (2) occupation of similar ecological niches in their native range, (3) tendency to invade similar habitats in their introduced range and (4) possibility of allelopathetical effects (Ahmed and Wardle 1994; Callaway and Ridenour 2004; Callaway and Vivanco 2007). Furthermore, they have both encountered polyploidisation in their native range leading to the presence of diploid and tetraploid genotypes. It seems that both genotypes have been introduced in the new range, but so far, only tetraploid genotypes have been found in their introduced range (Lafuma *et al.* 2003; Treier *et al.* in press). Due to this distribution pattern, *C. maculosa* and *S. inaequidens* serve as good models to study genetically and environmentally induced processes and their consequences on invasion success. Maps of sampled populations are presented in Appendix I.

Table 1: Principal characteristics of the two model species

	***Centaurea maculosa* Lam.**	***Senecio inaequidens* DC.**
Family	Asteraceae	Asteraceae
Taxonomical complex	*Centaurea stoebe* subsp. *stoebe* *Centaurea stoebe* subsp. *micranthos* *Centaurea stoebe* subsp. *serbica*	*Senecio madagascariensis* *Senecio harveianus* *Senecio inaequidens*
Native range	Europe	South Africa - Lesotho
Introduced range	North-America	Europe, Australia, South America
Geocytotypes	Native diploid (Nat 2n) Native tetraploid (Nat 4n) Introduced tetraploid (Int 4n)	Native diploid (Nat 2n) Native tetraploid (Nat 4n) Introduced tetraploid (Int 4n)
Reproduction	hermaphrodite	hermaphrodite
Inflorescences	capitula	capitula
Pollinisation mode	entomogamous, autogamous	entomogamous, autogamous
Dissémination mode	anemochore, epizoochore	anemochore, epizoochore
Fruits	achenes	achenes
Vegetal formations	hemicryptophytaie	hemicryptophytaie
Life Cycle	biennial or short-lived perennial	perennial
Seed production	Up to 40 000 seeds per plant	Up to 30 000 seeds per plant
Habitat (native range)	- well drained and disturbed soils - dry meadows, pasturelands, - dry and disturbed sites - debris, stony hills, upland rocky areas - open forest, pastures and rangelands - along roadsides - from 578 to 3,040 m	- well-drained and disturbed soils but can survive in most soils (even salty) - along roads and railways, river banks, wastelands - from coastal to mountain areas (up to 1900 m)
Habitat (introduced range)	- dry ruderal sites - dry sterile gravelly or sandy openings - roadsides, gravel pits, vacant lots - forest clearings - hayfields, pastures, old fields - well drained, deep soils - from sea level to 2000 m	- warm, dry ruderal sites - gravelly or sandy soils - railroad, railways, highways - logging areas, storm damages - forests - Near natural sites: rocky sites, coastal dunes, volcanic soils - from sea level to 600 m

Senecio inaequidens

Centaurea maculosa

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PART I

Comparison of native and invasive genotypes of invasive species

Chapter 2

Polyploidisation may determine invasion success through trade-offs among plant traits

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Submitted to Biological Invasions

Abstract

Many ecological and evolutionary hypotheses have been formulated to explain invasion success in new environments. Some of them are linked to an increase in fitness or competitive ability in the new range. Evolutionary changes in growth strategies including trade-offs among plant traits have received little attention so far in the context of biological invasions. We used two taxonomically related species, *Centaurea maculosa* Lam. and *Senecio inaequidens* DC., in a greenhouse experiment to test for changes in fitness, competitive ability and trade-offs among traits between native and introduced genotypes. For both species, diploid and tetraploid genotypes occur in the native range, whereas only tetraploids are present in the introduced range.

Our results show that despite the fact that the two model species are taxonomically related, they present two distinct growth strategies leading to distinct invasion strategies, which emphasizes the difficulty to find common traits responsible for invasiveness. For both species, polyploidisation in the native range seems to be linked to a specialisation towards higher competitive ability, which could allow the first step of invasion. Introduction in the new range seems to be related to a loss of specialisation through selection of traits allowing coping with different environments, which in turn can lead to successful spread.

Key-words: *Centaurea maculosa,* evolutionary processes, plant traits, *Senecio inaequidens*, trade-offs.

2.1. Introduction

Invasive species represent a threat to natural and managed ecosystems (Pimentel *et al.* 2000; Prieur-Richard *et al*. 2000). Understanding how they can invade ecosystems is a major challenge to ecologists (Richardson and Pysek 2006). So far, a large array of both ecological and evolutionary processes has been suggested to explain invasion success in new environments. In accordance with ecological processes, invasion success is the consequence of extrinsic changes in the new environment that favour the invading species, such as disturbance or creation of empty niches, release from competitively superior neighbours or natural enemies (Keane and Crawley 2002) or inhibition of resident species through the release of chemical compounds into the soil (Callaway *et al*. 2004). Evolutionary processes such as hybridization or polyploidisation (Amsellem *et al*. 2001; Pandit *et al.* 2006; Prentis *et al*. 2008; Soltis and Soltis 2000; Verlaque *et al*. 2002; Vila and D'Antonio 1998) have also been mentioned for explaining invader success. Indeed, they lead to intrinsic changes of the invasive species such as adaptive evolution of traits (Blossey and Notzold 1995; Bossdorf *et al.* 2004) which can lead to higher fitness (Hufbauer and Torchin 2007) or change in ecological breadth (Petit and Thompson 1999; Soltis and Soltis 2000; Weber and Schmid 1998), from the native range to the introduced one. However, until recently, evolutionary processes have received little attention in the framework of invasion ecology despite the fact that their study could give new insights into mechanisms of invasion (Bailey *et al.* 2007; Blair and Wolfe 2004; Blumenthal and Hufbauer 2007; Dietz and Edwards 2006; Facon *et al*. 2006; Keller and Taylor 2008; Lee 2002; Maron *et al*. 2004; Müller-Schärer *et al*. 2004; Prentis *et al*. 2008; Richards *et al*. 2008; Whitney and Gabler 2008; Williams *et al*. 2008).

Since invasive species are a minority among alien species (Williamson 1996), the process of invasion may be the result of a strong selection sequence leading to an assemblage of biological traits contributing to invasion success. The question of whether it is possible to determine a set of traits that predispose a species to be invasive remains controversial (Richardson and Pysek 2006) but also highly appealing since it would facilitate early diagnostic and preventive management of the most harmful potential invaders (Moles *et al.* 2008).

To be a successful invader, an individual must have a higher fitness than its native competitors in the new area (Colautti *et al*. 2006; Daehler 2003; Pyšek and Richardson 2007) and/or a high competitive ability (Daehler 2003). Competitive ability has two components: the competitive effect, which is related to resource acquisition, and the competitive response, based on the trade-off between resource use and conservation (Goldberg and Landa 1991; Keddy *et al*. 1998; Navas and Moreau-Richard 2005). Therefore, studying simultaneously traits related to fitness and competitive ability (*i.e.* competitive effect and competitive response) could give some insights in traits related to invasiveness. A trait is defined as a morphological, physiological or phenological feature of a plant, measurable at the individual level (Violle *et al.* 2007). While performance traits affect fitness directly via their effects on survival, growth and/or reproduction, functional traits can affect competitive ability (Violle *et al.* 2007). Indeed, vegetative traits such as plant height or lateral spread are known to be indicators of plant competitive effect since they give an advantage in space occupation and capture of light (Grime 1977), which in turn impedes the neighbouring species. Traits related to growth rate and resource acquisition and conservation, such as specific leaf area (SLA), leaf dry matter content (LDMC) or root-shoot ratio (RSR), can be used to assess competitive response (Navas and Moreau-Richard 2005). SLA and LDMC characterise the trade-off in plant functioning between production of biomass and conservation of nutrients (Diaz *et al*. 2004). While SLA is related to short leaf retention and fast growth rate (Cornelissen *et al.* 2003; Grotkopp *et al.* 2002), LDMC is related to defence against natural hazards and herbivory through high investment in leaf tissues (Cornelissen *et al*. 2003) and conservation of nutrients (Wright *et al*. 2004). RSR indicates preferential resource allocation and is also linked to nutrient acquisition as a low RSR reflects efficient soil nutrient uptake (Zou *et al.* 2007).

Classical studies in evolutionary ecology have often focused on variation in single trait. Only little attention has been paid to co-variation among characters, despite the fact that trade-offs have been recognized for their importance in explaining coexistence and local persistence (Silvertown 2004; Suding *et al*. 2003). The study of trade-offs among traits and their directional co-variation might thus provide insights into pre-adapted life-history strategies for invasiveness (Chun *et al*. 2007; Kuster *et al.* 2008).

Most of the work in plant invasion ecology has addressed inter-specific comparisons of traits between invasive and native or exotic non invasive congeners. Only few recent studies have concentrated on variation of traits between native and invasive genotypes of exotic species (Bastlova and Kvet 2002; Buschmann *et al.* 2005; DeWalt *et al*. 2004; Erfmeier and Bruelheide 2004; Erfmeier and Bruelheide 2005; Guesewell *et al*. 2006; Maron *et al*. 2007; Maron *et al.* 2004; Zou *et al*. 2007), despite the fact that intra-specific comparisons between native and invasive genotypes of an invasive species are critical to understanding invasion success. For a given species, the comparison of the ecology of the introduced genotypes with the one of the native genotypes provides a measure of changes in ecology which result from introduction in the new range as well as an understanding of the processes that enable exotics to dominate recipient communities (Dlugosch and Parker 2008; Hierro *et al.* 2005).

In this study, we used *Centaurea maculosa* Lam. and *Senecio inaequidens* DC. in a greenhouse experiment. These two species present diploid and tetraploid cytotypes in their native range but only tetraploid ones in the introduced range (Lafuma *et al.* 2003; Treier *et al*. in press). Due to this distribution pattern, these species are good models to study genetically and environmentally induced processes and their consequences on invasion success. Furthermore, there are taxonomical and ecological similarities between these two species: (1) they belong to the same family (Asteraceae), (2) they occupy similar ecological niches in their native range and

(3) they tend to invade similar habitats in their introduced range. If we define for convenience a geocytotype as a ploidy level in a given area (native or introduced range), both model species have three geocytotypes (native diploid, native tetraploid and invasive tetraploid).

All geocytotypes of both species were grown individually in pots to test for differences in fitness components, traits related to competitive ability and trait co-variation and trade-offs linked to growth and invasion strategies. Since species are taxonomically and ecologically related, we hypothesized that changes in traits linked to fitness and competitive ability following polyploidisation are the same between species. More precisely, we hypothesized that for both species (1) fitness increases following polyploidisation event in the native range as well as following introduction in the new range. Since several hypotheses regarding invasion success are linked to changes in traits related to competitive ability, we expected (2) both species' functional traits to change within tetraploid geocytotypes so that plants with introduced genotypes should be taller, have lower LDMC, higher SLA and lower RSR than native ones. Furthermore, as evolutionary and ecological processes can affect trait co-variations and trade-offs, which could result in changes in growth strategy leading to invasive success, we hypothesized that for both species (3) trait trade-offs occurred following polyploidisation within the native range as well as following introduction in the new range.

2.2. Material and Methods

2.2.1. Model species

Centaurea maculosa Lam. is a herbaceous biennial or short-lived perennial tap-rooted forb with a rosette of basal leaves and relatively thick flowering stems (Hook *et al.* 2004). Its native range spans from Western Asia to Western Europe. It is mainly found in continental xeric plant communities (Steinger and Müller Schärer 1992) but can also grow on sites ranging from semi-arid grasslands to open canopy forests, at low to mid elevation (Watson and Renney 1974), as well as on ruderal sites such as roadsides (Steinger and Müller Schärer 1992). *Centaurea maculosa* was introduced in the Pacific Northwest of the United States in the late 1800s (Watson and Renney 1974) and spread rapidly throughout north-western America and western Canada where it is now widespread in rangelands, pastures and on roadsides (Duncan 2001; Skinner *et al.* 2000). This species is known for its release of (±) catechine to the soil which may be allelopathic to other plants (Bais *et al.* 2002; Blair *et al.* 2006; Perry *et al.* 2005; Ridenour and Callaway 2001). It causes substantial economic damages through its low nutritive value which reduces forage quality (Campobasso *et al.* 1994), and its invasion of agricultural land, displacing forage species (Watson and Renney 1974).

Senecio inaequidens DC. is an erect perennial dwarf shrub native to South Africa and Lesotho. Originally found in grasslands from 1400 m a.s.l. to 2800 m a.s.l., it is now quite widespread in South Africa. It was accidentally introduced to Europe at the end of 1880s (Bornkamm 2002) where it is now widespread, even at lower altitudes. It is also found nowadays

Table 1: Details of the sampled *Centaurea maculosa* and *Senecio inaquidens* populations in their native and introduced range

Collection Site	Country	Ploidy	Elevation (m-asl)	Latitude	Longitude
Centaurea maculosa					
Native range (Europe)					
Hainburg	Austria	2n	326	49.90’N	16.58’E
Bovshev	Ukrainia	2n	296	49.13’N	24.42’E
Chutove	Ukrainia	2n	131	49.40’N	34.57’E
Czortova	Ukrainia	2n	305	49.24’N	24.40’E
Branson	Switzerland	2n	536	46.80’N	7.50’E
Conthey	Switzerland	2n	527	46.13’N	7.19’E
Basel	Switzerland	2n	298	47.33’N	7.39’E
Pècs	Hungary	4n	496	46.60’N	22.13’E
Khotyn	Ukrainia	4n	248	48.31’N	26.28’E
Introduced range (United States)					
Montana - USA	Montana	4n	1055	46.49’N	114.06’O
Montana - USA	Montana	4n	1146	47.00’N	113.23’O
Montana - USA	Montana	4n	804	47.19’N	114.18’O
Montana - USA	Montana	4n	1254	46.35’N	114.80’O
Montana - USA	Montana	4n	1384	45.50’N	113.56’O
Montana - USA	Montana	4n	1578	45.19’N	112.05’O
Montana - USA	Montana	4n	1630	46.10’N	110.05’O
Oregon - USA	Oregon	4n	50	45.40’N	121.31’O
Oregon - USA	Oregon	4n	762	44.34’N	121.25’O
Oregon - USA	Oregon	4n	1279	43.42’N	121.30’O
Senecio inaequidens					
Native Range (South Africa and Lesotho)					
Bultfontein	Free State - South Africa	2n	1340	28.16’S	26.08’E
Dealesville	Free State - South Africa	2n	1240	28.40’S	25.46’E
Memel	Free State - South Africa	2n	1741	27.41’S	29.35’E
God’s Window	Mpumalanga - South Africa	2n	1550	24.50’S	30.50’E
(Mpumalanga)	Mpumalanga - South Africa	2n	1450	24.58’S	30.49’E
Mt. Lebanon I	KwaZula-Natal - S-Africa	2n	1800	29.35’S	29.40’E
Joel’s Drift	Lesotho	2n	2000	28.46’S	28.25’E
Golden Gate I	Free State - South Africa	4n	2150	28.31’S	28.25’E
Golden Gate II	Free State - South Africa	4n	2050	28.31’S	28.25’E
Zastron	Free State - South Africa	4n	1448	30.15’S	27.10’E
Sterkfontein	KwaZula-Natal - S-Africa	4n	1649	28.34’S	29.02’E
Barkly East	Eastern Cape - S-Africa	4n	1789	30.58’S	27.36’E
Semonkong	Lesotho	4n	2200	29.49’S	28.03’E
Introduced range (Europe)					
Amersfoort	Netherlands	4n	5	52.09’N	5.23’E
Amsterdam	Netherlands	4n	0	52.21’N	4.54’E
Bremen	Germany	4n	15	53.05’N	8.48’E
Halle	Germany	4n	104	51.28’N	11.58’E
Hamburg	Germany	4n	11	53.33’N	10.00’E
Karlsruhe	Germany	4n	115	49.00’N	8.24’E
Kiel	Germany	4n	10	54.20’N	10.08’E
Köln (Germany)	Germany	4n	46	50.56’N	6.57’E
Weissenfels	Germany	4n	159	51.12’N	11.58’E
Basel	Switzerland	4n	254	47.33’N	7.36’E
Denges	Switzerland	4n	-	49.31’N	6.32’E
Laconnex	Switzerland	4n	-	48.09’N	6.02’E
Lieges	Belgium	4n	-	58.38’N	5.34’E
Mazamet	France	4n	-	43.29’N	2.22’E
Györ	Hungary	4n	-	47.40’N	17.38’E

in Australia and South America (see Ernst 1998 and Lafuma *et al.* 2003 for review). Due to its broad ecological range, it can be found in ruderal sites such as railroads and riverbeds (Garcia-Serrano 2004), as well as in disturbed pastures, wetlands and heathlands (Bornkamm 2002) and, more recently, also in undisturbed grassland ecosystems (Garcia-Serrano 2004). *Senecio inaequidens* contains pyrrolizidine alkaloids (Macel and Vrieling 2003), which are toxic to livestock (Noble *et al.* 1994). Therefore, when growing in pastures, *S. inaequidens* is refused by cattle, which is expected to lead to reduction of forage quality and thus to cause economical losses.

2.2.2. Seed collection

Seeds of *C. maculosa* were collected during summer 2005 throughout its native range in Europe as well as in Montana and Oregon, USA (Broennimann *et al*. 2007; Treier *et al*. in press). For this experiment, 19 populations were randomly taken from 97 available populations: 9 from native populations (Austria, Hungary, Switzerland and Ukraine) with unknown ploidy level and 10 from introduced populations (Montana and Oregon) known to be only tetraploids (Table 1). After ploidy analysis using flow cytometry, it came out that we had seven native diploids and two native tetraploids populations. Within populations, seeds were randomly collected among a pool of seeds gathered from 16 mother plants.

Seeds of *S. inaequidens* (mostly provided by Dr. Daniel Prati, University of Leipzig-Halle and Sandrine Maurice, Institut des Sciences de l'Evolution Montpellier) were collected in Western Europe, South Africa and Lesotho (Lafuma *et al.* 2003). For the experiment, 28 populations were randomly taken (Table 1): seven native diploids, six native tetraploids and 15 introduced tetraploids (across Belgium, France, Germany, Hungary, the Netherlands and Switzerland). For each population, seeds were randomly taken from two mother plants.

The unequal set of samples resulted from various constraints, in particular the limitation for field exploration and sampling (*C. maculosa*), the delivery of plant material by partners (*S. inaequidens*), and the *a posteriori* analysis of ploidy level (*C. maculosa*).

All seeds within each geocytotype of each species were then mixed together, since we wanted to compare geocytotypes and not individual populations.

2.2.3. Pot experiment

A 9-months pot experiment was set-up in the greenhouse of the University of Lausanne, Switzerland. On February 2006, 180 seeds of each model-species were sown in germination trays filled with sieved garden soil commonly used for seedbeds. For each species, half of the seeds came from invasive populations and half from native populations. As ploidy level of seeds of *S. inaequidens* was known prior to the experiment, we used seeds from 45 diploid and 45 tetraploid parents from the native range, and 90 seeds from tetraploid parents from the introduced range. Since ploidy of *C. maculosa* was unknown at the beginning of the experiment and was analysed later, it came out that among native seeds, 70 seeds were from diploid parents and 20 from tetraploid parents. The 90 seeds from the introduced range were from tetraploid parents.

After one month, surviving seedlings (223 individuals) were transplanted into 1 L pots. Each pot was filled with standard compost (Ricoter SA., Switzerland) and contained one individual. Pots were randomly arranged on tables and watered every 2-4 days. For bio-security reasons and in order to prevent seed set, capitula of all plants were cut regularly during the experiment. Nine months after sowing all living plants (212 individuals) were harvested and plant trait measured.

2.2.4. Measurements

Survival was measured on all 223 germinated plants. Other performance traits (shoot biomass and reproductive outputs) were measured on all the 212 plants living at the end of the experiment. Survival of transplanted seedlings, shoot biomass and flowering ability were determined at the end of the experiment. Flowering ability is a binary variable describing the ability of the individual to flower or not during the experiment. Number of days between sowing and the appearance of the first capitulum and the total number of capitula produced by flowering plants during the experiment were recorded to measure the onset of flowering and the capitulum production, respectively. Together, these performance traits characterise fitness.

Since the measurement of functional traits such as SLA, LDMC or RSR is time consuming, we randomly selected a subset of seven plants of each geocytotype of both species for measurements of traits related to competitive ability. Two distinct measures of vegetative size were done for the two species due to their distinct growth form: stem height for *S. inaequidens* and lateral extension of the canopy for *C. maculosa* (Grime 1977; Navas and Moreau-Richard 2005). On each individual, five leaves were taken randomly among the healthy, fully developed leaves for measurements of total leaf area and fresh biomass (Cornelissen *et al.* 2003). Total leaf area was measured using a LI-3100C Leaf Area Meter (Li-COR, Lincoln, Nebraska, USA). Leaves were dried at 60°C for 72 h and weighed for dry mass. Specific leaf area (SLA, leaf area per unit, leaf dry mass in m^2 kg^{-1}) and leaf dry matter content (LDMC, leaf dry mass per unit of leaf fresh mass in mg g^{-1}) were calculated for each plant as the mean of measurements of its five leaves. Selected plants were then separated into shoots and roots, dried at 35°C for seven days (to allow potential chemical analyses), and weighed together with the leaves collected for leaf trait measurements. Root-shoot ratio (RSR) was calculated as the ratio between root and shoot dry mass.

2.2.5. Statistical analyses

All the analyses were carried out with R 2.7.2 (R Development Core Team, 2008). Survival and flowering ability binary data were analysed with a generalised linear model fitted with a binomial distribution and a logit link function (Venables and Ripley 1999). Other performance traits were analysed with linear models which are most robust for unbalanced designs (Pinheiro and Bates 2000). All surviving plants were taken into account in the analysis of shoot biomass and flowering ability, whereas analysis of capitulum production and onset of flowering were only done on flowering individuals. Whenever factor effects were significant, Tukey post-hoc test were performed to assess significant differences between levels of factor (species and geocytotype). To reduce heteroscedasticity, shoot biomass and flowering potential were log-transformed.

LDMC, SLA and RSR were analysed using analysis of variance (ANOVA). To achieve the assumption of normality for analysis of variance, LDMC and SLA were log transformed. Since vegetative size does not correspond to the same measurement for the two model species, we used a linear mixed model with residual maximum likelihood estimation (Venables and Ripley 1999). Vegetative size was log transformed to reduce heteroscedasticity. Species was specified as a random factor and geocytotype as a fixed factor. Whenever interaction terms of ANOVA or linear mixed model were significant or marginally significant, separate ANOVA for each species were performed to test for differences between geocytotypes within species. We used Tukey post-hoc tests to assess significant or marginally significant differences between levels of factors. We considered marginally significant differences for the functional traits since the low number of replicates could show some trends that would need to be tested with more important samples.

To study the multivariate response of traits, we performed a redundancy analysis (RDA) which constrained performance and functional traits of the subset of individuals (*n* = 42) by species and geocytotype. Permutation tests were used to assess the significance of these multivariate regression models.

2.3. Results

2.3.1. Comparison of performance traits between species and among geocytotypes

Overall, the survival rate of transplanted seedlings was high, ranging from 77 % to 98 % which is not surprising considering optimal conditions of the experiment. No significant differences were detected between species and among the geocytotypes (Table 2).

Table 2: Results of the analyses of performance traits using Generalized Linear Models (§) and Linear Mixed Models (*n* = number of individuals taken into account for each analysis)

		Survival rate (§) (*n* = 223)		Shoot Biomass (*n* = 212)		Flowering ability (§) (*n* = 212)		Capitulum production (*n* = 148)		Onset of flowering (*n* = 148)	
	d.f.	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Species	1	0.146	0.702	48.057	**<0.001**	105.921	**<0.001**	64.293	**<0.001**	173.837	**<0.001**
Geocytotype	2	3.072	0.215	0.409	0.665	5.715	0.057	0.738	0.480	3.317	**0.039**
Species*Geocytotype	2	1.348	0.510	0.199	0.820	4.928	0.085	10.423	**<0.001**	5.884	**<0.001**

Senecio inaequidens produced more shoot biomass than *Centaurea maculosa* but there were no differences in shoot biomass between geocytotypes (Table 2, Figure 1a). Reproductive outputs were significantly different between species (Table 2, Figures 1b, 1c, 1d). During the nine months of growth, a higher proportion of *S. inaequidens* individuals flowered than *C. maculosa* (Table 2, Figure 1b). *Senecio inaequidens* flowered earlier and produced much more capitula than *C. maculosa* (Figures. 1c, 1d). The evolution of reproductive outputs according to geocytotypes differed between species as shown by the significant interaction terms (Table 2). Indeed, onset of flowering did not vary between geocytotypes of *C. maculosa* whereas introduced geocytotypes of *S. inaequidens* significantly flowered later than native diploid ones (Figure 1c). Both species showed evolution of capitulum production. However, while the number of capitula produced increased from native geocytotypes to introduced geocytotypes of *S. inaequidens*, it decreased following polyploidisation of *C. maculosa* (Figure 1d).

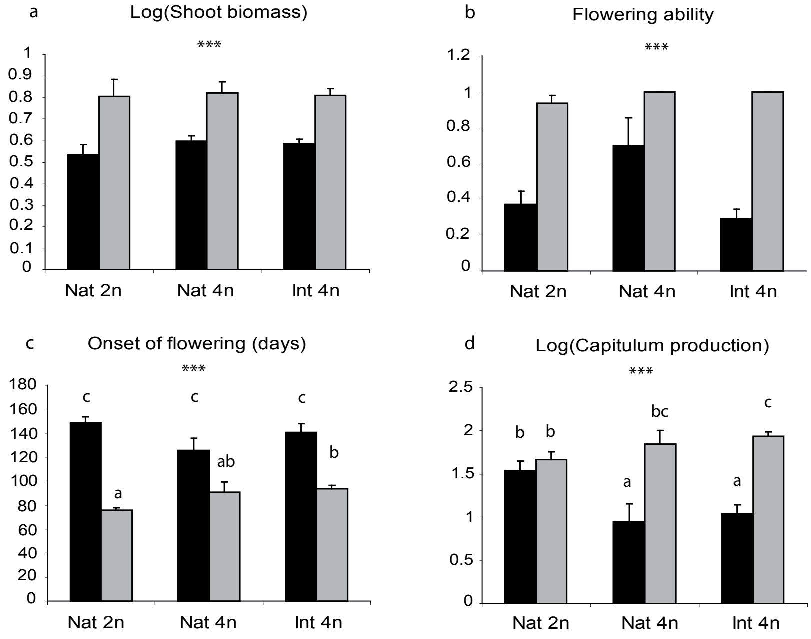

Figure 1: Performance traits of geocytotypes (native diploid "Nat 2n", native tetraploid "Nat 4n" and introduced tetraploid "Int 4n") of *Centaurea maculosa* (black) and *Senecio inaequidens* (grey). Shoot biomass and flowering ability were measured after nine months growth on surviving plants whereas onset of flowering and capitulum production were measured on flowering plant only. Bars indicate the standard error of the mean. Significant differences between species are indicated with stars (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Significant differences between geocytotypes are indicated with different letters (according to Tukey post-hoc tests)

2.3.2. *Comparison of functional traits related to competitive ability*

Species are significantly different regarding functional traits linked with competitive ability (Table 3). Although both species have SLA between 10 and 20 m^2 kg^{-1} and LDMC ranging from 150 to 250 mg g^{-1}, *Centaurea maculosa* has a significantly higher LDMC (Figure 2a) and lower SLA (Figure 2b) than *Senecio inaequidens*. It has also a higher RSR (Figure 2c), showing a higher allocation in roots than *S. inaequidens*.

Table 3: Analysis of variance on functional traits related to competitive ability of the subset of individuals (*n* = 42)

		LDMC		SLA		RSR		Vegetative size	
	d.f.	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Species	1	8.444	**0.006**	6.010	**0.019**	43.313	**< 0.001**	887.726	**<0.001**
Geocytotype	2	1.446	0.249	2.406	0.105	0.985	0.383	0.433	0.652
Species*Geocytotype	2	2.434	0.102	2.902	0.068	0.857	0.433	7.554	**<0.001**
Residuals	36								

The marginally significant interaction term between species and geocytotype for LDMC and SLA and the significant interaction term for vegetative size (Table 3) show that the two species do not seem to respond in the same way to polyploidisation in the native range as compared to the new range (Table 4). Indeed, among native geocytotypes of *C. maculosa,* tetraploid cytotypes had a lower LDMC (Fig. 2a) and a higher SLA (Figure 2b) than diploid ones. These traits did not evolve following polyploidisation of *S. inaequidens*. By contrast, among native cytotypes of *S. inaequidens* tetraploids were taller than diploids (Figure 2d). When comparing tetraploids of the native and introduced range, introduced geocytotypes did not significantly differ in functional traits from native ones, whatever the species considered.

Table 4: Analysis of variance on functional traits related to competitive ability of the three geocytotypes for both model species (*n* = 21 for each species)

		LDMC		SLA		Vegetative size	
	d.f.	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Centaurea maculosa	2	2.891	0.082	4.735	**0.022**	0.147	0.864
Residuals	18						
Senecio inaequidens	2	0.325	0.727	0.202	0.819	3.119	0.069
Residuals	18						

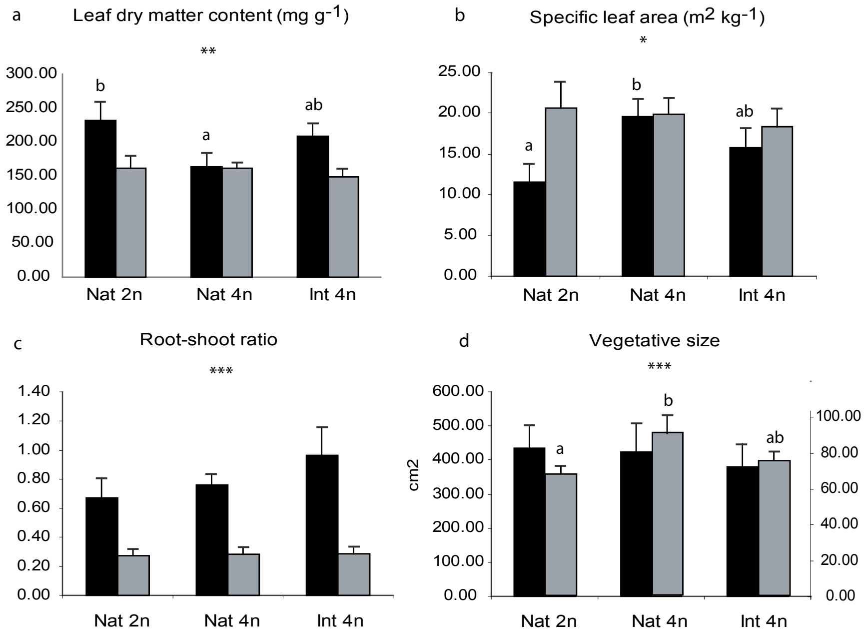

Figure 2: Functional traits of geocytotypes (native diploid "Nat 2n", native tetraploid "Nat 4n" and introduced tetraploid "Int 4n") of *Centaurea maculosa* (black) and *Senecio inaequidens* (grey). Vegetative size corresponds to lateral extension of the canopy (cm^2) for *C. maculosa,* and vegetative height (cm) for *S. inaequidens.* Bars indicate the standard error of the mean. Significant differences between species are indicated with stars (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Significant and marginally (up to 10%) significant differences between geocytotypes of each species are indicated with different letters (according to Tukey post-hoc tests)

2.3.3. *Comparison of trade-offs among traits of geocytotypes of both species*

The measured functional traits clearly separated the two species in the redundancy analysis (Figure 3) revealing two growth strategies (999 permutations, $p<0.001$). The first axis explains 28.25 % of total variation and represents the strategy of resource allocation. It separates *C. maculosa* with a high resource allocation to roots from *S. inaequidens* that invests preferentially into shoot development and flowering. The second axis, which holds 2.85 % of total variation, indicates the nutrient acquisition/conservation strategy of the plants. Since this axis is not significant, there is no clear differentiation of the species along this gradient. Within each species and each geocytotype there is a continuum from individuals with nutrient conservation strategy (high LDMC) to individuals with highly competitive strategy through resource investment in rapid growth (high SLA). Introduced tetraploid geocytotypes seem to have an “intermediate” strategy compared to native ones as shown by the position of centroids on the RDA biplot. RDA constrained by species only (not shown) indicates that species explains 29.7% of the total variation of traits, which was significant (F=2.953, 1 d.f., $p<0.005$), while RDA constrained by geocytoype (not shown) indicates that geocytotype explains only 2.5% of total trait variation, which is not significant (F=0.124, 2 d.f., $p=0.69$).

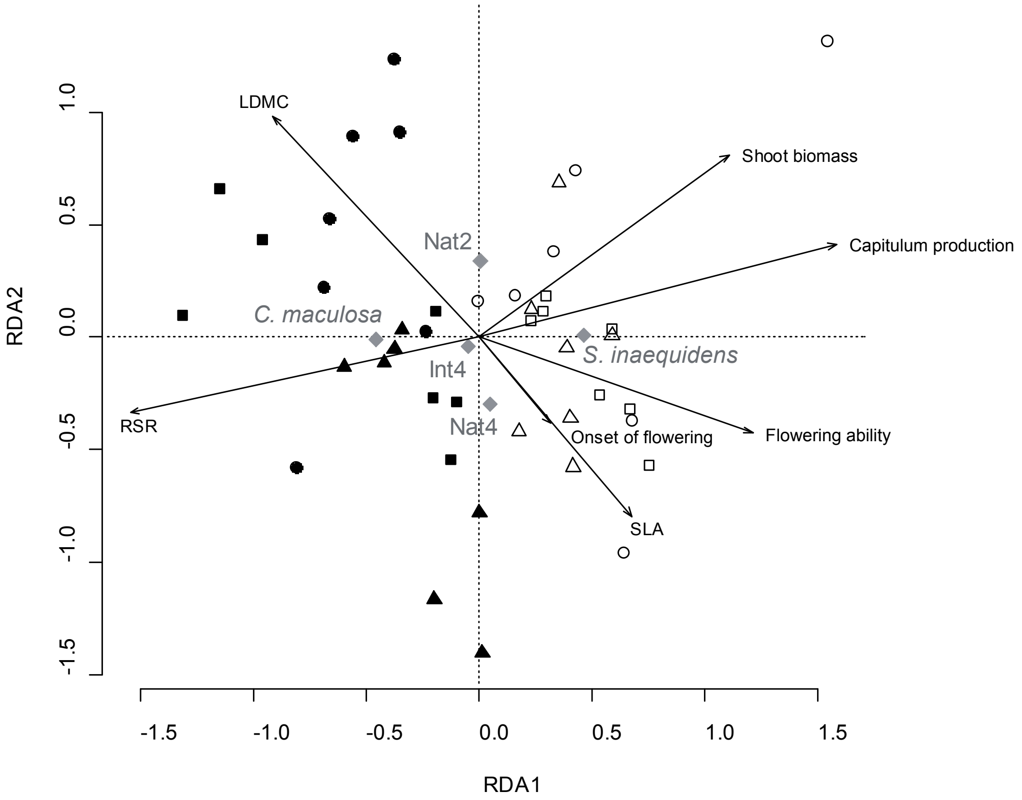

Figure 3: Redundancy Analysis (RDA) on performance and functional traits of *Centaurea maculosa* (black symbols) and *Senecio inaequidens* (white symbols). All geocytotypes are considered: native diploids (circles), native tetraploids (triangles) and introduced tetraploids (squares). Axis 1 and 2 represent respectively 28.25% (999 permutations, $P=0.005$) and 2.85% (999 permutations, $p=0.380$) of total trait variation ($n = 42$)

2.4. Discussion

2.4.1. Growth strategies differ between taxonomically related invasive species with similar ecology

Both species are characterised by high values of SLA and low values of LDMC, which is typical for species with fast relative growth rate in relation to fast acquisition of resources (Cornelissen *et al.* 2003; Navas and Moreau-Richard 2005; Poorter and De Jong 1999; Weiher *et al*. 1999). Thus, both species can be defined as exploitative species (Diaz *et al.* 2004; Grime 1979; Wright *et al.* 2004).

However, the two studied species have still a different set of traits values, which give them distinct growth strategies, as shown by the synthetic picture of the RDA. Indeed, *C. maculosa* is characterised by a higher resource-allocation to belowground parts (as shown by higher values of RSR) and a higher investment in leaf toughness (as shown by higher values of LDMC). This resource allocation to belowground parts and leaf tissues can be linked to resource conservation and resistance against physical hazards or herbivory (Cornelissen *et al.* 2003). *Senecio inaequidens* is characterised by a higher investment in shoot biomass and reproductive outputs, contributing to a higher competitive effect and leading to longer persistence in the environment (Goldberg and Landa 1991; Navas and Moreau-Richard 2005; Weiher *et al.* 1999). *Senecio inaequidens* is also associated with higher values of SLA, which characterise species with fast relative growth rate (Cornelissen *et al.* 2003; Grime 1979; Poorter and De Jong 1999; Weiher *et al.* 1999).

We highlight in this study that even among invasive species of the same family and furthermore living in relatively similar habitats, life strategies can be markedly different. This could explain the difficulties in finding key traits related to invasiveness at this taxonomic level (Muth and Pigliucci 2006) and stresses the need of selecting pertinent functional traits that can best reveal the invasive success.

2.4.2. Evolution of fitness following polyploidisation and introduction

Survival of seedlings *per se* may not be associated with invasiveness since in our experimental conditions all geocytotypes had an equally high survival rate, whatever the species considered. Neither polyploidisation in the native range, nor introduction into the new area led to an increase in shoot biomass. This shows that seedling survival and biomass may not be the discriminating traits for these two worldwide invasive species. However, conditions of the experiment do not necessarily correspond to the field reality where stress factors may become critical. Furthermore, as pointed out by Guesewell *et al.* (2006), the absence of difference in shoot biomass could be the consequence of the short duration of experiments.

Both species show changes in reproductive output following polyploidisation and introduction in the new range. However, we found these changes to be species specific, indicating two different strategies of invasion. Indeed, while the number of flower heads of *S. inaequidens* increases following polyploidisation in the native range and introduction in the new range, *C. maculosa* invests less in flowering potential in the introduced range than in the native one, as shown by the lower number of flower heads produced.

Invasiveness of *S. inaequidens* could be due to an increase in flowering potential. Indeed, by increasing capitulum production, introduced geocytotypes enhance propagule pressure in the new area, which has often been considered as one major component of invasion success (Kolar and Lodge 2001; Lockwood *et al.* 2005; Von Holle 2005; Williamson 1996), since it enhances chances of establishment and subsequent potential for spreading. By contrast, tetraploid geocytotypes of *C. maculosa* show a decrease in flowering potential, which argue in favour of another invasion strategy than propagule pressure. Allelopathy has received much attention in the last years and was thought to be partially responsible for invasion success of *C. maculosa* (Perry *et al*. 2005; Ridenour and Callaway 2001; Weir *et al*. 2003). However, recent studies have shown that the amount of catechine released in the soil by *C. maculosa* is too low to inhibit efficiently neighbouring plants (Blair *et al*. 2006).

2.4.3. Evolution of trade-offs among traits during invasion process

Studying traits of offsprings from the native and the introduced range growing in the same conditions allows to reveal traits controlled by genetic differentiation (Bossdorf *et al*. 2005; Willis *et al.* 2000). Invasion of both species may be controlled by genetic changes since traits related to competitive ability evolved already in the native range following polyploidisation event. Indeed, in the native range of *S. inaequidens*, polyploidisation was linked to higher stem height. Taller plants can quickly overgrow the surrounding vegetation and therefore better compete for solar irradiance in the early growing season (Menges 1987). Within the native range of *C. maculosa,* polyploidisation turned out to be a competitive advantage since tetraploid geocytotypes invest more resource in photosynthetic tissues (higher SLA) and less in defence components (lower LDMC). For both model species, the competitive advantage brought by this specialisation might explain the invasion success of the tetraploid genotypes (Lafuma *et al.* 2003; Treier *et al*. in press).

Following introduction, vegetative height of *S. inaequidens* tended to decrease and *C. maculosa* tended to reverse towards a more defence-oriented resource conservation strategy (lower SLA, higher LDMC). Therefore, it appears that a loss of specialisation was needed for invasive success. For *S. inaequidens*, we could argue that once the competitive advantage was acquired in the introduced range, selection led to a decrease in vegetative height as more resources were allocated to flowering. The trade-off between flowering and growth could therefore explain why introduced cytotypes are not taller than native ones. A similar post-invasion scenario could have applied to *C. maculosa,* with a selection for slow nutrient cycling in the introduced range. Again, the fast cycling strategy linked to high competitive ability could have been necessary in the first steps of invasion (Guesewell *et al.* 2006), but subsequent spread would have selected a slow-nutrient cycling strategy, with individuals more adapted to herbivory and perturbations, through unpalatable and resistant tissues. These results allow us to argue in favour of a two-phase invasion, as described by Dietz and Edwards (2006), where high competitive ability of native tetraploids would have permitted them to invade low stressed and rich habitats, and where evolutionary changes towards a less competitive and more stress tolerant strategy would have allowed the second phase of invasion into harsher and poor habitats.

Such evolutionary adjustments could explain the time lag between the species' first introduction and the beginning of invasion (Richards *et al.* 2006). This also could explain the niche shift experienced by *C. maculosa* in its introduced range (Broennimann *et al.* 2007; Treier *et al.* in press). Indeed, the loss of specialisation in the introduced range seems to allow for an intermediate growth strategy in introduced geocytotypes as compared to strategies adopted by diploid and tetraploid geocytotypes within their native range (Figure 3). With such an intermediate strategy, introduced tetraploid geocytotypes could have a higher potential to deal with various or changing environments since they could be able to shift their growth strategy between conservative defence-oriented and exploitative-competitive characteristics. This ability of introduced genotypes to perform well in all sorts of environments as opposed to native geocytotypes, which are specialised to one kind of environment, has been mentioned as a Jack-of-all-Trades strategy (Richards *et al.* 2006) and could also explain the invasive success on our study species.

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Chapter 3

What makes *Centaurea maculosa* and *Senecio inaequidens* successful invaders: Inherent superiority, disturbance benefit or evolutionary changes?

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Abstract

Exotic plant invasions are considered as one of the most important threat to biodiversity and represent a danger to natural and managed ecosystems. Understanding what predisposes a species to become an invader is a major challenge to ecologists. Different hypotheses have been formulated to explain invasion success in new environments. While invaders have been firstly thought to be inherently superior to natives, ecological and evolutionary hypotheses have later been suggested as an explanation for invader success. According to ecological processes, invaders could benefit from an extrinsic change of the community, like a resource release following disturbance. Due to evolutionary processes, such as polyploidisation, invaders could also benefit from an intrinsic change for their establishment success.

We used two taxonomically related species, *Centaurea maculosa* Lam. and *Senecio inaequidens* DC., in a two-year field experiment to disentangle the effects of inherent superiority from polyploidisation and resource release following disturbance to explain invasion success. For both species, diploid and tetraploid populations occur in the native range, whereas only tetraploids are present in the introduced range.

Our results highlight the fact that the effects of polyploidisation and response to disturbance are species-specific since the effects of polyploidisation and release from competition did not affect the same traits for *C. maculosa* and *S. inaequidens*. Introduced genotypes of *C. maculosa* had an inherent superiority to cope with competition for light, which might allow a reinvestment of resources from aboveground growth to belowground growth and allelochemical production. Polyploidisation of *S. inaequidens* might lead to an evolutionary ability to take advantage of an ecological process such as a release from competition for light. This ability could allow a reinvestment of resources into reproductive output, leading to an increase in capitulum production and therefore a higher propagule pressure. From a management point of view, mowing, by limiting survival, growth and reproductive output, should be considered as a useful tool to limit spread of both *C. maculosa* and *S. inaequidens* in natural grasslands.

Keywords: Competition release, management, polyploidisation, traits trade-offs

3.1. Introduction

Exotic plant invasions are considered as one of the most important threat to biodiversity (Heywood 1989). They represent a danger to natural and managed ecosystems (Pimentel *et al*. 2000; Prieur-Richard *et al*. 2000) and cause substantial economical losses in plant and animal agricultural production (Lonsdale 1999). Understanding what predisposes a species to become an invader is a major challenge to ecologists (Richardson and Pysek 2006).

Since Elton (1958), a large array of hypotheses has been suggested to explain invasion success in new environments. The first hypothesis was the inherent superiority of the invader compared to the native species of the community they invade (Elton 1958). According to this hypothesis, invaders may be superior competitors for community resources (Sax and Brown 2000). This superiority can also occur through the release of allelopathic compounds that alter plant-soil interactions as proposed by the novel weapon hypothesis (Callaway and Ridenour 2004). Since these compounds are novel to the native inhabitants of the community, they are more efficient than in the native range of the invader where neighbouring species have co-evolved with these compounds. Invasiveness has also been explained by ecological and evolutionary processes. In accordance with ecological processes, invasion success would be the consequence of extrinsic changes in the new environment that favour the invading species such as disturbance or creation of empty niches (Elton 1958; Hierro *et al.* 2006), release from competitively superior neighbours or natural enemies (Keane and Crawley 2002). By contrast, evolutionary processes, such as hybridization or polyploidisation, lead to intrinsic changes of the invasive species (Amsellem *et al*. 2001; Pandit *et al.* 2006; Prentis *et al.* 2008; Soltis and Soltis 2000; Verlaque *et al.* 2002; Vila and D'Antonio 1998). Genome changes can lead to an adaptive evolution of traits (Blossey and Notzold 1995; Bossdorf *et al.* 2004) which can improve fitness (Hufbauer and Torchin 2007) or widen ecological breadth (Petit and Thompson 1999; Soltis and Soltis 2000; Weber and Schmid 1998), from the native range to the introduced one. However, until recently, evolutionary processes have received little attention in the framework of invasion ecology despite the fact that their study could give new insights into the mechanisms of invasion (Bailey *et al.* 2007; Blair and Wolfe 2004; Blumenthal and Hufbauer 2007; Dietz and Edwards 2006; Facon *et al.* 2006; Keller and Taylor 2008; Lee 2002; Maron *et al*. 2004; Müller-Schärer *et al*. 2004; Prentis *et al.* 2008; Richards *et al.* 2008; Whitney and Gabler 2008; Williams *et al.* 2008). Understanding invasion processes and disentangling the importance of inherent superiority, ecological processes and evolutionary changes responsible for species invasion may improve the efficiency of management options.

Intra-specific comparisons between native and invasive genotypes of an invasive species are necessary to understand invasion success (Hierro *et al.* 2005). Comparing the ecology of introduced populations of a species with its native populations provides a measure of changes in ecology which result from introduction in the new range as well as an understanding of the processes that enable exotics to dominate recipient communities (Dlugosch and Parker 2008; Hierro *et al.* 2005). Plant traits can give clues on the underlying mechanisms that allow invasion. A trait is a morphological, physiological or phenological feature of a plant, measurable at the individual level (Violle *et al*. 2007). Only few recent studies have concentrated on the variation of traits between native and invasive populations of exotic species (Bastlova and Kvet 2002; Buschmann *et al.* 2005; DeWalt *et al.* 2004; Erfmeier and Bruelheide 2004; Erfmeier and Bruelheide 2005; Guesewell *et al.* 2006; Maron *et al.* 2007; Maron *et al.* 2004; Zou *et al.* 2007). A successful invader has a higher fitness than its native competitors in the new area (Colautti *et al.* 2006; Daehler 2003; Pyšek and Richardson 2007) and/or a higher competitive ability (Daehler 2003). Therefore, studying simultaneously traits related to fitness and competitive ability could give some insights in traits related to invasiveness. Fitness depends on survival, growth and reproduction. Competitive ability is the combination of the competitive effect, which is related to resource acquisition, and the competitive response, based on the trade-off between resource use and conservation (Goldberg and Landa 1991; Keddy *et al.* 1998; Navas and Moreau-Richard 2005). The competitive effect can be measured through vegetative traits such as plant height or lateral spread as these give an advantage in capture of light and/or space occupation (Grime 1977). The competitive response can be assessed through traits related to growth rate, such as shoot or root biomass (Gaudet and Keddy 1988; Navas and Moreau-Richard 2005), and resource acquisition and conservation such as root-shoot ratio (Navas and Moreau-Richard 2005; Zou et al. 2007).

In this study, we investigated the invasion success of two species, *Centaurea maculosa* and *Senecio inaequidens*, through a two-year field experiment. These two species present diploid and tetraploid cytotypes in their native range but only tetraploid ones in the introduced range (Lafuma *et al.* 2003; Treier *et al.* in press). Due to this distribution pattern, these species are good models for studying genetically and environmentally induced processes and their consequences on invasion success. Furthermore, there are taxonomical and ecological similarities between these two species: (1) they belong to the same family (Asteraceae), (2) they occupy similar ecological niches in their native range, (3) they tend to invade similar habitats in their introduced range and (4) they both may have allelopathic effects (Ahmed and Wardle 1994; Callaway and Ridenour 2004). If we define for convenience a geocytotype as a ploidy level in a given area (native or introduced range), both model species have three geocytotypes (native diploid, native tetraploid and introduced tetraploid).

All geocytotypes of both species were grown in a semi-natural grassland to investigate the relative importance of inherent superiority, ecological processes and evolutionary changes in the invasive success. Growing native and introduced genotypes of an invasive species in a new community will clarify whether the species itself, independently of the genotype, is inherently superior to native species forming the recipient community. Since both species present diploid and tetraploid genotypes, comparison of fitness and competitive ability of both genotypes allows testing for effects of evolutionary changes (*i.e.* polyploidisation) on invasive success. A disturbance treatment was applied in the field to simulate an extrinsic change in order to assess the importance of ecological processes. We compared fitness and competitive ability of all geocytotypes of both species under two different disturbance treatments to test whether (1) invasive species will be inherently superior competitors, (2) competition release following disturbance will favour the establishment of the invasive species, (3) tetraploid genotypes will have a higher establishment success than diploid ones and (4) tetraploid genotypes will be more favoured by a change in the community (*i.e.* competition release following disturbance) than diploid ones.

3.2. Material and methods

3.2.1. Model species

Two worldwide invasive species were used in this experiment as model species. *Centaurea maculosa* Lam. is a herbaceous biennial or short-lived perennial tap-rooted forb with a rosette of basal leaves and relatively thick flowering stems (Hook *et al.* 2004). Its native range spans from Western Asia to Western Europe. *Centaurea maculosa* was introduced in the Pacific Northwest of the United States in the late 1800s (Watson and Renney 1974) and expanded rapidly throughout north-western America and western Canada where it is now widespread in rangelands, pastures and on roadsides (Duncan 2001; Skinner *et al.* 2000). Its low nutritive value reduces forage quality and makes it often avoided by cattle (Campobasso *et al.* 1994). Seeds of *C. maculosa* were collected during summer 2005 throughout its native range in Europe as well as in Montana and Oregon, USA (Broennimann *et al.* 2007; Treier *et al.* in press). On the whole, 22 populations were randomly chosen for this study from 97 available populations: 6 from native diploid populations, 6 from native tetraploid populations and 10 from introduced populations known to be only tetraploids. Within populations, seeds were randomly chosen among a pool of seeds from 16 mother plants.

Senecio inaequidens DC. is an erect perennial dwarf shrub native to South Africa and Lesotho. Originally found in grasslands from 1400 m a.s.l. to 2800 m a.s.l., it is now quite widespread in South Africa. It was accidentally introduced to Europe at the end of 1880s (Bornkamm 2002) where it is now widespread, even at lower altitudes. It is also found nowadays in Australia and South America (Ernst 1998; Lafuma *et al.* 2003). *Senecio inaequidens* contains pyrrolizidine alkaloids (Macel and Vrieling 2003), which are toxic to livestock (Noble *et al.* 1994). Therefore, when growing in pastures, *S. inaequidens* is avoided by cattle, which is expected to lead to reduction of forage quality and thus to cause economical loss. Seeds of *S. inaequidens* were collected in Western Europe, South Africa and Lesotho (Lafuma *et al.* 2003). Additional seeds were collected in two populations in Switzerland. Of 89 available populations, 18 were randomly chosen: 6 native diploids, 3 native tetraploids and 9 introduced tetraploids. For each population, seeds were randomly taken from two mother plants.

C. maculosa and *S. inaequidens* individuals will hereafter be referred as target individuals, whatever the geocytotype concerned.

3.2.2. Seed germination

Seeds of both model species were sown in peat pellets and placed in a germination room under daily controlled conditions, *i.e.* 14 hours at 24°C and 10 hours at 18°C. After one week, seedlings were put outside to acclimatise to natural field conditions. Two-week-old target individuals were used for transplantation in the experimental field site.

3.2.3. Experiment setup

A two-year field experiment was set up in May 2006 at the Swiss federal research station of Changins (ACW Agroscope Changins-Wädenswil, 46°23'47 N; 6°13'51 E) in Switzerland. A 15 m x 15 m enclosure was constructed in a semi-natural mown grassland. The experimental site was mown prior to seedling transplantation and divided into 16 blocks of $2m^2$ (Figure 1a). A management treatment, simulating unselective («mowing») and selective disturbance («grazing»), was randomly assigned to the blocks (Figure 1a) and applied twice a year. In 8 blocks, all aboveground vegetation, including the target individuals, was mown to ground level (*i.e.*mowing). In the 8 other blocks, only the aboveground vegetation surrounding the target individuals was mown to simulate grazing (Figure 1b). Each block was further divided into 8 plots of 30cm x 30cm (Figure 1c). Geocytotypes of both model species were randomly assigned to the plot within each block. Target seedlings (n = 96) were transplanted with their peat pellet directly into the soil. Two additional plots, one with a blank peat pellet and one control (no transplantation) were added to test for disturbance due to the experimental setup (Figure 1d).

3.2.4. Measurements

Survival of transplanted seedlings, vegetative size and reproductive output were determined at the end of each vegetation season. Due to differences in growth form between *S. inaequidens* and *C. maculosa,* we measured for each species, respectively, height of the plant and lateral extension of the canopy (*i.e.* lateral spread) (Gaudet and Keddy 1988; Grime 1977; Navas and Moreau-Richard 2005). Reproductive output was represented by the flowering ability and capitulum production of the targets. Flowering ability is a binary variable which describes the ability of the individual to flower or not during each year of the experiment. Capitulum production was recorded as the total number of capitula produced by flowering plants during each vegetation season. These performance traits characterise fitness and competitive effects of the geocytotypes.

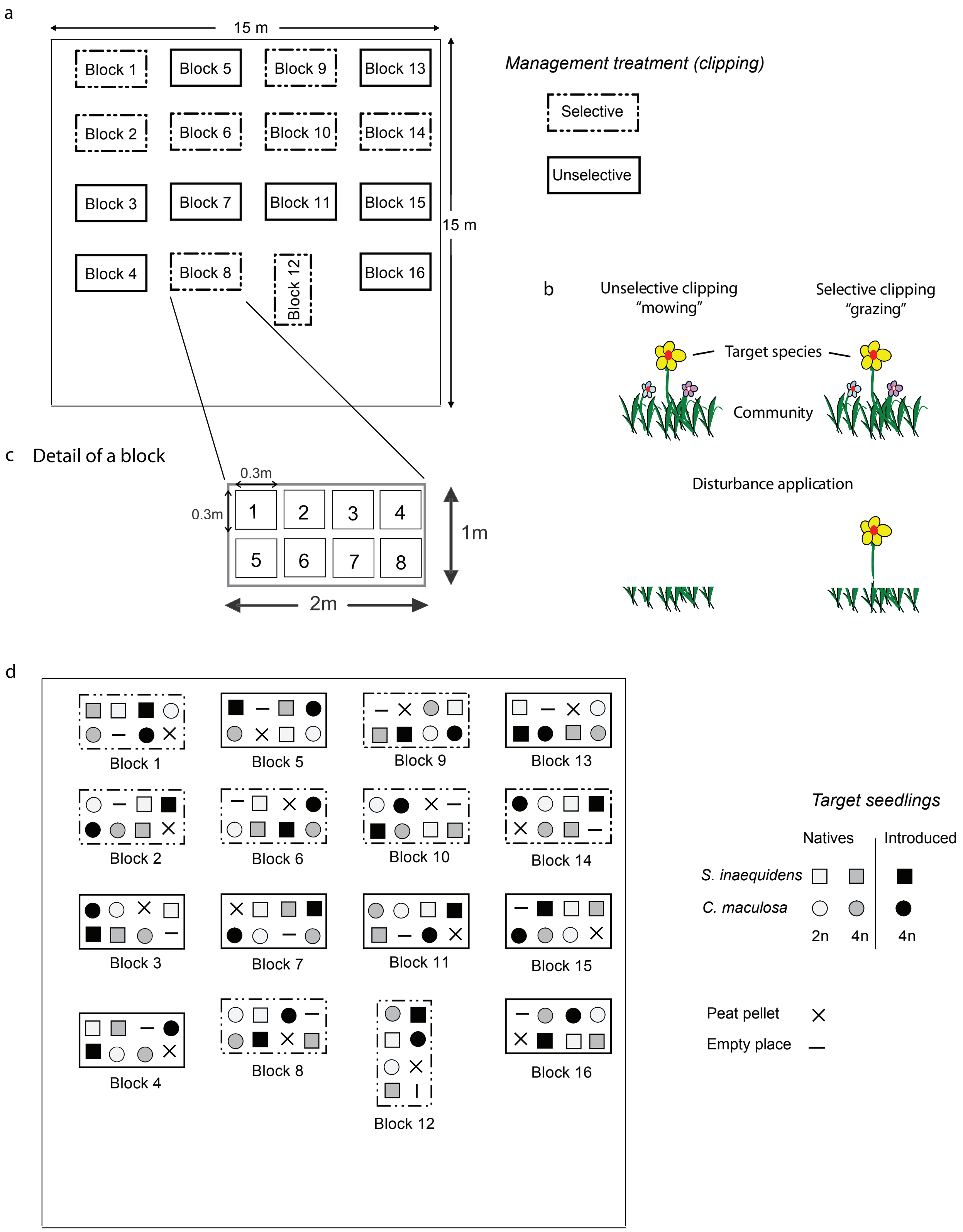

Figure 1: Experimental design: a management treatment (a), *i.e* unselective or selective clipping (b), was randomly assigned to 16 blocks. Each block contained 8 plots (c) in which geocytotypes of model species were randomly assigned (d).

The competitive response was assessed through root and shoot biomass measurements at the end of the experiment on all surviving targets. Plants were separated into shoots and roots, dried at 60°C for 72 hours and weighed to get respectively shoot biomass and root biomass (Cornelissen *et al.* 2003). Root-shoot ratio (RSR) was calculated as the ratio between root and shoot dry mass. For bio-security reasons and in order to prevent seed set, capitula of all plants were cut regularly during the experiment.

3.2.5. Statistical analyses

Whenever possible, data were analysed using linear mixed effects models specifying management, species and geocytotype as fixed effects and plot nested into block as random factors. Thus, the management effect was tested against the block-level mean square as error term whereas effects of species, geocytotypes and all the interactions were tested at the plot level, *i.e.* against the plot-level mean square as error term (Pinheiro and Bates 2000). Because the test of interactions is not straightforward with generalised linear mixed model (GLMM), survival and flowering ability binary data, were analysed using generalised linear models (GLM) fitted with a binomial distribution and a logit link. For the same reason, the number of capitula per flowering plant were analysed with a GLM fitted with a poisson distribution. As terms are added sequentially in GLMs, we specified management as the first factor to be tested, in order to keep the block structure of the experiment. Separate analyses were done for the vegetative height of *S. inaequidens* and the lateral spread of *C. maculosa*. Shoot biomass, root biomass and RSR of surviving individuals were analysed using linear mixed effects models (LME), with plot nested into block specified as a random factor. Each year of the experiment was analysed separately. As the survival in the second year was very low under unselective disturbance (2 surviving plants out of 48), reproductive output and competitive ability were analysed under selective clipping only. Consequently, linear models were used instead of linear mixed effect models since the management treatment was not tested anymore. Vegetative size, capitulum production, shoot biomass, root biomass and root-shoot ratio were log-transformed to reduce heteroscedasticity. All the analyses were carried out with R 2.7.2 (R Development Core Team, 2008).

3.3. Results

3.3.1. Fitness: survival and reproductive output

Survival of both species was high under all combinations of species, geocytotypes and management treatment after the first growing season. Survival ranged from 75 to 100% for *Centaurea maculosa* and from 62.5 to 100% for *Senecio inaequidens*. There was no difference in survival between species, geocytotypes or management type (Table 1). At the end of the second vegetation season survival was in all cases lower than 40% (Figure 1). Survival was significantly influenced by the management treatment but no differences occurred between species and geocytotypes (Table 1). Under unselective clipping only two targets out of 48 survived after two vegetation season, whereas there were 14 surviving target under selective clipping.

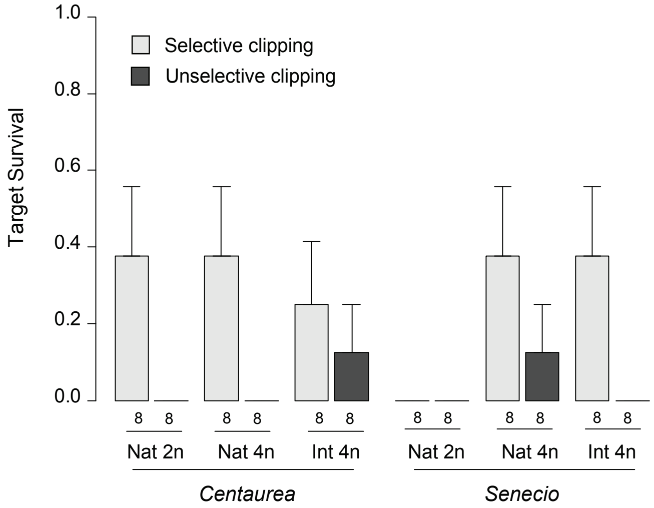

Figure 1: Survival after the second vegetation season of geocytotypes (Nat 2n: native diploids, Nat 4n: native tetraploids and Int 4n: introduced tetraploids) of *C. maculosa* and *S. inaequidens* under selective (light grey) and unselective (black) clipping. Numbers on the x-axis indicate the number of observations used for statistical analyses. Bars indicate the standard error of the mean.

Only *S. inaequidens* flowered during the first year. The proportion of flowering individuals of *S. inaequidens* was significantly higher under selective clipping than under unselective clipping, but there were no significant differences among geocytotypes (Table 1, Figure 2a). Only tetraploid genotypes of *S. inaequidens* produced more capitulum under selective than under unselective clipping as shown by the significant interaction between geocytotype and management (Table 1; Figure 2b). Under unselective clipping, capitulum production was not different among geocytotypes. During the second year of experiment and under selective clipping, there were no differences in the flowering ability of *C. maculosa* and *S. inaequidens* ($F_{1,9}$=6.028, p=0.278) and there were no significant differences in the flowering ability among geocytotypes ($F_{2,9}$=2.773, p=0.196). Differences in the capitulum production among geocytotypes were species-specific as shown by the significant interaction between species and geocytotype. Introduced genotypes of *C. maculosa* produced less capitula than native ones, whereas there were no differences in the capitulum production among the geocytotypes of *S. inaequidens* (Table 1, Figure 2c).

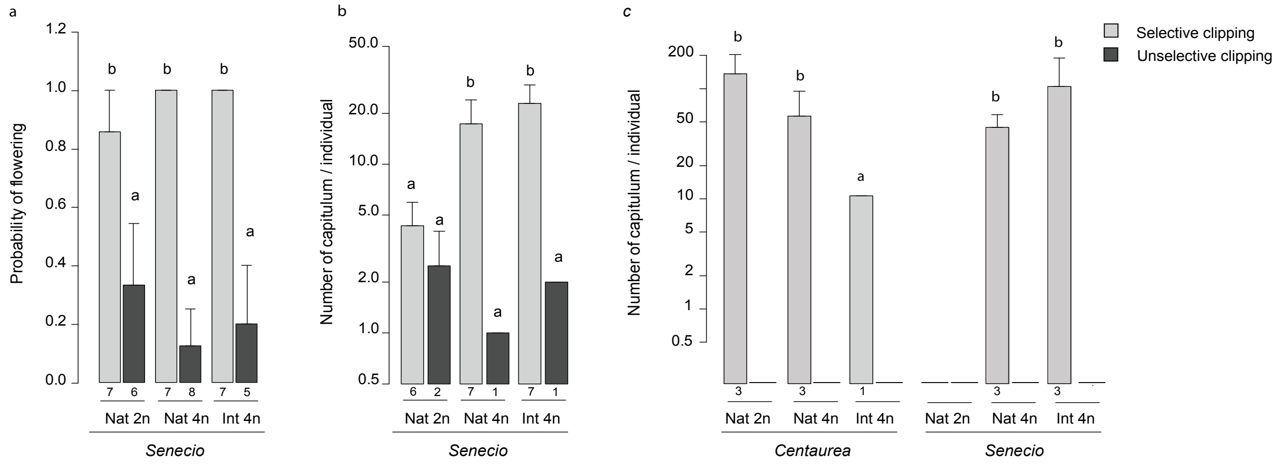

Figure 2: Flowering ability at the end of the first vegetation season (a) and capitulum production at the end of the first (b) and second (c) vegetation period of geocytotypes (Nat 2n: native diploids, Nat 4n: native tetraploids and Int 4n: introduced tetraploids) of *S. inaequidens* and *C. maculosa* according to the management treatment, *i.e* selective (light grey) or unselective (black) clipping. Numbers on the x-axis indicate the number of observations. Bars indicate the standard error of the mean. Letters indicate significant differences according to Tukey post-Hoc tests. Number of capitulum is on a logarithmic scale.

Table 1: Results of the generalised linear models fitted with a binomial (§) or a poisson (¶) distribution testing effects of management (unselective *vs*. selective clipping), species (*C. maculosa* vs. *S. inaequidens*), geocytotype (native diploid, native tetraploid or introduced tetraploid) and their interactions on survival, flowering ability and capitulum production at the end of the first (year 1) and the second vegetation season (year 2).

	Survival (§)					Flowering ability (§)			Capitulum production (¶)					
	Year 1			Year 2		Year 1			Year 1			Year 2		
	Df	*F*-value	*P*-value	*F*-value	*P*-value	Df	*F*-value	*P*-value	Df	*F*-value	*P*-value	Df	*F*-value	*P*-value
Management (M)	1	0.383	0.536	11.931	**0.001**	1	14.893	**<0.001**	1	66.36	**<0.001**			
Species (Sp)	1	1.556	0.212	0.339	0.561	1	58.018	**<0.001**				1	0.10	0.750
Geocytotype (G)	2	2.427	0.297	2.348	0.309	2	0.148	0.929	2	84.51	**<0.001**	2	234.02	**<0.001**
M*Sp	1	0.226	0.635	0.070	0.791	1	< 0.001	1.000						
M*G	2	0.802	0.670	0.787	0.675	2	3.038	0.219	2	7.52	**0.02**			
Sp*G	2	4.485	0.106	4.682	0.096	2	< 0.001	1.000				2	170.16	**<0.001**
M*Sp*G	2	3.740	0.154	2.958	0.228	2	0.000	1.000						
Residuals	84					72			18			8		

3.3.2. Competitive ability: competitive effects and competitive response

The vegetative height of all geocytotypes of *S. inaequidens* was significantly lower under unselective than under selective clipping (Figure 3a, Table 2) after the first growing season. By contrast, only native genotypes of *C. maculosa* showed a smaller lateral expansion of their basal rosette under unselective than under selective clipping (Figure 3b) as shown by the significant interaction between geocytotype and management (Table 2). Contrarily to the unselective treatment, there were no differences in vegetative size of *C. maculosa* under the selective treatment (Figure 3b). None of the species showed significant differences in their vegetative size among geocytotypes after the second vegetation period ($F_{1,4}$=2.280, p=0.206 and $F_{2,5}$ =0.597, p=0.585 for vegetative height of *S. inaequidens* and lateral spread of *C. maculosa* respectively). There were no significant differences between species and geocytotypes for shoot biomass, root biomass and root-shoot ratio at the end of the second vegetation season (Table 3).

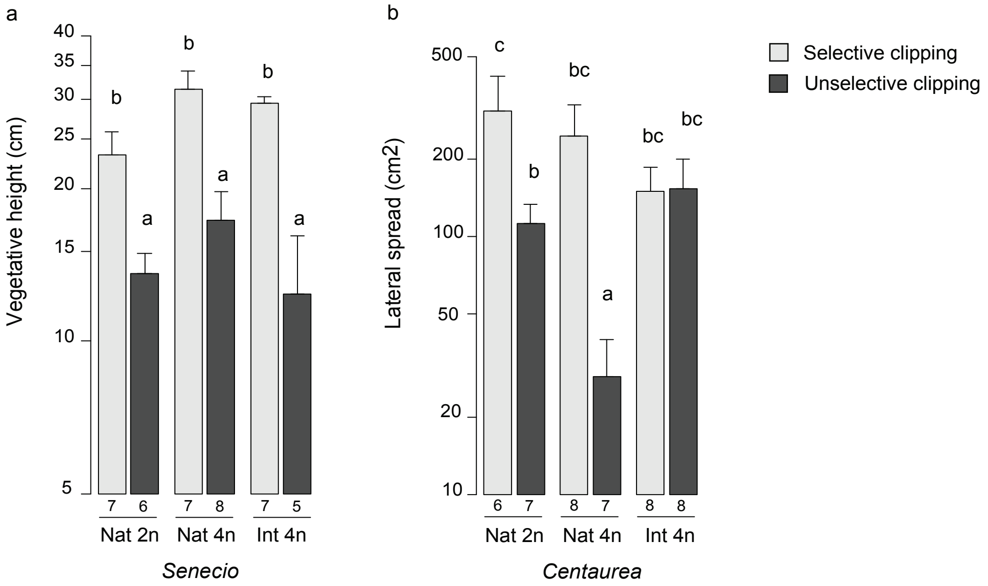

Figure 3: Vegetative size of geocytotypes after the first growing season (Nat 2n: native diploids, Nat 4n: native tetraploids and Int 4n: introduced tetraploids) of *S. inaequidens* and *C. maculosa* according to the management treatment, *i.e* selective (light grey) or unselective (black) clipping. Vegetative size corresponds to vegetative height (cm) for *S. inaequidens* (a) and lateral extension of the canopy (cm^2) for *C. maculosa* (b). Numbers on the x-axis indicate the number of observations. Bars indicate the standard error of the mean. Letters indicate significant differences ($p < 0.05$) according to Tukey post-Hoc tests. Response variables are on a logarithmic scale.

Table 2: Results of the linear mixed models testing for the effects of management (selective *vs*. unselective clipping), geocytotype (native diploid, native tetraploid or introduced tetraploid) and their interaction on vegetative size, at the end of the first vegetation season

	Year 1		
	Df	*F*-value	*P*-value
Centaurea maculosa			
Block level			
Management	1	8.564	**0.011**
Residual	14		
Plot level			
Geocytotype	2	3.390	**0.051**
Geocytotype:Management	2	6.689	**0.005**
Residuals	24		
Senecio inaequidens			
Block level			
Management	1	21.923	**<0.001**
Residual	14		
Plot level			
Geocytotype	2	1.676	0.212
Geocytotype:Management	2	2.375	0.119
Residuals	20		

Table 3: Results of the linear models testing the effects of species (*C. maculosa* vs. *S. inaequidens*), geocytotype (native diploid, native tetraploid or introduced tetraploid) and their interaction on shoot biomass, root biomass and root-shoot ratio at the end of the second vegetation season

		Shoot biomass		Root biomass		Root-shoot ratio	
	Df	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Species	1	0.385	0.551	0.847	0.381	0.550	0.477
Geocytotype	2	0.280	0.762	0.040	0.961	1.185	0.349
Species:Geocytotype	1	0.019	0.893	0.000	0.985	0.002	0.967
Residuals	9						

Overall, during the first vegetation season, all geocytotypes of *S. inaequidens* were affected by the management treatment whereas only native genotypes of *C. maculosa* responded to the management treatment (Figure 4, tables 1 and 2). Furthermore, tetraploid genotypes of *S. inaequidens* tended to be more affected by the management treatment than diploid ones.

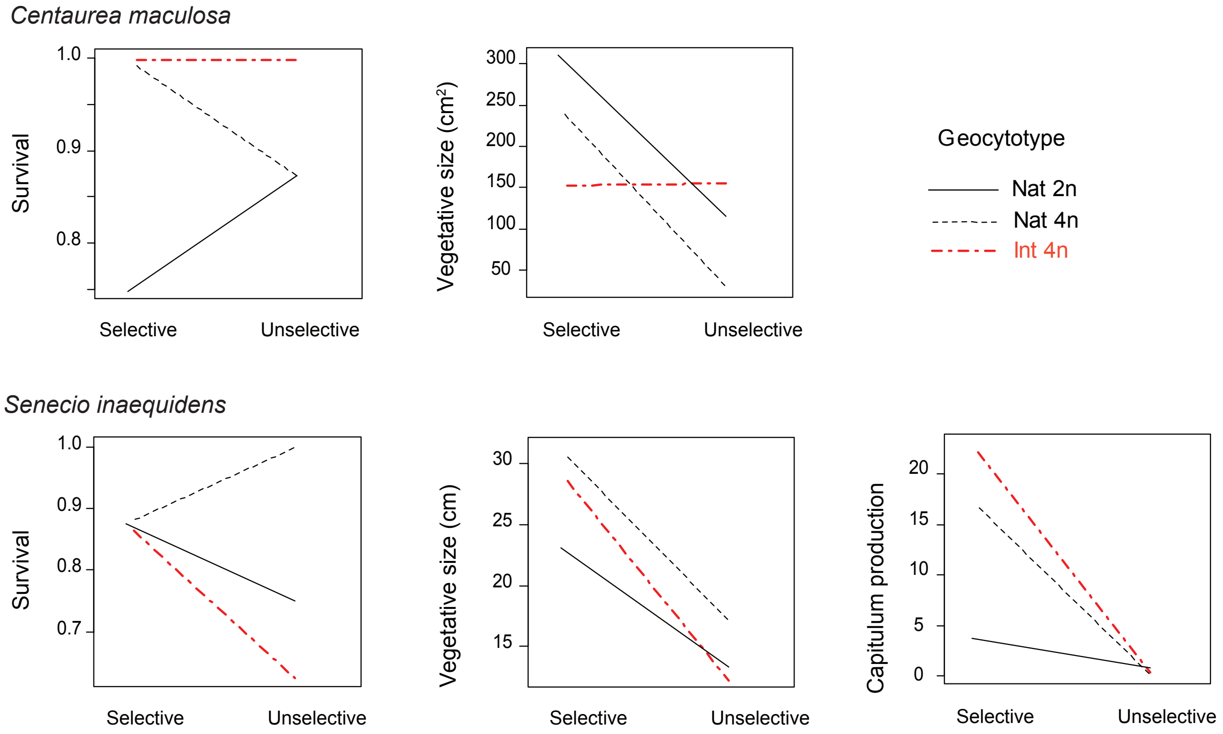

Figure 4: Interaction plots of survival, vegetative size and capitulum production at the end of the first vegetation season for native diploid (Nat 2n: solid lines), native tetraploid (Nat 4n: dashed line) and introduced (Int 4n: red dotdashed line) genotypes of *C. maculosa* (top) and *S. inaequidens* (bottom) according to disturbance treatment (selective *vs.* unselective clipping). Vegetative sizes of *C. maculosa* and *S. inaequidens* are respectively the lateral expansion of the canopy (cm^2) and the vegetative height (cm).

3.4. Discussion

The aim of the experiment was to disentangle inherent superiority of exotic species from the effects of polyploidisation and release from competition following a disturbance on invasion success of two worldwide invasive species, *Centaurea maculosa* and *Senecio inaequidens*. Our study shows that polyploidisation and competition release following disturbance increased the performance of both species but through effects on different traits.

Mowing the neighbouring vegetation around the target species (*i.e.* selective disturbance), by releasing for a while the target individuals from neighbouring competition enhanced survival, growth and reproductive output of all geocytotypes of *S. inaequidens*. This result indicates that ecological processes, and more precisely a release of resource such as light, have a predominant effect on early establishment success of *S. inaequidens* (Milbau *et al.* 2003; Naeem *et al.* 2000), as compared to evolutionary processes. This is in accordance with the fluctuating resource theory hypothesis, which argues that a species has the ability to establish in a community whenever a resource release coincides with the availability of propagules, independently of the species invasiveness (Davis *et al.* 2000). Thus, a stochastic event like a temporary release from competition for light can promote fitness of *S. inaequidens* and be determinant for its further development and spread in the recipient community (Davis *et al*. 2000; Davis and Pelsor 2001; Emery and Gross 2007; Milbau *et al.* 2005). The increase in vegetative height and capitulum production following mowing of the neighbouring vegetation (*i.e.* selective disturbance) was higher for tetraploid genotypes of *S. inaequidens* than diploid ones. Since propagule pressure seems to be critical for the invasion of *S. inaequidens* (Thébault *et al.* submitted), this asymmetric response to competition release supports the idea that the invasive success of this species is likely to be explained by an evolutionary ability to take advantage from an ecological process such as the release from competition for light (Bossdorf *et al.* 2005; Richards *et al.* 2006). This ability could allow a reinvestment of resources into reproductive output, leading to an increase in capitulum production and therefore a higher propagule pressure.

Geocytotypes of *C. maculosa* benefited differently from disturbance as revealed by the interaction between management treatment and geocytotypes for lateral extension of the basal rosette. Only native genotypes benefited in terms of growth and survival from a release from competition following the clipping of neighbouring vegetation (Figure 4). This result suggests that the introduced genotypes of *C. maculosa* were not influenced by light competition, contrarily to the native ones. An inherent superiority of introduced genotypes of *C. maculosa* to cope with neighbouring competition might therefore explain their invasive success, rather than an ecological process such as stochastic resource release. Since clipping neighbouring vegetation released *C. maculosa* from light competition, we suggest that introduced genotypes had the ability to cope with competition for light, which may be particularly useful for such a plant with basal rosettes that has low access to light. This ability to cope with aboveground competition might allow a reinvestment of resources from aboveground growth to reproduction or belowground growth. Under selective clipping, introduced genotypes of *C. maculosa* produced significantly less capitulum than native ones. This result confirms the finding that *C. maculosa* invests less in reproduction in the introduced range than in the native one (Thébault *et al.* submitted). Therefore, it seems more probable that the reinvestment of resources is oriented towards higher resource allocation to belowground tissues. Accordingly to the already described allelopathic ability of *C. maculosa* (Perry *et al.* 2005; Ridenour and Callaway 2001; Weir *et al.* 2003), this trade-off from aboveground growth and reproduction to investment in belowground tissues could be associated with the release of exudates which may affect neighbouring vegetation as described in the novel weapon hypothesis (Callaway and Ridenour 2004; Callaway and Vivanco 2007). However, only a precise study of compounds released into the soil could confirm this hypothesis.

During this two-vegetation season experiment, unselective clipping was more damaging for target fitness than selective clipping. From a management point of view, mowing, by limiting survival, growth and reproductive output, should be considered as a useful tool to limit spread of both *C. maculosa* and *S. inaequidens*.

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Chapter 4

Relationships among range, polyploidisation, plant traits and rhizosphere microorganisms of invasive species *Centaurea maculosa* and *Senecio inaequidens*

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Abstract

Invasive plant species represent a threat to terrestrial ecosystems but their effects on the soil biota and the mechanisms involved are not yet well understood. We studied the effects of different genotypes and ranges (diploid native, tetraploid native and introduced) of *Centaurea maculosa* and *Senecio inaequidens* on microbial biomass carbon, total DNA content, and bacterial communities (T-RFLP profiles and Shannon-diversity index) in relation to 1) polyploidisation, 2) introduction in the new range and 3) plant functional traits.

There was no difference in microbial biomass between species and genotypes. Rhizosphere bacterial diversity was higher in *C. maculosa* than in *S. inaequidens*. The variation of total DNA content and bacterial diversity according to range and polyploidisation was species specific. Indeed, rhizosphere DNA content of *S. inaequidens* decreased with polyploidisation in the native range but did not vary for *C. maculosa*. By contrast, rhizosphere bacterial diversity increased 1) with polyploidisation in the native range and 2) following introduction in the new range for *C. maculosa* whereas there was no significant change for *S. inaequidens*. The total bacterial communities structure differed significantly between the two species and was affected by polyploidisation for *C. maculosa* only.

Plant traits of *S. inaequidens* only were correlated to the rhizosphere biota. Bacterial diversity and total DNA content were positively correlated with resource allocation to belowground growth, while microbial biomass carbon was negatively correlated to investment in reproduction. Despite differences in leaf traits and bacterial diversity among *C. maculosa* genotypes, there were no correlation between plant traits and soil biota.

To our knowledge, this study is the first attempt to link plant traits with their rhizosphere biota, in the framework of biological invasion and polyploidisation.

Keywords: bacterial community structure, invasive species, microbial biomass carbon, plant-soil interactions, T-RFLP

4.1. Introduction

Invasive species represent a major threat to natural and managed ecosystems (Pimentel *et al*. 2000; Prieur-Richard and Lavorel 2000). Therefore understanding how they can invade ecosystems is a major challenge to ecologists (Richardson and Pysek 2006). Most work on invasion ecology has focused on the aboveground compartment (Levine *et al.* 2003 but see references in Batten *et al.* 2006), although it is recognised that soil organisms play an important role in regulating ecosystem-level processes (Wardle *et al.* 2004) and that the aboveground and belowground compartments are tightly linked (Batten *et al.* 2008). Indeed, soil microorganisms can strongly influence plant community dynamics and may contribute to the coexistence of competitive plant species or to the competitive dominance of one plant species over another (Batten *et al.* 2008). Thus changes in the soil biota could affect the resistance of plant communities to invasive species, through for example change in productivity, plant community composition or ecosystem functions (Bever 2003).

Invasive plants can also alter soil biota through diverse mechanisms such as alteration of litter quantity or quality, changes in soil physical properties and secretion of root exudates (Wardle *et al.* 2004). Recently, allelopathy has been intensively studied in the context of biological invasion as a possible mechanism through neighbouring plant inhibition (Bais *et al.* 2006; Callaway and Ridenour 2004; Hierro and Callaway 2003; Inderjit *et al.* 2006). Allelopathy is the phytotoxicity of a compound released from one plant to other susceptible plants. These compounds can be released either by root exudation, volatilization or residue composition. Since these compounds may be new in the ecosystem (*i.e.* not experienced before in the ecosystem), they may alter the soil biota (Kourtev *et al.* 2002) and impact competitive interactions both aboveground and belowground (Callaway and Ridenour 2004; Callaway and Vivanco 2007). This mechanism has been defined in the novel weapon hypothesis (Callaway and Ridenour 2004).

Many invasive species have undergone polyploidisation in the native range (Pandit *et al.* 2006; Verlaque *et al.* 2002). As an evolutionary process, polyploidisation can lead to changes in traits trade-offs (Blossey and Notzold 1995; Bossdorf *et al.* 2004) and could affect plant-soil interactions. Polyploidisation has been shown to lead to an increase in secondary metabolites in many plants species (De Jesus-Gonzalez and Weathers 2003; Dhawan and Lavania 1996; Kim *et al.* 2004). It can therefore be hypothesised that polyploidisation could affect plant soil interactions so as to give a competitive advantage to invasive species through inhibition of neighbouring plants and/or modification of the soil biota *via* increased releases of secondary compounds. By studying simultaneously native and introduced genotypes as well as diploid and tetraploid genotypes, it is possible to disentangle the effect of polyploidisation on soil biota from the effect of novelty of compounds on invasion success as suggested by the novel weapon hypothesis (Callaway and Ridenour 2004). To our knowledge, no study has attempted to link plant polyploidisation to communities of soil organisms.

Plant functional traits are known to influence ecosystem processes (Chapin *et al.* 2000; Loreau *et al.* 2001). Aboveground functional diversity has been shown to affect belowground processes (Scherer-Lorenzen 2008). Indeed, plants can strongly influence the activity of soil organisms (Hobbie 1992) *via* their ecophysiological traits such as photosynthetic rate, nutrient use, leaf properties and carbon allocation strategy (Cornelissen 1996; Cornelissen *et al.* 2003; Scherer-Lorenzen 2008). While plants with rapid growth, high rate of nutrient acquisition and high turnover of tissues promote decomposers (Cornelissen 1996), plants that allocate much of their carbon to secondary metabolites and grow more slowly have the potential to influence strongly microbial communities through release of these metabolites (Wardle 2002). Since a plant allocates resources either to vegetative growth (belowground or aboveground) or reproduction, measuring shoot biomass, root biomass, root-shoot ratio and flowering output (time of initial flowering and flowering potential) allows assessing carbon investment strategy. Specific leaf area (SLA) and leaf dry matter content (LDMC) characterise the trade-off in plant functioning between production of biomass and conservation of nutrients (Diaz *et al.* 2004). While SLA is related to short leaf retention, fast growth rate and high turnover of tissues (Cornelissen *et al.* 2003; Grotkopp *et al.* 2002), LDMC is related to defence against natural hazards and herbivory through high investment in leaf tissues (Cornelissen *et al.* 2003) and conservation of nutrients (Wright *et al.* 2004). It can therefore be expected that patterns of plant leaf traits such as SLA and LDMC are related to patterns of microbial communities and related processes in the rhizosphere. To our knowledge, no study has attempted to link plant traits to communities of soil organisms.

In this study, we used two invasive species, *Centaurea maculosa* Lam. and *Senecio inaequidens* DC., which we defined as taxonomically related since (1) they belong to the same family (Asteraceae), (2) they occupy similar ecological niches in their native range, (3) they tend to invade similar habitats in their introduced range and (4) they may both have allelopathetical effects (Ahmed and Wardle 1994; Callaway and Vivanco 2007). Furthermore, these two species have undergone polyploidisation in their native range leading to the presence of diploid and tetraploid genotypes, while only tetraploid genotypes have been observed in the introduced range (Lafuma *et al.* 2003; Treier *et al.* in press). If we define for convenience a geocytotype as a ploidy level in a given area (native or introduced range), both model species have three geocytotypes: 1) native diploid, 2) native tetraploid, and 3) introduced tetraploid. Due to this distribution pattern, these species are good models to study genetically and environmentally induced processes and their consequences on invasion success.

We performed a nine months greenhouse experiment to test for effects of different geocytotypes on rhizosphere microbial communities. We hypothesised that differences between rhizosphere characteristics (microbial biomass carbon, total DNA content, bacterial diversity as expressed as Shannon-diversity index) and bacterial communities (bacterial T-RFLP profiles) are related to polyploidisation in the native range and introduction in the new range. Since plants traits are known to influence ecosystem functioning and could lead to changes in soil microbial composition, we further hypothesised that differences in rhizosphere characteristics and bacterial community profiles among geocytotypes are explained by plant functional traits (Figure 1).

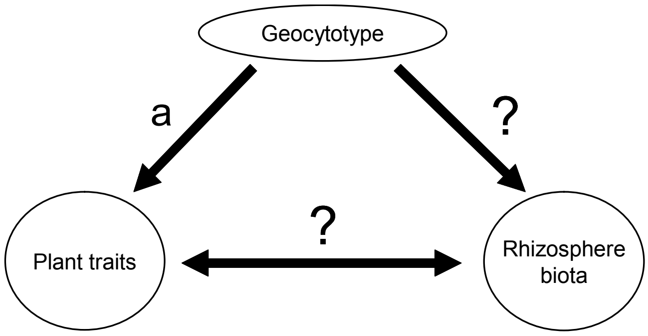

Figure 1: Theoretical linkages between geocytotypes, plant traits and rhizosphere biota of invasive species. The bidirectional arrow between plant traits and rhizosphere biota indicates possible mutual influences. The relationship between geocytotypes and plant traits (a) is described in Thébault *et al.* (*submitted*), Chapter 2.

4.2. Material and Methods

4.2.1. Model species

Centaurea maculosa Lam. is a herbaceous biennial or short-lived perennial tap-rooted forb with a rosette of basal leaves and relatively thick flowering stems (Hook *et al.* 2004). Its native range spans from Western Asia to Western Europe. The species was introduced in the Pacific Northwest of the United States in the late 1800s (Watson and Renney 1974) and expanded rapidly throughout north-western America and western Canada where it is now widespread in rangelands, pastures and on roadsides (Duncan 2001; Skinner *et al.* 2000). *Centaurea maculosa* is known for its release of (±) catechine to the soil which may be allelopathic to other plants (Bais *et al.* 2002; Callaway and Vivanco 2007; Perry *et al.* 2005; Ridenour and Callaway 2001).

Senecio inaequidens DC. is an erect perennial dwarf shrub native to South Africa and Lesotho. Originally found in grasslands from 1400 m a.s.l. to 2800 m a.s.l., it is now quite widespread in South Africa. It was accidentally introduced to Europe at the end of 1880s (Bornkamm 2002) where it is now widespread, even at lower altitudes. *Senecio inaequidens* contains pyrrolizidine alkaloids (Macel and Vrieling 2003), which are toxic to livestock (Noble *et al.* 1994). No studies have been done so far to test for impacts of *S. inaequidens* on soils communities. Despite no allelopathy effect has been mentioned for *S. inaequidens*, we can strongly believe in the allelopathetic potential of this species since it has already been demonstrated for another *Senecio* species (Ahmed and Wardle 1994).

4.2.2. Seed collection

Seeds of *C. maculosa* were collected during summer 2005 throughout its native range in Europe as well as in Montana and Oregon, USA (Broennimann *et al.* 2007; Treier *et al.* in press). On the whole, 18 populations were randomly chosen for this study from 97 available

Table 1: *Centaurea maculosa* and *Senecio inaquidens'* populations sampled in their native and introduced range used for this study. Populations not used in the T-RFLP analyses are marked with an *.

Collection Site	Country	Habitat	Ploidy	Elevation (m-asl)	Latitude	Longitude
Centaurea maculosa						
Native range (Europe)						
Hainburg	Austria	Natural dry meadow	2n	326	49.9'N	16.58'E
Bovshev	Ukrainia	Natural dry meadow	2n	296	49.13'N	24.42'E
Chutove *	Ukrainia	Natural dry meadow	2n	131	49.4'N	34.57'E
Czortova	Ukrainia	Natural dry meadow	2n	305	49.24'N	24.4'E
Basel	Switzerland	Railway tracks	2n	298	47.33'N	7.39'E
Pècs	Hungary	Roadside	4n	496	46.6'N	22.13'E
Khotyn	Ukrainia	Roadside/Pasture	4n	248	48.31'N	26.28'E
Introduced range (United States- US)						
Montana	Montana – US	unknown	4n	1146	47.00'N	113.23'O
Montana *	Montana – US	unknown	4n	804	47.19'N	114.18'O
Montana	Montana – US	unknown	4n	1384	45.5'N	113.56'O
Oregon	Oregon – US	unknown	4n	762	44.34'N	121.25'O
Oregon *	Oregon - US	unknown	4n	1279	43.42'N	121.3'O
Senecio inaequidens						
Native Range (South Africa (SA) and Lesotho)						
Bultfontein	Free State - SA	Roadside	2n	1340	28.16'S	26.08'E
Memel *	Free State - SA	Roadside	2n	1741	27.41'S	29.35'E
God's Window	Mpumalanga - SA	Roadside	2n	1550	24.50'S	30.50'E
Graskop	Mpumalanga - SA	Residential area	2n	1450	24.58'S	30.49'E
Mt. Lebanon I *	KwaZulu-Natal - SA	Pasture	2n	1800	29.35'S	29.40'E
Joel's Drift	Lesotho	Roadside/Pasture	2n	2000	28.46'S	28.25'E
Golden Gate I	Free State - SA	River Bed	4n	2150	28.31'S	28.25'E
Golden Gate II	Free State - SA	Rocks	4n	2050	28.31'S	28.25'E
Semonkong	Lesotho	River Bed	4n	2200	29.49'S	28.03'E
Introduced range (Europe)						
Hamburg	Germany	Car park	4n	11	53.33'N	10.00'E
Basel	Switzerland	Railway tracks	4n	254	47.33'N	7.36'E
Weissenfels *	Germany	Railway tracks	4n	159	51.12'N	11.58'E
Denges	Switzerland	Ruderal vegetation	4n	-	49.31'N	6.32'E
Lieges	Belgium	unknown	4n	-	58.38'N	5.34'E
Mazamet	France	unknown	4n	-	43.29'N	2.22'E

populations: eight from native populations (six from diploid and two from tetraploid populations) and 10 from introduced populations known to be only tetraploids. Within populations, seeds were randomly chosen among a pool of seeds gathered on 16 mother plants.

Seeds of *S. inaequidens* (mostly provided by Dr. Daniel Prati, University of Leipzig-Halle and Sandrine Maurice, Institut des Sciences de l'Evolution Montpellier) were collected in Western Europe, South Africa and Lesotho (Lafuma *et al.* 2003). For the experiment, 28 populations were randomly taken from 89 available populations: seven native diploids, six native tetraploids and 15 introduced tetraploids. For each population, seeds were randomly taken from two mother plants. All seeds within each geocytotype of each species were then mixed together.

4.2.3. Pot experiment

A nine-months pot experiment was set-up in the greenhouse of the University of Lausanne, Switzerland. On February 2006, 180 seeds of each model species were sown in germination trays filled with sieved garden soil commonly used for seedbeds, half of the seeds coming from invasive populations and half from native populations. After one month, surviving seedlings (330 out of 360) were transplanted into 1 L pots. Each pot was filled with standard compost (Ricoter SA., Switzerland) and contained one individual. Pots were randomly arranged on tables and watered every 2-4 days. For bio-security reasons and in order to prevent seed set, capitula of all plants were cut regularly during the experiment. Nine months after sowing seven replicates of each geocytotype were selected randomly among the pool of living individual to test for differences in rhizosphere characteristics. It turned out that for *Centaurea maculosa*, we had 5 populations of native diploid, 2 populations of native tetraploid and 5 populations of introduced tetraploid genotypes. For *Senecio inaequidens*, we ended with 6 populations of native diploid, 3 populations of native tetraploids and 3 populations of introduced tetraploid genotypes (Table 1).

4.2.4. Plant trait measurements

The detailed study on plant traits was presented elsewhere (Thébault *et al.* submitted). These data are included in this study to assess the relationships among geocytotype, rhizosphere characteristics and plant traits. Two reproductive traits were monitored during the experiment to measure the *onset of flowering* and the *capitulum production*, respectively: 1) the number of days between sowing and the appearance of the first capitulum and 2) the total number of capitula produced by flowering plants during the experiment. On each individual, five leaves were taken randomly among the healthy, fully developed leaves for measurements of total leaf area and fresh biomass (Cornelissen *et al.* 2003). Total leaf area was measured using a LI-3100C Leaf Area Meter (Li-COR, Lincoln, Nebraska, USA). Leaves were dried at 60°C for 72 h and weighed for dry mass. *Specific leaf area* (*SLA*, leaf area per unit of leaf dry mass in m^2 kg^{-1}

and *leaf dry matter content* (*LDMC*, leaf dry mass per unit of leaf fresh mass in mg g^{-1}) were calculated for each plant as the mean of measurements on the five harvested leaves. Selected plants were then entirely harvested and separated into *shoot* and *root*, dried at 35°C for seven days (to allow potential chemical analyses), and weighed together with the leaves collected for leaf trait measurements. *Root-shoot ratio* (*RSR*) was calculated as the ratio between root and shoot dry mass.

4.2.5. Microbial measurements

Microbial carbon biomass was assessed using chloroform fumigation- K_2SO_4 extraction (Brookes 1985; Vance *et al.* 1987). After chloroform fumigation, microbial biomass carbon was determined by extraction of 25 mg fresh soil by 25 ml of a 0.5M solution of K_2SO_4. Briefly, soil samples were fumigated with $CHCl_3$ for 24 h at 25 °C. After removal of the $CHCl_3$, soluble C was extracted from fumigated and un-fumigated samples with 25 ml 0.5 M K_2SO_4 for an hour on an orbital shaker. Total organic C in filtered extracts (Whatman No. 1) was determined using a total organic carbon analyser (Shimadzu TOC 5000). Microbial C flush (difference between extractable C from fumigated and un-fumigated samples) was converted to microbial biomass C using a k_{EC} conversion factor of 0.45 (Wu *et al.* 1990).

4.2.6. Rhizosphere soil DNA

Nine months-old plants were taken from planting pots and shaken carefully to remove the nonadhering soil. A brush was used to remove gently the adhering rhizosphere soil from plant roots, which was passed through a 1mm sieve and stored in DNA extraction buffer (Frey *et al.* 2006) at -20 C until analysis. Soil DNA of rhizosphere samples were prepared by a bead beating procedure (Frey *et al.* 2008). Briefly, 0.5 g of soil (dry weight) was subjected to three repeated extractions using a bead beater (FP 120; Savant Instruments, NY). Supernatants from all three extractions were pooled and subsequently purified with a chloroform-isoamyl alcohol (24/1) extraction. DNA was recovered after ethanol precipitation and re-suspended in TE buffer, pH 8 (10 mM Tris-HCl, 0.1 mM EDTA, pH 8). DNA concentrations were determined using a fluorometric assay with PicoGreen (Molecular Probes, Eugene, OR). DNA concentration was adjusted to 10 ng ul^{-1} with Tris-EDTA. Before PCR, soil DNA was pretreated with bovine serum albumin to bind humic acids and other PCR-inhibiting substances.

4.2.7. Amplification of bacterial 16S rRNA gene fragments.

Bacterial 16S ribosomal RNA genes were amplified by the PCR using fluorescently labelled (6-FAM) forward primer 27f and unlabeled reverse primer 1378r (Heuer *et al.* 1997) in a total volume of 50 ul reaction mixture containing 20 ng of template DNA, 1 x PCR-buffer (Qiagen, Hilden, Germany), 2mM $MgCl_2$, 0.2 uM of each primer, 0.4mM deoxynucleoside triphosphate (Promega), 0.6 mg ml^{-1} BSA (Fluka, Buchs, Switzerland), and 2 U HotStar *Taq* polymerase (Qiagen). PCR amplification was performed with the following cycling conditions: an initial activating step for HotStar Taq-polymerase (15 min at 95°C), followed by 35 cycles with denaturation for 45 s at 94°C, annealing for 45 s at 48°C, and extension for 2 min at 72°C, with final extension for 5 min at 72°C. The PCR amplification was then ended by an additional final extension step at 72°C for 5 min. Amplified DNA was verified by electrophoresis of aliquots of PCR mixtures (5 ul) on a 1% agarose gel in 1% TAE buffer.

4.2.8. Terminal restriction enzyme fragment length polymorphism analysis (T-RFLP)

Following confirmation of successful PCR reaction by agarose gel electrophoresis, the PCR products were purified with the Montage PCR purification cleanup kit (Millipore Corporation, Billerica, MA). Purified PCR products were digested with 2U of the restriction endonuclease *MspI* or *HaeIII* (Promega) and incubated overnight at 37°C. Aliquots (5 ul) of digestion products were verified on a 2% agarose gel in 1% TAE buffer. Prior to the T-RFLP analysis, digests were desalted with Montage SEQ96 sequencing reaction cleanup kit (Millipore Corporation, Billerica, MA), according to the manufacturer's instructions. T-RFLP analyses were performed according to Frey *et al.* (2006). Two microliters of digested PCR products was analyzed along with 0.2 ul of internal size standard ROX500 (Applied Biosystems, Foster City, USA) and 12 ul HiDi formamide (Applied Biosystems, Foster City, CA) on an ABI Prism 310 Genetic Analyzer (Applied Biosystems) with 36-cm capillaries filled with POP-4 polymer. T-RFLP profiles were analyzed using Genotyper v3.7 NT (Applied Biosystems) with a signal threshold of 50 relative fluorescence units. Normalization of T-RFLP profiles was performed according to Blackwood *et al.* (2003).

We used the Shannon-Weaver index (H) to assess bacterial diversity based on TRF peaks using number and size of TRF peaks for each profile according to the equation:

$$H = C/N \,.\, (N.\mathrm{Log}_{10} N - \sum n_i .\, \mathrm{Log}_{10} n_i) \qquad (1)$$

where C = 2.3, N = sum of peak heights, n_i= height of TRF i and i= number of TRFs in each T-RFLP profiles (Brodie *et al.* 2003).

4.2.9. Numerical analyses

All the analyses were carried out with R 2.7.2 (R Development Core Team, 2008). In a first step, we looked for outliers using boxplots and principal component analysis. One outlier was removed for *C. maculosa* microbial carbon. Due to time constraints, analyses of DNA content and T-RFLP were only done on 13 individuals of *C. maculosa* (respectively 4 native diploids, 4 native tetraploids and 5 introduced tetraploids) and 17 individuals of *S. inaequidens* (respectively 5 native diploids, 6 native tetraploids and 6 introduced tetraploids). Despite an unbalanced design, we decided to keep all individuals in the statistical analyses.

Microbial biomass carbon, DNA content and Shannon-diversity index were analysed using

Analysis of Variance (ANOVA). Since we wanted to disentangle the effects of polyploidisation from the effects of introduction in the new range, ploidy and range were specified as main factors. Whenever results of ANOVA were significant, Tukey post-hoc tests were performed to assess significant differences between factor levels. Analyses were done first on both species together to test for species differences as well as interactions between species and range and species and ploidy. Since effects of polyploidisation or introduction in the new range can be species specific, we then performed separate analyses for each model species. To reduce heteroscedasticity, rhizosphere total DNA content and Shannon-diversity index were log-transformed.

To study the multivariate response of T-RFLP profiles, we first performed a redundancy analysis (RDA) on the entire dataset to get an overview of the main factors constraining rhizosphere bacterial T-RFLP profiles. A series of redundancy analyses which constrained bacterial profile by species, ploidy, range, combinations of species and ploidy, and species and range were performed to assess differences in bacterial profiles according to these factors. Permutation tests were used to assess the significance of these multivariate regression models. Since species appeared to be a main factor of change, we then studied both species separately, using non-metric multidimensional scaling analyses (NMDS) on chord distance matrix to look at distribution of species' rhizosphere bacterial T-RFLP profiles. NMDS was chosen since it preserves ordering relationships among objects (Legendre and Legendre 1998) while representing them in a few dimensions. Redundancy analyses and subsequent permutation tests were performed to test effects of geocytotype, range and ploidy on rhizosphere bacterial T-RFLP profiles of the two species.

Lastly, we performed multifactorial analyses (MFA) to get an overview of the correlations between plant identity, plant traits, rhizosphere characteristics and rhizosphere bacterial T-RFLP profiles of invasive species. We built four data matrices. One matrix contained information about individuals' identity (species and geocytotype). A second matrix contained data on vegetative (shoot and root biomass, root-shoot ratio, SLA, LDMC) and reproductive (onset of flowering, capitulum production) traits of the individuals growing in the pot. The two last matrices contained information on individual's rhizosphere characteristics. While one matrix contained results from T-RFLP bacterial profiles, the other one contained quantitative data on rhizosphere microbial biomass carbon, total DNA content and the bacterial diversity as expressed by the Shannon index. We first considered all individuals in the multifactorial analysis to assess global correlations between groups of variables. We used only the plant traits and rhizosphere biota matrices, adding plant identity matrix afterwards as passive information. In a second step, we did separate MFA for each model species, keeping plant identity (reduced to geocytotype in this case) as passive information. Significance of the correlations between the matrices was tested using Mantel tests. Since we found a correlation between plant traits of *Senecio inaequidens* and biota characteristics of the rhizosphere, we performed a RDA constraining microbial biomass carbon, DNA content and Shannon-diversity index by plant traits. We performed a forward selection and used AIC criteria to get the best model.

4.3. Results

4.3.1. Microbial biomass carbon, total DNA content and bacterial diversity in the rhizosphere

Microbial biomass carbon in the rhizosphere ranged from 0.9 to 1.5 mg C g^{-1} dry soil. There were no significance differences between species, ranges, or ploidy levels (Table 2 and 3, Figure 2a). Rhizosphere DNA content ranged from 200 to 450 µg DNA g^{-1} dry soil. Overall, the DNA content was significantly lower in the rhizosphere of tetraploid plants than in the rhizosphere of diploid plants in the native range (Table 2).

Table 2: Analysis of variance on rhizosphere microbial biomass carbon (n=41), total rhizosphere DNA content (n=30) and bacterial diversity represented by Shannon-diversity index (n=30). DNA content and bacterial diversity were log-transformed prior to the analyses.

		Microbial biomass C			DNA content			Bacterial diversity	
	d.f.	*F*-value	*P*-value	d.f.	*F*-value	*P*-value	d.f.	*F*-value	*P*-value
Species	1	1.141	0.293	1	0.037	0.850	1	4.519	**0.044**
Ploidy	1	0.093	0.762	1	4.571	**0.043**	1	0.000	0.999
Range	1	3.274	0.079	1	1.486	0.235	1	1.303	0.265
Species*Ploidy	1	0.306	0.584	1	1.871	0.184	1	2.459	0.130
Species*Range	1	0.264	0.611	1	1.423	0.244	1	0.030	0.864
Residuals	35			24			24		

However, when analyses were done separately on each species, only *Senecio inaequidens* presented a decrease in rhizosphere DNA content following polyploidisation in the native range (Table 3, Figure 2b). Whatever the species considered, there was no variation in rhizosphere DNA content according to range (Tables 2 and 3). The rhizosphere bacterial Shannon-diversity index was higher for polyploid and introduced plants than for native diploid ones for *C. maculosa*, but not for *S. inaequidens* (Table 3, Figure 2c).

Table 3: Analysis of variance on microbial biomass carbon, DNA content and rhizosphere bacterial diversity (Shannon-diversity index) for *C. maculosa* and *S. inaequidens*. DNA content and bacterial diversity were log-transformed prior to the analyses.

		Microbial biomass C			DNA content		Bacterial diversity	
	d.f.	*F*-value	*P*-value	d.f.	*F*-value	*P*-value	*F*-value	*P*-value
Centaurea maculosa								
Ploidy	1	0.042	0.841	1	0.713	0.418	7.063	**0.024**
Range	1	2.522	0.131	1	0.027	0.872	4.404	**0.062**
Residuals	17			10				
Senecio inaequidens								
Ploidy	1	0.415	0.527	1	4.012	**0.065**	0.028	0.871
Range	1	0.913	0.352	1	1.955	0.184	1.018	0.330
Residuals	18			14				

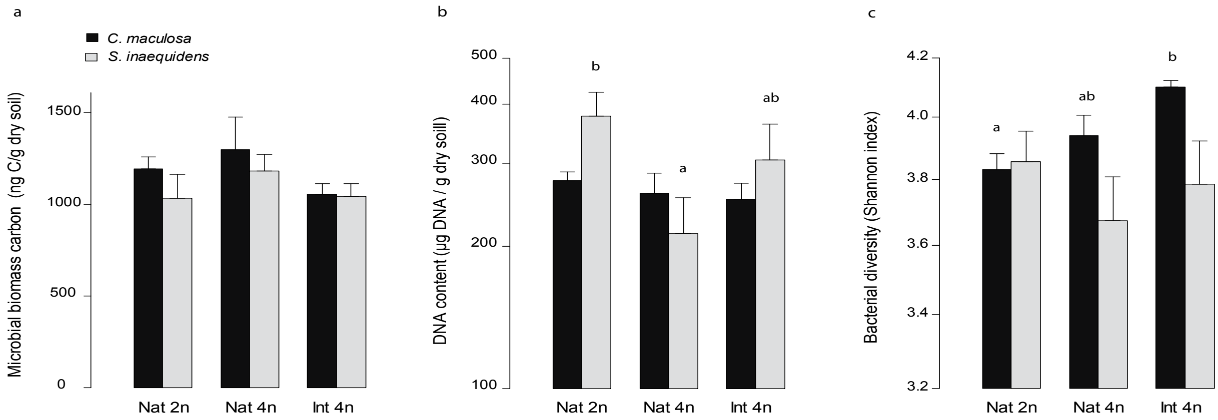

Figure 2: Microbial biomass carbon (2a), total DNA content (2b) and bacterial diversity (2c) in the rhizosphere of geocytotypes (native diploid "Nat 2n", native tetraploid "Nat 4n" and introduced tetraploid "Int 4n") of *C. maculosa* (black) and *S. inaequidens* (grey). Bars indicate the standard errors of the means. DNA content is represented on a logarithmic scale.

4.3.2. T-RFLP profiling

Rhizosphere bacterial communities differ significantly between the two plant species as shown by the RDA (Table 4). Furthermore, the differentiation of T-RFLP profiles according to polyploidisation is species specific, as indicated by the significant interaction term between plant species and ploidy (Table 4). By contrast, the interaction between species and range is not significant.

While T-RFLP profiles of *C. maculosa* are clearly differentiated according to geocytotype and polyploidisation (Figure 3a) along the first dimension of the NMDS, the pattern is less clear for *S. inaequidens* (Figure 3b). This is confirmed by the results of permutation tests performed on RDAs constraining T-RFLP profiles by geocytotype, ploidy or range, which show that the differentiation of bacterial profiles according to geocytotype or ploidy is significant for *C. maculosa* but not for *S. inaequidens* (Table 5). Whatever the species considered, the range (native *vs.* introduced) does not influence T-RFLP profiles significantly (Table 5).

Factor	Model *P*-value	% var. explained	*F*-value	*P*-value
Species	0.04	5.48	F(1,28) = 1.6325	**0.03**
Ploidy	0.29	3.75	F(1,28) = 1.0946	0.28
Range	0.29	3.75	F(1,28) = 1.0911	0.28
Sp x Ploidy	0.03	9.9	F(2,27) = 1.4214	**0.04**
Sp x Range	0.09	12.8	F(3,26) = 1.2156	0.11

Table 4: Result of RDAs' Monte-Carlo permutation tests (1999 permutationtests) foreach constraining variables and interactions.

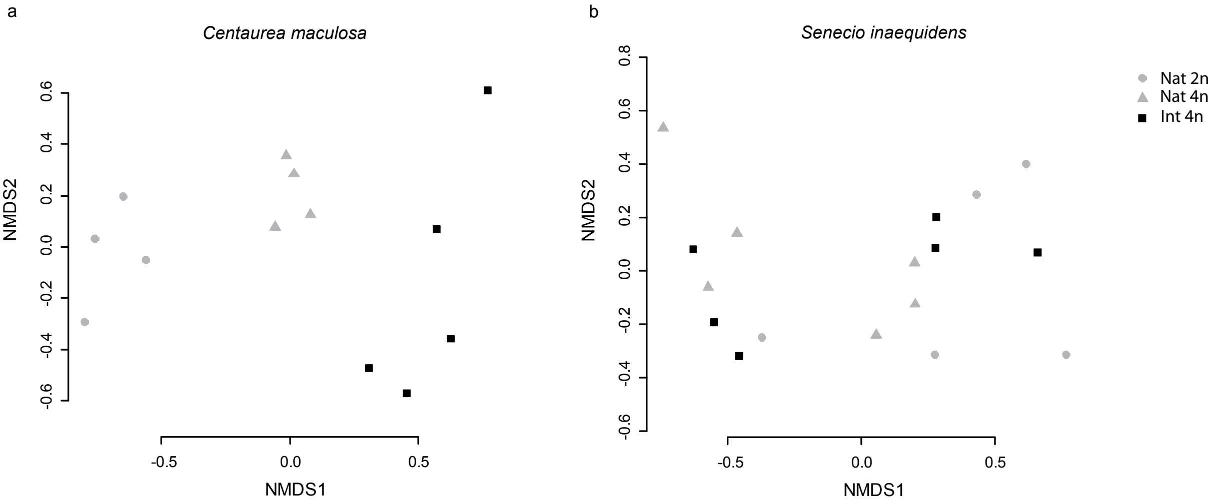

Figure 3: Non-metric multidimensional scaling analysis of T-RFLP profiles of native diploid (“Nat 2n”), native tetraploid (“Nat 4n”) and introduced tetraploid (“Int 4n”) geocytotypes of *C. maculosa* (3a) and *Senecio inaequidens* (3b). Stress values are respectively 3.74 and 10.94.

Table 5: Results of Monte-Carlo permutation tests (1999 permutations) following redundancy analysis done on T-RFLP profiles of each species, testing one factor each time.

Factor	Model *P-value*	% var. explained	*F-value*	*P-value*
Centaurea maculosa (n=13)				
Geocytotype	0.02	23.43	F(2,10) = 1.5312	**0.02**
Ploidy	0.02	13.73	F(1.11) = 1.7433	**0.03**
Range	0.09	11.27	F(1.11) = 1.394	**0.09**
Senecio inaequidens (n=17)				
Geocytotype	0.45	12.4	F(2,14) = 0.0995	0.45
Ploidy	0.35	6.44	F(1,15) = 1.0328	0.37
Range	0.59	5.67	F(1,15) = 0.903	0.58

4.3.3. Correlations among plant traits, rhizosphere biota characteristics and plant identity

The multiple factorial analysis (MFA) and redundancy analysis (RDA) combining species traits and rhizosphere biota characteristics are presented here. The detailed results on plant traits are presented elsewhere (Thébault *et al.* submitted). In the MFA combining bacterial T-RFLP profiles, microbial biomass carbon, DNA content, bacterial diversity, plant traits and plant individual identity (species and geocytotype), *Centaurea maculosa* and *Senecio inaequidens* were well separated, but the differentiation according to geocytotype was less clear (not illustrated). Nevertheless, plant identity (species and geocytotype) is significantly correlated to plant traits and rhizosphere bacterial community (Table 6). However, rhizosphere T-RFLP profiles and other biota characteristics are not correlated, indicating that changes in bacterial profiles do not lead to changes in microbial biomass carbon, DNA content or bacterial diversity. Plant traits were significantly correlated with rhizosphere characteristics such as microbial biomass carbon, DNA content or bacterial diversity but not to rhizosphere bacterial T-RFLP profiles (Table 6).

	T-RFLP profiles	Rhizosphere characteristics	Plant traits	Plant identity
T-RFLP profiles	1.000			
Rhizosphere characteristics	0.112	1.000		
Plant traits	0.107	**0.225 ****	1.000	
Plant identity	**0.210 ***	**0.169 ***	**0.322 *****	1.000

Table 6: Table of correlations between matrices used in the MFA. Significance of correlation is indicated as follows : * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

As for the global analysis, we observed no correlations between bacterial T-RFLP profiles and other rhizosphere biota characteristics, or between species traits and bacterial T-RFLP profiles for either *C. maculosa* or *S. inaequidens* (Table 7). In the MFA of *C. maculosa,* plant traits were neither correlated to geocytotypes nor to rhizosphere bacterial communities (Table 7). In agreement with results presented above, rhizosphere T-RFLP profiles were significantly correlated to geocytotype (Table 7), but there were no correlations between other rhizosphere biota characteristics and geocytotype. By contrast, *S. inaequidens* plant traits were significantly correlated to rhizosphere biota characteristics such as microbial carbon biomass, DNA content or bacterial communities, but there were no significant correlation between geocytotypes and plant traits, rhizosphere T-RFLP profile or microbial content (Table 7). In the RDA on rhizosphere microbial content, plant traits explained 54.3 % of the variance ($F_{5,11}$= 2.61, p=0.014, 999 Monte-Carlo permutations). Bacterial diversity and DNA content of the rhizosphere were positively correlated with late flowering and high allocation of resource to root biomass and weakly also to capitulum production for *S. inaequidens*. By contrast, capitulum production of *S. inaequidens* was negatively correlated with microbial biomass carbon (Figure 4). Microbial biomass carbon was also positively correlated to LDMC and negatively to root-shoot ratio.

	T-RFLP profiles	Rhizosphere characteristics	Plant traits	Geocytotype
Centaurea maculosa				
T-RFLP profiles	1.000			
Rhizosphere characteristics	0.110	1.000		
Plant traits	0.220	0.047	1.000	
Geocytotype	**0.754** ***	0.190	0.174	1.000
Senecio inaequidens				
T-RFLP profiles	1.000			
Rhizosphere characteristics	0.268	1.000		
Plant traits	0.162	**0.297** *	1.000	
Geocytotype	0.223	0.127	0.063	1.000

Table 7: Table of correlations between matrices used in the Multiple Factorial Analyses on *C. maculosa* and *S. inaequidens*. The significance of correlations is indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

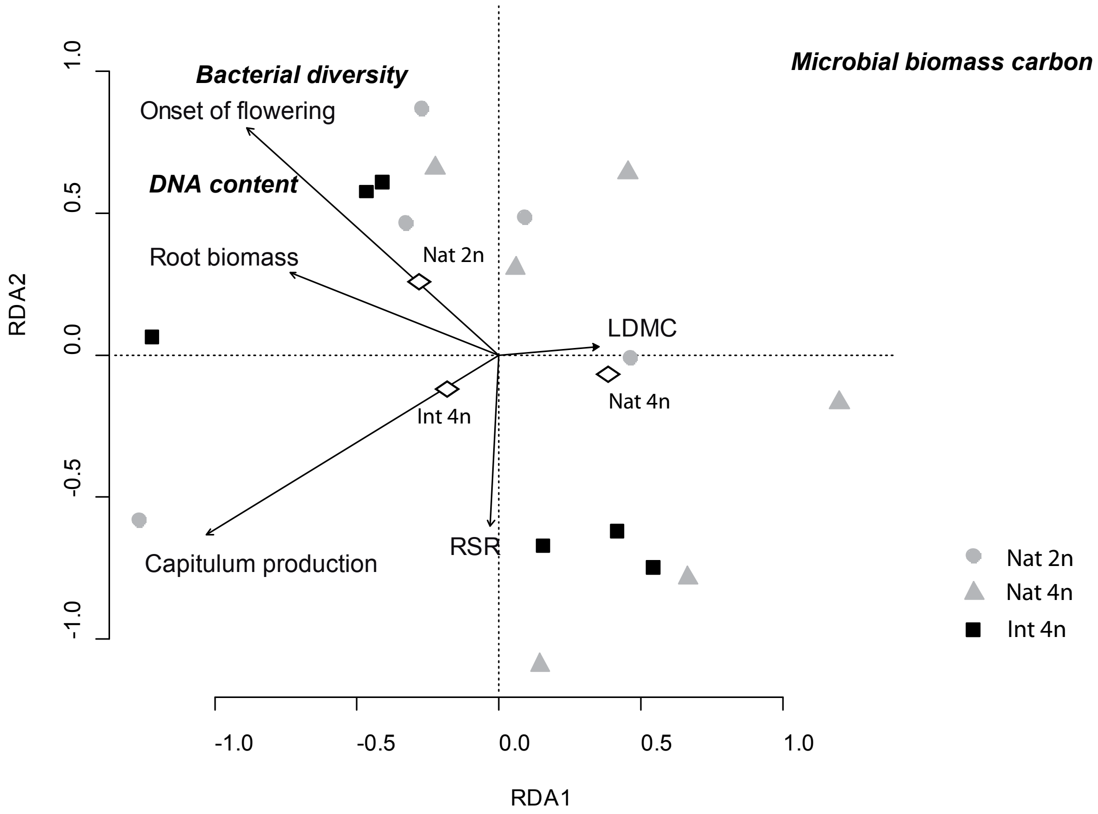

Figure 4: Redundancy analysis biplot of *S. inaequidens* rhizosphere biota characteristics constrained by plant traits. Symbols represent native diploid ("Nat 2n"), native tetraploid ("Nat 4n") and introduced tetraploid ("Int 4n") geocytotypes. Diamonds represent centroids of geocytotypes. Axes 1 and 2 hold respectively 35.9 % ($F_{1,11} = 8.64$, $p = 0.029$) and 15.5 % ($F_{1,11} = 3.74$, $p = 0.27$) of explained variance.

4.4. Discussion

The main goal of this study was to test for effects of different geocytotypes on rhizosphere communities of *Centaurea maculosa* and *Senecio inaequidens*. We hypothesised that differences between rhizosphere characteristics (microbial biomass carbon, total DNA content and bacterial diversity expressed as Shannon-diversity index) and rhizosphere bacterial communities (bacterial T-RFLP profiles) are related to polyploidisation in the native range and introduction in the new range. We further hypothesised that differences in rhizosphere characteristics and bacterial community profiles among geocytotypes are explained by plant functional traits.

We did not observe any difference in microbial biomass carbon between species or geocytotypes. The absence of variation in microbial biomass carbon linked to geocytotypes growth shows that polyploidisation does not affect overall rhizosphere microbial biomass carbon and that introduced genotypes do not inhibit overall microbial growth as compared to native ones. However, this result does not give information on potential effects on the structure of bacterial communities.

We observed significant effects of polyploidisation on bacterial communities. To our knowledge, this is the first time that polyploidisation of vascular plants has been shown to induce changes in rhizosphere bacterial community structure. However, these effects were species-specific. Indeed, polyploidisation led to a decrease in rhizosphere DNA content in the rhizosphere of *S. inaequidens*, while in the case of *C. maculosa* it led to change in bacterial T-RFLP profiles and increase in Shannon-diversity index. This suggests that polyploidisation affected mainly rhizosphere bacterial communities in the case of *C. maculosa*, whereas it probably affected other microbes (*e.g* fungi, protists) more than bacteria in the case of *S. inaequidens*.

Since polyploidisation has been shown to increase the production of secondary metabolites (De Jesus-Gonzalez and Weathers 2003; Dhawan and Lavania 1996; Kim *et al.* 2004), we tentatively interpret the observed changes in bacterial communities as the results of increased production of secondary metabolites. We acknowledge that only precise assessment of volume, composition, and concentration of leachates (either from root exudates or tissues decomposition) for each geocytotype could confirm this interpretation but this was beyond the scope of this study.

In our study we observed species-specific responses of bacterial T-RFLP profiles (Figures 3a and 3b), which highlights the difficulties to draw generalisations. The fact that among tetraploid genotypes, plants do not induce highly different bacterial communities may argue in favour of the novel weapon hypothesis (Callaway and Ridenour 2004). Indeed, since introduced genotypes do not alter bacterial community in a different way than native one, invasion success could be explained by a lack of resistance of the recipient community in the introduced range, where soil organisms are exposed to a new set of metabolites. The novel weapon hypothesis has been proposed first for *C. maculosa* (Hierro and Callaway 2003), however, it has been recently disputed by Blair *et al.* (2006) who argue that the amount and the concentrations released can not be responsible for inhibitive effect on neighbouring plants.

The novel weapon hypothesis has never been tested so far for *S. inaequidens*. Since bacterial communities do not seem to change significantly according to geocytotypes, one could argue that invasion success of *S. inaequidens* is controlled by aboveground mechanisms rather than by belowground ones. However, although bacteria are likely to be the first affected by changes in the quantity or quality of root exudates, other soil biota and/or soil physical properties might have been affected by polyploidisation, as suggested by the decrease in total DNA content of the rhizosphere.

To our knowledge, this study is the first attempt in the framework of biological invasion to link plant traits with their rhizosphere bacterial communities, and polyploidisation. Assessing correlations between plant traits and rhizosphere biota characteristics can give insight on the pathway involved in invasion success, taking into account plant attributes.

We did not find any significant correlations between plant traits and changes in bacterial community profiles of the rhizosphere, leading to the conclusions that the subset of traits we studied does not affect rhizosphere bacterial community structure. Although, plant traits were significantly correlated to other rhizosphere biota characteristics (total DNA content, bacterial diversity and microbial biomass carbon) in the global analysis, it appeared that traits were correlated to the plant rhizosphere biota only for *S. inaequidens,* which is precisely the species for which no difference in plant traits among geocytotypes was found while differences were found for *C. maculosa* (Thébault *et al.* submitted). These results illustrate the importance of inter-species differences.

The reproductive output of *Senecio inaequidens* was correlated to changes in their soil rhizosphere biota. The fact that capitulum production was negatively correlated to microbial biomass carbon but positively correlated to total DNA content and bacterial diversity suggests that plant investment in reproduction may lead to a shift in soil biota functional groups (bacteria, fungi, nematodes, protozoa, rotifera…). Moreover, tetraploid individuals of the native range are characterised by higher values of leaf dry matter content (LDMC) than diploid ones, indicating a more conservative strategy and therefore lower decomposition rate. Plants with high values of LDMC are defined as slow growing plant, with low rate of nutrient recycling (Cornelissen *et al.* 2003) that favour the growth of fungi and their consumers, by contrast to fast growing plants that stimulates growth and activity of bacteria and their consumers (Bardgett 2005). Since higher values of LDMC are correlated with higher microbial biomass carbon and lower bacterial diversity, we could hypothesize that the shift in rhizosphere biota functional group following polyploidisation might be an indication for a shift from a bacterial-dominated towards a fungal-dominated food-web (Bardgett 2005).

Lastly, plant root biomass being positively correlated to Shannon-diversity index, resource allocation to belowground tissues may lead to increase in bacterial diversity of the rhizosphere. This would agree with the theory of accumulation of local pathogens, according to which plants are able to accumulate pathogens in their rhizosphere by amplifying a subset of the bacterial communities (Eppinga *et al.* 2006; Mangla *et al.* 2008). The accumulation of pathogens is thought to be more noxious for neighbouring plants than for the exotic plant itself, which gives it a competitive advantage in the community.

Surprisingly, despite the observed change in leaf traits according to geocytotypes of *C. maculosa* (Thébault *et al.* submitted) as well as a change in bacterial diversity, there were no correlation between plant traits and soil biota. Moreover, even if some traits changed according to geocytotypes of *S. inaequidens* (Thébault *et al*. submitted), these same traits were not correlated to changes in rhizosphere biota characteristics of corresponding plants. This pattern suggests that changes in the rhizosphere biota were genetically induced in the case of *C. maculosa*, while they were physiologically induced for *S. inaequidens* (Figure 5).

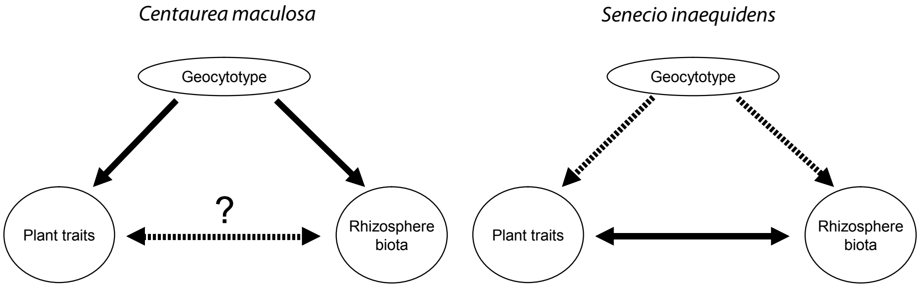

Figure 5: relations between geocytotype, plants traits and rhizosphere biota of the two model species *C. maculosa* and *S. inaequidens* according to results of the study.

This study highlights the difficulties to draw generalisations on invasion mechanisms since even taxonomically related invasive species tend to present different invasion mechanism. We are aware that this experiment has to be considered as a preliminary sketch of the potential effects of polyploidisation and plant functional traits on rhizosphere biota. Since our results come from a pot experiment, extrapolation to natural conditions is risky. However our results suggest that study of belowground mechanisms and interactions between aboveground and belowground compartments could improve knowledge on invasion success. Since polyploidisation is recognised as a common attribute of invasive species, research linking evolutionary changes to rhizosphere characteristics needs deeper studies in the next years. This experiment should be considered as a starting point for more comprehensive and realistic studies.

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PART II

Patterns of community resistance

Chapter 5

Effects of spatial pattern and community diversity on invasibility

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In preparation

Abstract

Invasive plant species represent a threat to terrestrial ecosystems. Understanding how and why they invade a given ecosystem is of major importance. Despite many controversial results, plant community diversity is thought to affect invasibility. Spatial distribution of species within a community can deeply affect growth and reproduction of plants by changing the outcome of interspecific competition. We studied the combined effects of community diversity and spatial pattern on survival, growth and reproductive output of native and introduced genotypes of *Centaurea maculosa* and *Senecio inaequidens*.

We experimentally established artificial communities of intra-specifically aggregated and randomly dispersed plant species with two diversity levels, in which we added seedlings of native and introduced genotypes of the two invasive species. Our results showed that species' survival, growth and reproductive output were affected by community diversity and spatial pattern. Survival and growth were enhanced in aggregated compared to randomly assembled communities. Growth increased with community diversity regardless of the spatial pattern of the community. By contrast, the increase in reproductive output of *S. inaequidens* following the increase in community diversity depended on the spatial pattern. The response of native and introduced genotypes to experimental factors was species-specific. Introduced and native genotypes of *S. inaequidens* both responded to spatial arrangement and diversity in terms of aboveground biomass. However, introduced genotypes showed a higher ability to take advantage of favourable conditions than natives ones. By constrast, while native genotypes of *C. maculosa* were affected by experimental factors, introduced genotypes were almost insensitive to the same factors.

According to these results, we argue that the two model species adopted two different strategies to deal with community spatial pattern and diversity changes. *Centaurea maculosa* was characterised by an ability to cope with unfavourable environments and can be defined as a Jack-of-all-Trades invader. By contrast, *S. inaequidens* was characterised by a Jack-and-Master strategy, where native and introduced genotypes were both affected by community changes, but introduced genotypes were more able to cope with unfavourable environments and to take advantage of favourable conditions than native ones.

Keywords: Invasion success, spatial aggregation, species richness, *Centaurea maculosa*, *Senecio inaequidens*, Jack-of-all-Trades, Jack-and-Master

5.1. Introduction

Invasive species are a threat to natural and managed ecosystems (Prieur-Richard and Lavorel 2000). Known to displace native species (Levine *et al.* 2003; Reinhart *et al.* 2006; Walker and Vitousek 1991) and to cause substantial losses to plant and animal agricultural production (Pimentel *et al.* 2000), they are nowadays considered as a major cause of ecosystem disturbance (Clavero and Garcia-Berthou 2005; Rejmanek and Richardson 1996). Therefore, understanding how and why they invade a given ecosystem is of major importance (Richardson and Pysek 2006).

Invasion success is the consequence of the ability of an exotic species to invade (*i.e.* invasiveness) and of the lack of resistance of the recipient community (*i.e.* invasibility). So far, invasiveness and invasibility have been hardly studied together, despite the growing evidence that only their combined study can lead to reliable understanding of invasion success (Hierro *et al.* 2005). Many hypotheses have been formulated to explain invasion success. Invasiveness is thought to be influenced by ecological and evolutionary processes. In accordance with ecological processes, invasion success is the consequence of extrinsic changes in the new environment that favour the invading species, without any intrinsic change of the invasive species (Callaway and Aschehoug 2000; Eppinga *et al.* 2006; Hierro *et al.* 2005; Keane and Crawley 2002; Mack *et al.* 2000; Maron and Vila 2001). By constrast, evolutionary processes lead to intrinsic changes of the invasive species, which favour their invasive success, for example through evolution of traits (Blossey and Notzold 1995; Bossdorf *et al.* 2004; Ellstrand and Schierenbeck 2000; Pandit *et al.* 2006; Prentis *et al.* 2008; Soltis and Soltis 2000; Suarez and Tsutsui 2007; Verlaque *et al.* 2002).

Biotic resistance seems to be important to explain invasibility (Elton 1958; Levine *et al.* 2004). Since Elton's hypothesis (Elton 1958), according to which invasive species succeed because some niches are left empty, few new hypotheses have been suggested. Among them, diversity has been mentioned as a driver of ecosystem stability and therefore also of invasibility (Chapin *et al.* 1997; Levine and D'Antonio 1999). However, different studies led to different results on the relationship between diversity and invasibility (Gilbert and Lechowicz 2005; Lanta and Leps 2008; Levine *et al.* 2004; Maron and Marler 2007), suggesting that the direction of the relationship could be scale dependent (Byers and Noonburg 2003; Wardle *et al.* 2008). Moreover, some authors argue that the diversity-invasibility relationship is just the consequence of a sampling effect since the more diverse a community is, the more likely is the presence of a highly competitive species which is able to limit invasion (Levine and D'Antonio 1999; Palmer and Maurer 1997; Tilman 1997). Since the presence of one particular species, such as a highly competitive species, can influence the outcome of the invasion, species identity is thought to be more important than species diversity (Crawley *et al.* 1999; Emery 2007; Emery and Gross 2007; Lyons and Schwartz 2001; Meiners *et al.* 2004). Lastly, functional diversity may be important too because the presence of a species from the same guild as an invader could limit the invader success by occupying a particular niche (Fargione *et al.* 2003; Xu *et al.* 2004).

Spatial patterns of plant species within communities have received little attention in the context of invasion ecology. Spatial distribution of species can deeply affect growth and reproduction of plants by changing the outcome of interspecific competition (Idjadi and Karlson 2007; Monzeglio and Stoll 2005; Monzeglio and Stoll 2008; Murrell *et al.* 2001; Stoll and Prati 2001). Indeed as spatial intra-specific aggregation increases, strong competitors suffer from intra-specific competition while weak competitors benefit from segregation from stronger competitors (Stoll and Prati 2001). Moreover, spatial aggregation leads to a decrease in resource uptake and complementarity (De Boeck *et al.* 2006; Mokany *et al.* 2008). Since spatial pattern of the community affects competitive outcomes, resource use and complementarity within the community, one might think that it could also affect invasibility (De Boeck *et al.* 2006). To our knowledge, effects of spatial pattern of plant species within communities on invasive success have never been studied.

In this study, we tested the effects of intra-specific aggregation and community diversity on invasive success of two invasive species. We used native and introduced genotypes of two worldwide invasive species, *Centaurea maculosa* and *Senecio inaequidens*, to test whether spatial aggregation and diversity have different impacts according to the genotype. We considered these two model species since (1) they belong to the same family (Asteraceae), (2) they occupy similar ecological niches in their native range and (3) they tend to invade similar habitats in their introduced range. We experimentally established artificial communities of intra-specifically aggregated and randomly dispersed plant species in which we added seedlings of native and introduced genotypes of the two invasive species. Our aim was to test the following hypotheses: (1) since intra-specific aggregation is known to lower competitive potential of strong competitors, invasive success should be higher in aggregated compared to randomly dispersed species assemblages. (2) In accordance with the diversity-invasibility theory, invasive success should be higher in communities with low compared to high diversity. (3) Since intra-specific aggregation promotes species coexistence, the positive effects of spatial aggregation on invasive success should be more important in communities with low compared to high diversity, as diversity is promoted by aggregation.

5.2. Material and Methods

5.2.1. *Invasive and resident species*

Two worldwide invasive species were used as model species. *Centaurea maculosa* Lam. is a herbaceous biennial or short-lived perennial tap-rooted forb with a rosette of basal leaves and relatively thick flowering stems (Hook *et al.* 2004). Its native range spans from Western Asia to Western Europe. *Centaurea maculosa* was introduced in the Pacific Northwest of the United States in the late 1800s (Watson and Renney 1974) and expanded rapidly throughout north-western America and western Canada where it is now widespread in rangelands, pastures and on roadsides (Duncan 2001; Skinner *et al.* 2000). Seeds of *C. maculosa* were collected during summer 2005 throughout its native range in Europe as well as in Montana and Oregon, USA (Broennimann *et al.* 2007; Treier *et al.* in press). On the whole, 15 populations from 97 available populations were randomly chosen for this study: 9 from native tetraploid populations and 6 from introduced populations known to be only tetraploids. Within populations, seeds were randomly chosen among a pool of seeds gathered from 16 mother plants.

Senecio inaequidens DC. is an erect perennial dwarf shrub native from South Africa and Lesotho. Originally found in grasslands from 1400 m a.s.l. to 2800 m a.s.l., it is now widespread in South Africa. It was accidentally introduced to Europe at the end of 1880s (Bornkamm 2002) where it is now widespread, even at lower altitudes. It is also found nowadays in Australia and South America (Ernst 1998; Lafuma *et al.* 2003). Seeds of *S. inaequidens* (mostly provided by Dr. Daniel Prati, University of Leipzig-Halle) were collected in Western Europe, South Africa and Lesotho (Lafuma *et al.* 2003). Additional seeds were collected from two populations in Switzerland. Of 89 available populations, 23 were randomly chosen: 14 native tetraploids and 9 introduced tetraploids. For each population, seeds were randomly taken from two mother plants.

Eight species commonly found in European grasslands were selected to build artificial communities. Seeds of community species were provided by FENACO SA. (Switzerland). The eight species were chosen so as to belong to 3 functional groups: grasses (*Arrhenaterum elatius*, *Agrostis capillaris*, *Bromus erectus*, *Festuca pratensis* and *Lolium perenne)*, legumes (*Lotus corniculatus* and *Trifolium repens)* and forbs (*Achillea millefolium)*. In the following sections, invasive species will be called target species, whatever the range considered, while species of the community will be adressed as resident species.

5.2.2. Community assemblage - Experimental setup

On April 2008, we sow seeds of the resident species in peat seedbed cubes of 3.0*3.5*4.0 cm^3. Seeds of target species (native and introduced genotypes) were sown two weeks later in the same manner. All seeds were put in a dark germination chamber for 10 days before being put in a greenhouse. The communities were assembled one month after the first seed sowing, at the beginning of May 2008. All seedbed cubes were placed on a homogeneous substrate made of sand (67%) and compost (33%), in a randomized split-split plot design (Figure 1). Spatial pattern and diversity varied at the plot level, species at the subplot level and range at the sub-subplot level. All combinations of diversity and spatial patterns were randomly assigned to plots. Each treatment was replicated 4 times yielding 16 plots arranged in 4 blocks. The main plots of 60*60 cm were subdivided into 2 subplots of 30 * 60 cm, each one containing one of the target species. Each subplot was further subdivided into 2 sub-subplots of 30*30 cm containing 81 cubes (Figure 1). While one sub-subplot contained native genotypes of the target species, the other one contained introduced genotypes of the same target species. Each target

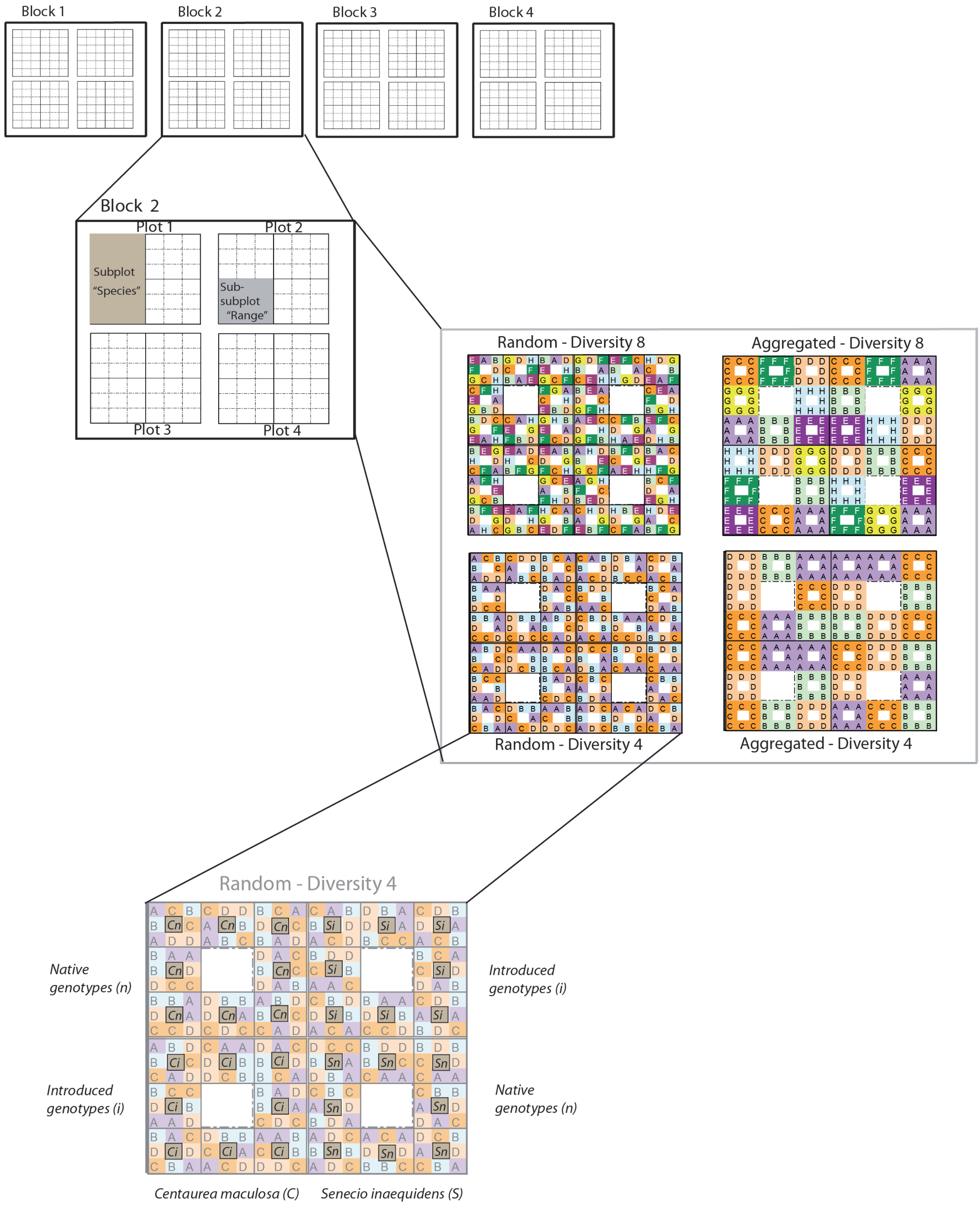

Figure 1: The experimental design: each of 4 blocks contained 4 plots with either random or aggregated distribution and low (4 species) or high (8 species) diversity. Each plot was subdivided into 2 subplots, each containing one invasive target species. Each subplot was further divided into 2 sub-subplots, containing either seedlings of the native range, or seedlings of the introduced range. Letters A-H represent resident species.

species was surrounded by 8 seedlings of resident species. In the aggregated treatment, target species were surrounded by one type of resident species, each target of a sub-subplot being surrounded by a different resident species. In the random pattern, target species were growing in the middle of a mixture of 4 or 8 resident species according to the diversity level considered. In the 4-diversity treatment, each resident species appeared twice around a given target species, while in the 8-diversity treatment each resident species appeared only once around the target species (Figure 1). Each sub-subplot contained 8 target individuals from the same range for a combination of spatial pattern and diversity.

5.2.3. Measurements

Survival was measured 15 days after the community assemblage to test for treatment effects on establishment. First harvest of target species was done 1.5 months after community assemblage. Aboveground parts were harvested, dried at 60°C during 3 days and weighted. A second harvest was done 4 months after community assemblage. As for the first harvest, aboveground biomass was dried at 60°C during 3 days before being weighted. During the experiment, number of flowering and non-flowering target individuals (flowering ability) were assessed. Capitula of target species (capitulum production) were counted weekly and cut since we wanted to prevent seed dispersion. At each harvest, resident communities were mown, sorted to the functional group level, dried at 60°C for 3 days and weighted.

5.2.4. Statistical analysis

Whenever possible, data were analysed using linear mixed effects models specifying spatial pattern, diversity, species and range as fixed effects and sub-subplot nested into subplot nested into plot as random factors. Thus, the main effects (spatial pattern and diversity) and their interactions were tested against the plot-level mean square as error term. The species effect was tested at the plot level, *i.e.* against the subplot-level mean square as error term and the range effect was tested against sub-subplot mean square as error term. When the interactions were significant, we used the likelihood-ratio test to select for the best or minimal model. Since test of interactions is not yet implemented for generalized mixed effects models (GLMM), we used general linear models (GLM) specifying block as a first factor. Significances of main effects were similar using either GLMM or GLM. Binary data (*i.e.* survival and flowering ability) were analysed by fitting a generalized linear mixed model with a binomial error distribution. Count data (capitulum production) were analysed by fitting a generalized linear mixed model with a poisson error distribution. Residual deviance was larger then residual degrees of freedom, revealing overdispersion. Consequently, the model was re-fitted with a quasipoisson distribution (Crawley 2005). Since *C. maculosa* did not flower during the experiment, reproductive output (flowering ability and capitulum production) were analysed for *S. inaequidens* alone and at the first harvest only because very few targets flowered after resprouting. Target biomass was analysed with linear mixed effects models. Mean biomass at the sub-subplot level was log-transformed to reduce heteroscedasticity. Resident biomass was harvested twice, at the same time as targets. Analysis of variance with plot nested into block as error term was used to test for differences in total standing biomass, which was log-transformed to reduce heteroscedasticity. Generalized linear model fitted with a binomial error distribution were used to analyze proportions of grasses, percentage of legumes and percentage of forbs between plot level treatments (*i.e.* diversity and spatial pattern). Since block effect might be important and GLM are additive models, block was specified as first factor to remove its effect before testing for diversity and spatial pattern effects. All the analyses were carried out with R 2.7.2 (R Development Core Team, 2008).

5.3. Results

5.3.1. Resident biomass

Resident total standing biomass of the plots was equal at both harvests (325.5 (±27.3) g.m^{-2} at the first harvest and 392.2 (± 29.2) g.m^{-2} at the second one). Whatever the harvest, there were no significant changes in the total standing biomass or the proportions of grasses and legumes between treatments. Diversity effect on forbs proportion was not tested since there were no forbs in the low diversity communities.

5.3.2. Target survival

Target survival at the first harvest ranged from 67 to 86%. There were no differences between species, range, diversity level and spatial patterns. By contrast, survival after resprouting only ranged from 4 to 27% (Figure 2). Survival of *S. inaequidens* was higher than survival of *C. maculosa* ($F_{1,54}$ = 11.57, p=0.001). Spatial pattern affected target survival ($F_{1,54}$ = 7.23, p=0.007) with a lower survival in randomly assembled communities (19%) than in aggregated ones (33%).

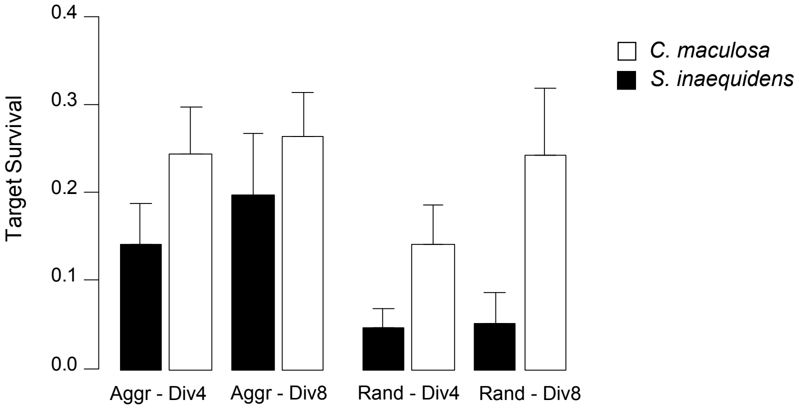

Figure 2: Survival of *C. maculosa* (black) and *S. inaequidens* (white) after resprouting, *i.e.* survival after 4 months, according to combinations of diversity (4 *vs.* 8 species) and spatial pattern (aggregated “Aggr” *vs.* random “Rand”).

5.3.3. Target biomass

Diversity and spatial pattern had significant effects on targets' early growth ($F_{1,10}$=10.19, p=0.01 and $F_{1,10}$=6.36, p=0.03 respectively). At the first harvest, target species produced more biomass in 8-species communities as compared to 4-species communities. Whatever the diversity level, targets produced more aboveground biomass in aggregated compared to random communities (Figure 3).

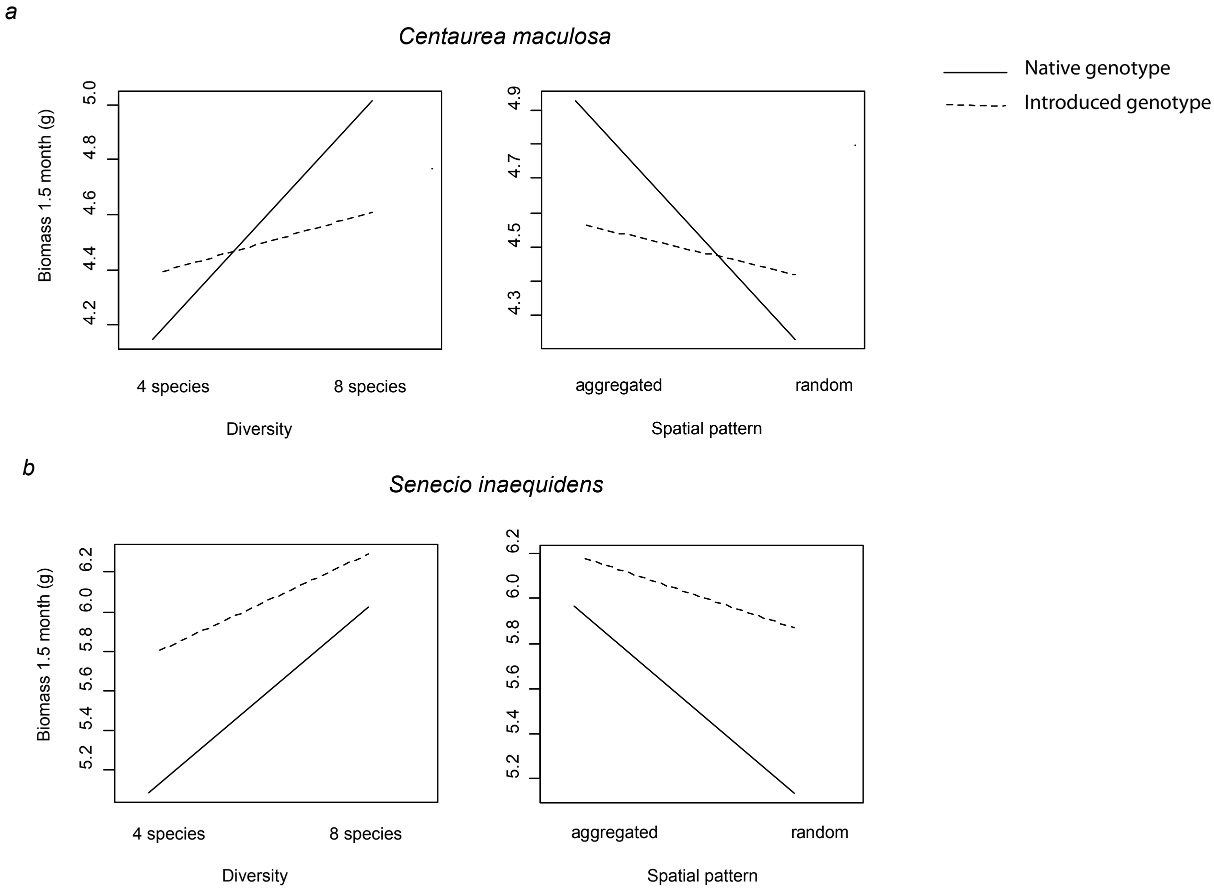

Figure 3: Interaction plot of standing biomass after 1.5 months of native (solid line) and introduced (dashed line) genotypes of *C. maculosa* (top) and *S. inaequidens* (bottom) according to diversity (4 *vs.* 8 resident species) and spatial pattern (aggregated *vs.* randomly assembled) of the community.

The two model species showed the same trends in response to changes in community spatial pattern and increased diversity at the first (Figures 4a and 4c) and second harvest (Figures 4b and 4d). At both harvest, *S. inaequidens* produced significantly more aboveground biomass than *C. maculosa* ($F_{1,15}$=67.30, p<0.001 and $F_{1,10}$=10.05, p=0.01 at first and second harvest respectively).

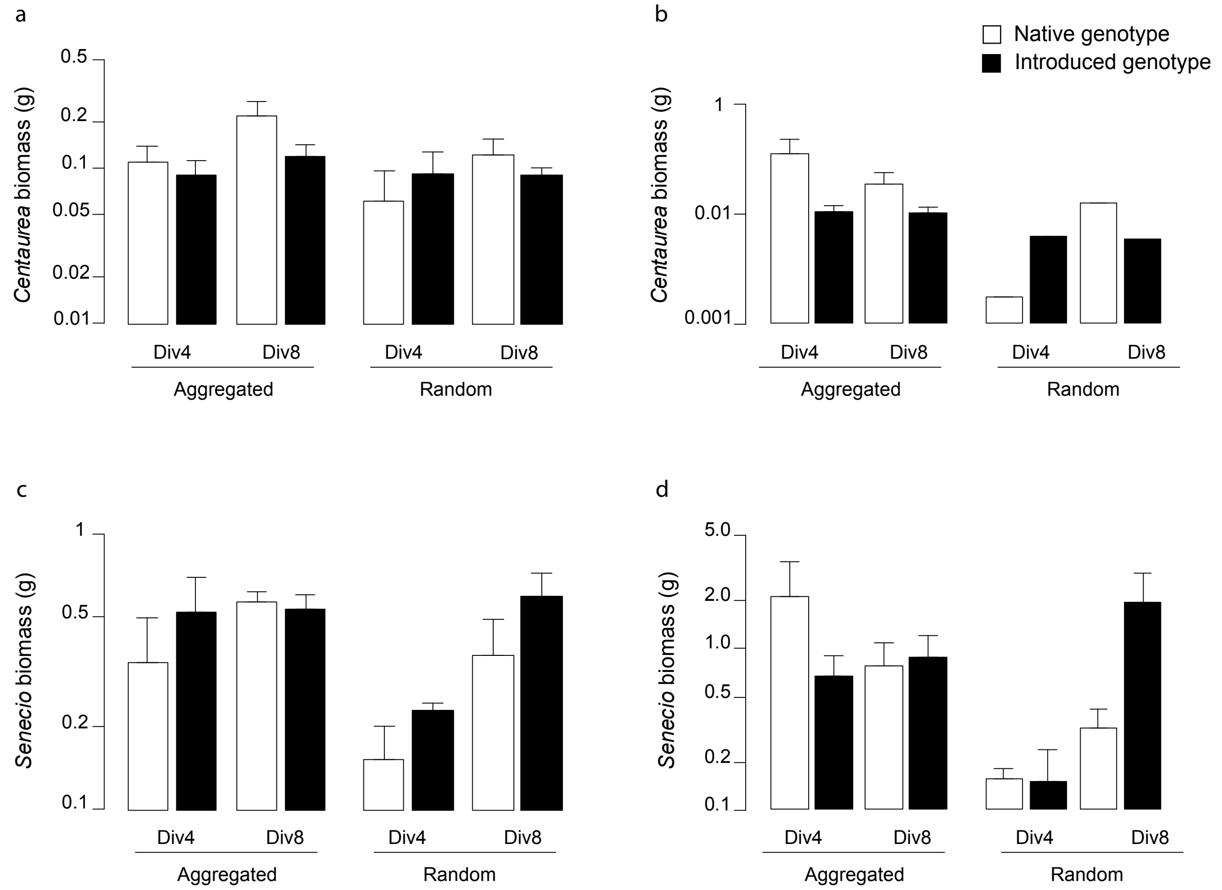

Figure 4: Standing biomass of native (white) and introduced (black) genotypes of *C. maculosa* (top) and *S. inaequidens* (bottom) respectively after 1.5 months (4a and 4c) and after resprouting (4b and 4d). Standing biomass is represented on a logarithmic scale.

Diversity influenced native and introduced genotypes' early growth of *C. maculosa* differently (Table 1, Figure 3a). Introduced genotypes biomass was not affected by diversity, whereas biomass of native genotypes increased with community diversity regardless of the spatial pattern considered (Figure 3a). The same trend, but not significant ($F_{1,13} = 3.483$, $p=0.085$), appeared in response to spatial pattern (Figure 3a and 4a). After resprouting, biomass of *C. maculosa* was lower in communities with random patterns than in communities with aggregated pattern, whatever the genotype and the diversity level considered (Figure 4b).

By contrast to *C. maculosa*, native and introduced genotypes of *S. inaequidens* were both affected by community spatial pattern and diversity (Figure 3b). Moreover, biomass of introduced genotypes was marginally higher than biomass of native genotypes, whatever the community considered (Table 1, Figure 4c). Biomass of both genotypes was higher in aggregated spatial pattern and high diversity communities (Figure 4c). As for *C. maculosa*, standing biomass of *S. inaequidens* after resprouting seemed to be lower in randomly organised communities compared to aggregated ones (Figure 4d) although not significantly (Table 1).

Table 1: Results of linear mixed effect models testing effects of diversity (4 *vs.* 8 species), spatial pattern (random *vs.* aggregated), range (native *vs.* introduced) and their interactions on *C. maculosa* and *S. inaequidens* standing biomass at the first (1.5 months) and second (4 months) harvest. Non significant interactions have been removed on the basis of likelihood-ratio.

		Biomass 1.5 months			Biomass 4 months	
	d.f.	*F*-value	*P*-value	*df*	*F*-value	*P*-value
Centaurea maculosa						
Block/Plot						
Diversity	1	4.672	0.056	1	0.301	0.606
Spatial pattern	1	2.860	0.122	1	6.558	**0.051**
Residuals	10			5		
Sub-subplot level						
Range	1	0.319	0.582	1	3.247	0.213
Diversity :Range	1	4.702	**0.049**	1	3.092	0.221
Spatial pattern :Range	1	3.483	0.085	1	7.067	0.117
Residuals	13			2		
Senecio inaequidens						
Block/Plot						
Diversity	1	8.206	**0.017**	1	1.763	0.214
Spatial pattern	1	5.205	**0.046**	1	1.452	0.256
Residuals	10			10		
Sub-subplot level						
Range	1	4.269	0.057	1	0.233	0.641
Residuals	15			9		

5.3.4. Reproductive output

Senecio inaequidens' flowering ability was higher in 8 species (20.6 %) compared to 4 species communities (7.6%, $F_{1,25}$=9.05, p=0.003). There was difference neither in response to resident community spatial pattern, nor between target range.

Community spatial pattern and target range significantly influenced *S. inaequidens*' capitulum production (Table 2). Introduced genotypes produced significantly more capitula than native ones whatever the community assemblage (Figure 5). The effect of spatial pattern on capitulum production was diversity-dependant as shown by the significant interaction term between spatial pattern and diversity (Table 2). Capitulum production was higher in low diverse communities as compared to highly diverse ones in the aggregated spatial pattern, whereas there were no differences of capitulum production according to community diversity in randomly assembled communities (Figure 5).

Table 2: results of generalized linear model fitted with a quasipoisson distribution to test effects of diversity (4 *vs.* 8 species), spatial pattern (random *vs.* aggregated), range (native *vs.* introduced) and their interactions on *S. inaequidens*'s capitulum production during the first 1.5 months. Non significant interactions have been removed on the basis of likelihood-ratio.

		Capitulum production	
	d.f.	*F*-value	*P*-value
Block	3	12.66	**0.001**
Diversity	1	3.96	0.078
Spatial pattern	1	7.62	**0.022**
Range	1	35.23	**< 0.001**
Diversity : Spatial pattern	1	33.60	**< 0.001**
Residuals	9		

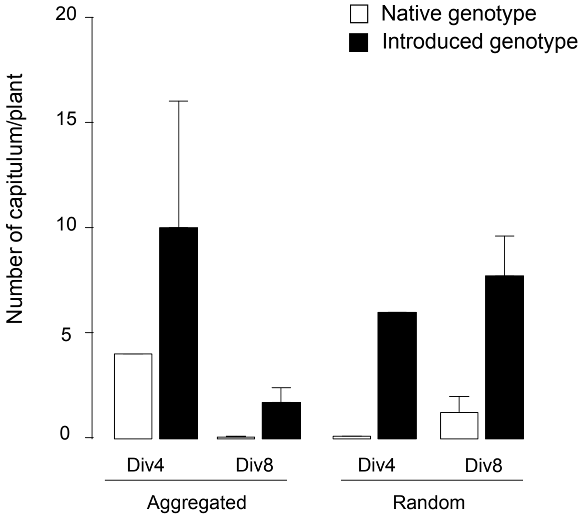

Figure 5: Capitulum production of native (white) and introduced (black) genotypes of *S. inaequidens* after 1.5 months. Capitulum production is the number of capitula produced by flowering plant.

5.4. Discussion

In this study, we used two worldwide invasive species, *Centaurea maculosa* and *Senecio inaequidens*, which are taxonomically related and have a close ecology. However, the two invasive species tested are quite different regarding their life strategies. Indeed, *Senecio inaequidens'* invasion strategy is based on aboveground productivity and flowering, while *C. maculosa* invests more in belowground tissues which seems also to be related to allelopathy (Chapter 1). In this experiment, we showed that *S. inaequidens* had a higher survival, particularly after resprouting, a higher aboveground biomass and a higher reproductive output than *C. maculosa*. However, despite these differences in growth strategies, we found some common patterns in the response of the two invasive species to spatial pattern and diversity of the resident community.

Invasive species' survival and early growth were enhanced in aggregated spatial patterns compared to random ones. Since resource use and complementarity decrease with intra-specific aggregation (De Boeck *et al.* 2006; Mokany *et al.* 2008), the availability of resources may enhance survival and growth in such aggregated communities. If we consider invasibility of the community as the lack of resistance to the arrival of a new individual, regardless of the invasive potential of this new species, invasibility could be promoted by community intra-specific aggregation. This is in agreement with the recent theory arguing that intra-specific aggregation promotes species coexistence within the community by limiting competitive effect of the most dominant species (Idjadi and Karlson 2007; Monzeglio and Stoll 2005; Monzeglio and Stoll 2008; Murrell *et al.* 2001; Stoll and Prati 2001).

Early growth was enhanced by community diversity. The positive correlation between invasibility and diversity highlights the ongoing debate on the nature of this relation. In small scale microcosm experiments, diversity has often been shown to limit invasibility (Brown and Peet 2003; Levine 2000; Lyons and Schwartz 2001; Naeem *et al.* 2000; Symstad 2000). Our results are in contradiction to the negative diversity-invasibility relationship at small scale. As community productivity was not different according to diversity in our experiment, this positive relationship can not be explained by a confounding factor such as productivity, which has often been criticised in studies that found a negative relationship between invasibility and diversity (Wardle 2001). However, since there were no forbs in low diverse communities by contrast to diverse ones, the positive effect of diversity on invasibility could be due to the increase either in species richness or in functional groups richness.

Spatial heterogeneity has been said to drive the positive relationship between invasibility and diversity (Davies *et al.* 2005). We did not found any interaction between spatial pattern and diversity for species growth, but effect of community diversity on reproductive output depended on the spatial pattern of the community. Indeed, while community diversity promoted capitulum production of *S. inaequidens* in randomly assembled communities, it limited the capitulum production in aggregated patterns. Since capitulum production may be the key traits responsible for invasive success of exotic species, spatial heterogeneity may play a central role in community invasibility and deserves more studies. In the case of *S. inaequidens*, spatial heterogeneity could drive the positive relationship between invasibility and diversity since the more diverse the community is, the higher the capitulum production is, which in turn increases the propagule pressure and the invasion success probability.

Native and introduced genotypes of *C. maculosa* were not equally affected by community pattern, native genotypes being more affected by community patterns than introduced ones. By contrast, while introduced genotypes of *S. inaequidens* had higher fitness than native ones, through higher growth and capitulum production, their response to community changes were not significantly different from native genotypes. These differences in genotypes fitness changes in response to favourable and unfavourable environments can be linked with phenotypic plasticity.

Indeed, phenotypic plasticity, the property of a genotype to express different phenotypes in different environments (Bradshaw 1965), has recently been suggested to explain invasiveness (Richards *et al.* 2006) since it could enhance niche breadth as plastic response may allow organisms to cope with a broader range of environments (Bradshaw 1965; Richards *et al.* 2008). Richards *et al.* (2006) argue that invaders may benefit from plasticity through three strategies. According to the Jack-of-all-Trades strategy, the invader is able to maintain fitness in unfavourable environments, by contrast to the opportunist Master-of-Some strategy which states that the invader is able to increase its fitness in favourable environments. Since these two strategies are not mutually exclusive, the Jack-and-Master strategy is a combination of the former ones, where invader is doing well in all kind of environments and is also able to take advantage from favourable conditions. Based on our results, we argue that the two model species adopted two different strategies to deal with community spatial pattern and diversity changes. Indeed, introduced genotypes of *C. maculosa* were better able to cope with “unfavourable” environments, such as spatially randomly assembled or species poor communities, than native ones (Figure 3a). This is characteristic of a Jack-of-all-Trades strategy, as introduced genotypes are not affected by community changes. By contrast, native and introduced genotypes of *S. inaequidens* were both affected by community changes but introduced genotypes had always higher growth than native ones (Figure 3b), which could be defined as a Jack-and-Master strategy. Therefore, according to these two strategies, the invasive success of *C. maculosa* in the introduced range could be due to the ability of North American genotypes to deal with all kind of stressful environments. By contrast, European genotypes’ fitness seems to decrease along a stress gradient, which could explain the lack of success of *C. maculosa* in its native range. The invasive success of *S. inaequidens* may be more likely explained by a better ability of introduced genotypes compared to native ones to cope with all kind of environments and to take advantage of favourable environments, such as spatially aggregated or diverse communities.

5.5. Acknowledgments

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Chapter 6

Dominant species identity and turnover may limit invasion success

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(In preparation)

Preliminary remarks

This chapter presents the preliminary results of a two-year field experiment conducted in 2006 and 2007 at the Federal Research Station of Changins (Agroscope Changins-Wädenswil).

In this experiment, two-weeks old seedlings of native and introduced genotypes of *Senecio inaequidens* and *Centaurea maculosa* were transplanted in a traditionally mown meadow.

During two vegetation seasons, survival and growth of target species were monitored, as well as evolution of the resident community. Through periodic floristic releves, we aimed at assessing diversity (*sensu lato*), competitive ability and dynamics of the resident community. Using various statistical approaches (regression trees, linear models, principal component analysis, multiple factorial analysis), we aimed at assessing effects of community diversity, competitive ability and composition and functional stability on seedlings survival, growth and reproductive output.

Preliminary results indicate that vegetative height of the community limits survival. Furthermore, growth and reproduction of both invasive species was affected by dominant species identity. *Centaurea maculosa* was influenced by proportion of grasses whereas *Senecio inaequidens* was affected by proportion of legumes and rosettes in the recipient community. Growth of *C. maculosa* was also affected by dominant species turnover and the consequent variation in resident community competitive ability.

We acknowledge that these preliminary results deserve deeper analyses. However, the original approach presented in this chapter will certainly provide a good starting point for discussions on the interaction between species invasiveness and community invasibility

6.1. Introduction

Invasive species represent a threat to natural and managed ecosystems (Pimentel *et al.* 2000; Prieur-Richard *et al.* 2000). Understanding how they can invade ecosystems is a major challenge to ecologists (Richardson and Pysek 2006). Until now, studies on biological invasions have concentrated on the understanding of either what predisposes a species to become an invader or what predispose a community to be invaded (Facon *et al.* 2006). This separation of respectively invasiveness and invasibility aspects of biological invasions could explain the difficulty to find general patterns in biological invasions. Since invasions represent a match between a species and an ecosystem (Bazzaz 1986; Shea and Chesson 2002), rather than an intrinsic property of either one, it seems necessary to focus on both aspects together.

A wide set of studies has focused on the relationship between invasibility and diversity since Elton's work in 1958. Elton's hypothesis, based on the theory of competitive exclusion and niche displacement, predicts that the most diverse communities are the least invaded, as empty niches are scarce and resources completely used. Controversial results highlight the fact that this relationship is not straightforward (reviewed in Foster *et al.* 2002; Wardle 2001). Several authors proposed that rather than species richness *per se*, functional diversity, dominant species identity or evenness could better explain invasibility (Emery 2007; Emery and Gross 2007; Fargione *et al.* 2003). Based on niche occupancy, functional diversity could limit invasion success since an exotic species might have more difficulties to invade a community containing species of the same guild (Fargione *et al.* 2003; Xu *et al.* 2004). A possible explanation for such a result holds in the stochastic niche theory based on resource partitioning (Tilman 2004). This theory predicts that once a species is established within a community, the probability that a similar species is successful in invading is low. Functional groups present in the community could also influence invasibility. Legumes for example, by fixing atmospheric nitrogen, might be expected to increase levels of available resources over time and consequently facilitate invasion (Palmer and Maurer 1997). High evenness, by increasing efficiency of resource use in the community is though to limit invasibility (Tracy and Sanderson 2004; Wilsey and Polley 2002). Consequently, low evenness*, i.e.* dominance, should promote invasibility. However, according to the dominant species, invasibility could be limited since the dominant species might have the same requirements as the invasive species (Emery 2007; Emery and Gross 2007). Therefore, quantifying traits relative to competitive ability of the dominant species might be useful to understand community invasibility. The competitive ability of an individual can be assessed through its competitive effect, *i.e.* its ability to suppress neighbours (Goldberg and Landa 1991). Vegetative traits such as plant height or lateral spread are known to be indicators of plant competitive effect since they give an advantage in space occupation and capture of light (Grime 1977), which in turn impedes the neighbouring species. Specific leaf area (SLA) and leaf dry matter content (LDMC) characterise the trade-off in plant functioning between production of biomass and conservation of nutrients (Diaz *et al.* 2004). SLA is related to short leaf retention and fast growth rate (Cornelissen *et al.* 2003; Grotkopp *et al.* 2002) whereas LDMC is related to defence against natural hazards and herbivory through high investment in leaf tissues (Cornelissen *et al.* 2003) and conservation of nutrients (Wright *et al.* 2004). According to the biomass ratio hypothesis (Grime 1998), traits of dominant species have the most important impact on ecosystem properties (Lavorel and Garnier 2002). At the community scale, any trait of dominant species can be weighted by species abundances to give an aggregated value of this trait at the community level (Garnier *et al.* 2007). Therefore, quantifying vegetative height, SLA and LDMC of dominant species of the community allows assessing community competitive effect and strategy of resource use.

The debate on diversity-invasibility relationship could profit from insights on temporal dynamics of the resident community, *i.e.* the consistency over time of community membership and species abundance in response to perturbation (Foster *et al.* 2002). This compositional stability is thought to be positively related to species richness (Bakker *et al.* 2003; Tilman 1996) and since species richness has often been thought to be linked to invasibility, compositional stability might be involved also in community resistance. To our knowledge, only few studies tried to link compositional stability with invasibility (Foster *et al.* 2002; Schoolmaster and Snyder 2007). Since the biomass ratio hypothesis predicts that traits of dominant species have the most important impacts on community functioning (Grime 1998), changes in dominant species of a community might affect community properties and therefore invasibility. On the basis of compositional stability, we define functional stability as the consistency of aggregated trait values at the community scale. To our knowledge, this study is the first attempt to link functional stability to invasibility.

Intra-specific comparisons between native and invasive genotypes of an invasive species are necessary to understand invasion success (Hierro *et al.* 2005). Comparing the ecology of introduced populations of a species with its native populations provides a measure of changes in ecology which result from introduction in the new range as well as an understanding of the processes that enable exotics to dominate recipient communities (Dlugosch and Parker 2008; Hierro *et al.* 2005). In this study, we investigated the effect of biotic and abiotic factors on invasion success of two species, *Centaurea maculosa* and *Senecio inaequidens*, through a two-year field experiment. These two species present diploid and tetraploid cytotypes in their native range but only tetraploid ones in the introduced range (Lafuma *et al.* 2003; Treier *et al.* in press). Due to this distribution pattern, these species are good models for studying genetically and environmentally induced processes and their consequences on invasion success. Furthermore, there are taxonomical and ecological similarities between these two species: (1) they belong to the same family (Asteraceae), (2) they occupy similar ecological niches in their native range, (3) they tend to invade similar habitats in their introduced range, (4) they are often avoided by cattle and (5) they both may have allelopathic effects (Ahmed and Wardle 1994; Callaway and Ridenour 2004). If we define for convenience a geocytotype as a ploidy level in a given area (native or introduced range), both model species have three geocytotypes (native diploid, native tetraploid and introduced tetraploid).

All geocytotypes of both species were grown in a semi-natural grassland. Since both species tend to invade agricultural area and are avoided by cattle, a management treatment was applied. In the unselective treatment, all the vegetation was cut whereas in the selective treatment, avoidance of invasive species was simulated by cutting all neighbouring vegetation, letting the invasive species intact. We analysed fitness (survival, growth and reproductive output) of geocytotypes of both species under two different management treatments to assess effects of abiotic (management treatment) and biotic (community diversity, community competitive effect and community stability) factors on geocytotypes' fitness during two vegetation seasons.

6.2. Material and methods

6.2.1. Model species

Two worldwide invasive species were used in this experiment as model species. *Centaurea maculosa* Lam. is a herbaceous biennial or short-lived perennial tap-rooted forb with a rosette of basal leaves and relatively thick flowering stems (Hook *et al.* 2004). Its native range spans from Western Asia to Western Europe. *Centaurea maculosa* was introduced in the Pacific Northwest of the United States in the late 1800s (Watson and Renney 1974) and expanded rapidly throughout north-western America and western Canada where it is now widespread in rangelands, pastures and on roadsides (Skinner *et al.* 2000; Duncan 2001). Its low nutritive value reduces forage quality and makes it often refused by cattle (Campobasso *et al.* 1994). Seeds of *C. maculosa* were collected during summer 2005 throughout its native range in Europe as well as in Montana and Oregon, USA (Broennimann *et al.* 2007; Treier *et al.* in press). On the whole, 22 populations out of 97 available populations were randomly chosen for this study: 6 from native diploid populations, 6 from native tetraploid populations and 10 from introduced populations known to be only tetraploids. Within populations, seeds were randomly chosen among a pool of seeds from 16 mother plants.

Senecio inaequidens DC. is an erect perennial dwarf shrub native to South Africa and Lesotho. Originally found in grasslands from 1400 m a.s.l. to 2800 m a.s.l., it is now quite widespread in South Africa. It was accidentally introduced to Europe at the end of 1880s (Bornkamm 2002) where it is now widespread, even at lower altitudes. It is also found nowadays in Australia and South America (see Ernst 1998; Lafuma *et al.* 2003 for review). *Senecio* *inaequidens* contains pyrrolizidine alkaloids (Macel and Vrieling 2003), which are toxic to livestock (Noble *et al.* 1994). Therefore, when growing in pastures, *S. inaequidens* is refused by cattle, which is expected to lead to reduction of forage quality and thus to cause economical losses. Seeds of *S. inaequidens* were collected in Western Europe, South Africa and Lesotho (Lafuma *et al.* 2003). Additional seeds were collected in two populations in Switzerland. Among the 89 available populations, 18 were randomly chosen: 6 native diploids, 3 native tetraploids and 9 introduced tetraploids. For each population, seeds were randomly taken from two mother plants.

C. maculosa and *S. inaequidens* individuals will hereafter be referred as target individuals, whatever the geocytotype concerned.

6.2.2. Seed germination

Seeds of both model species were sown in peat pellets and placed in a germination room under daily controlled conditions, *i.e.* 14 hours at 24°C and 10 hours at 18°C. After one week, seedlings were put outside to acclimatise to natural field conditions. Two-weeks-old target individuals were used for transplantation in the experimental field site.

6.2.3. Experiment setup

A two-year field experiment was set up in May 2006 at the Swiss federal research station of Changins (Agroscope Changins-Wädenswil, 46°23'47 N; 6°13'51 E) in Switzerland. A 15 m x 15 m enclosure was constructed in a semi-natural, mown grassland. The experimental site was mown prior to seedling transplantation and divided into 16 blocks of $2m^2$ (Figure 1a). A management treatment, simulating unselective («mowing») and selective disturbance («grazing»), was randomly assigned to the blocks (Figure 1a) and applied twice a year. In half of the blocks, all aboveground vegetation, including the target individuals, was mown to ground level (*i.e.* mowing). In the other half of the blocks, only the aboveground vegetation surrounding the target individuals was mown to simulate grazing (Figure 1b). Each block was further divided into 8 plots of 30cm x 30cm (Figure 1c). Geocytotypes of both model species were randomly assigned to the plot within each block. Seedlings (n = 96) were transplanted with their peat pellet directly into the soil. Two additional plots, one with a blank peat pellet and one control (no transplantation), were added to test for disturbance due to the experimental setup (Figure 1d).

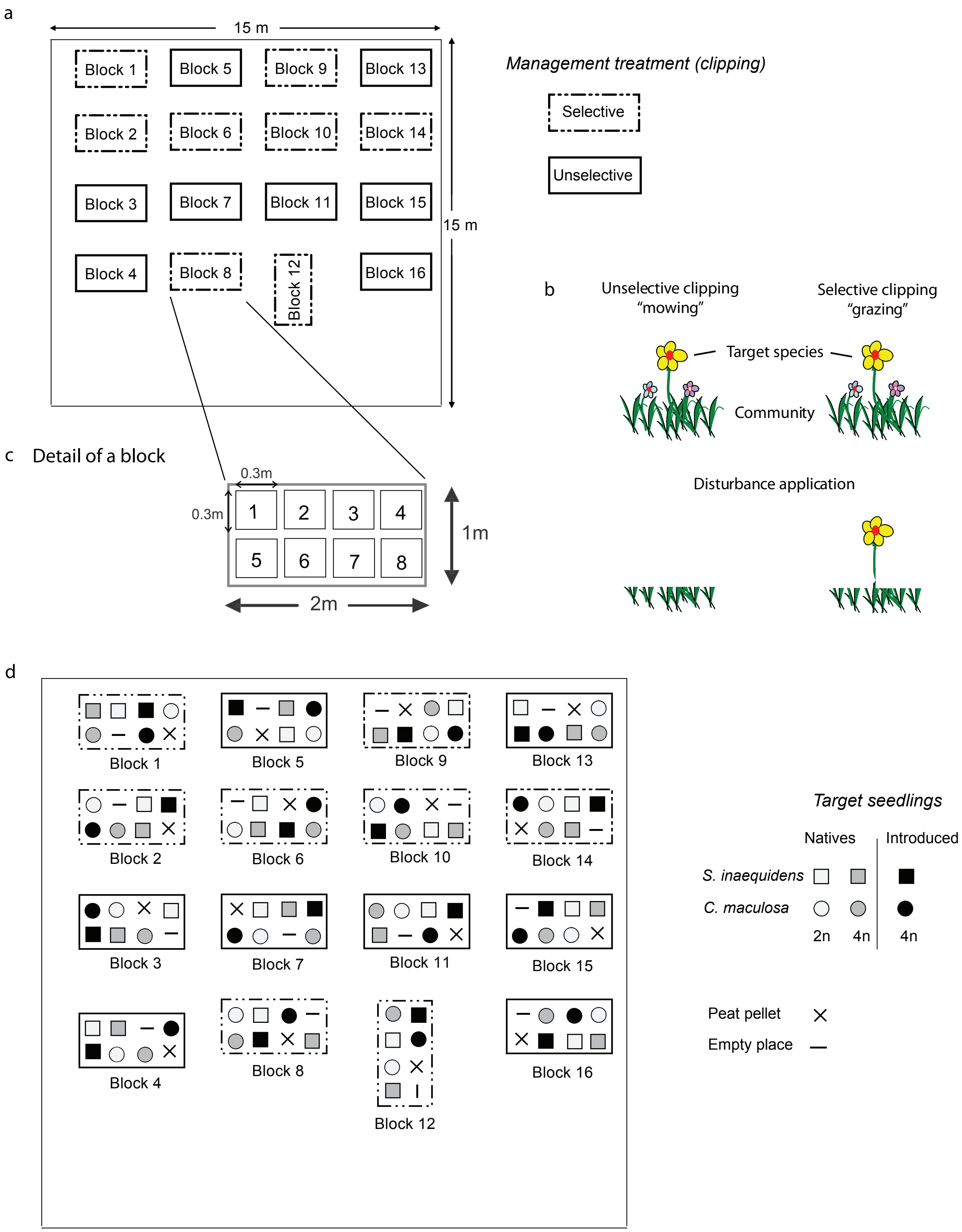

Figure 1: Experimental design: a management treatment (a), *i.e* unselective or selective clipping (b), was randomly assigned to 16 blocks. Each block contained 8 plots (c) in which targets (seedlings of both model species and geocytotypes, including peat pellets and empty places as controls) were randomly assigned (d).

6.2.4. Measurements

6.2.4.1. Target plants

Survival of transplanted seedlings, vegetative size and reproductive output were determined at the end of each vegetation season. Due to differences in growth form between *S. inaequidens* and *C. maculosa,* vegetation height and lateral extension of the canopy (*i.e.* lateral spread) were measured for each species respectively (Gaudet and Keddy 1988; Grime 1977; Navas and Moreau-Richard 2005). Reproductive output was assessed by the capitulum production of the targets, which is the total number of capitula produced by surviving plants during each vegetation season.

Root and shoot biomass were measured at the end of the second vegetation season on all surviving targets. Plants were separated into shoots and roots, dried at 60°C for 72 hours and weighed to get respectively shoot biomass and root biomass (Cornelissen *et al.* 2003). Root-shoot ratio (RSR) was calculated as the ratio between root and shoot dry mass. For bio-security reasons and in order to prevent seed set, capitula of all plants were cut regularly during the experiment.

6.2.4.2. Resident community

Floristic releves using Londo scale were done three times (*i.e.* periods) per vegetation season (2006 and 2007), in spring, summer and autumn. At each date, all resident species were recorded in all 128 plots of the experiment and Simpson diversity index, evenness and proportion of legumes, grasses and forbs in each plot were calculated. At the end of the first vegetation season, species accounting for 80% of total abundance were identified and selected for trait measurements. Vegetative height, leaf dry matter content (LDMC) and specific leaf area (SLA) were measured on the selected dominant species in summer 2007. Leaf traits were measured on 10 individuals of selected dominant species and vegetative height on 20 individuals. All these measurements followed standardized protocols (Cornelissen *et al.* 2003). For each plot and each survey period, a community aggregated trait value was calculated using the trait value of each dominant species weighted by its relative abundance in the plot (Garnier et al. 2007).

Lastly, based on floristic releves of each period and community aggregated trait values, dissimilarity matrices of Jaccard were calculated to assess turnover between following periods (*i.e.* spring-summer 2006, summer-autumn 2006, spring-summer 2007 and summer-autumn 2007) and between years (spring 2006 - spring 2007, summer 2006 - summer 2007, autumn 2006 - autumn 2007) as an estimate of compositional and functional stability respectively. The mean of periodic turnover was calculated to get mean turnover within year and the mean of yearly turnover gave turnover between years.

6.2.5. Statistical analyses

In a first step, impact of experimental setup on community floristic composition was tested by analyses of redundancy (RDA) constraining community floristic composition matrix by management (unselective *vs.* selective clipping), target (*C. maculosa* and *S. inaequidens vs* controls as peat pellets and no transplant) and geocytotype (native diploid, native tetraploid or introduced tetraploid) for each period (spring, summer, autumn) of the two vegetation seasons. Significance of RDAs was analysed with mantel tests on 999 permutations of Monte Carlo.

Regression trees were used to assess the main community variables affecting species survival after two growing seasons. Separate analyses were done on *C. maculosa* and *S. inaequidens* since the variables affecting each species might differ. Fifty multiple cross validations were selected to get the best model.

To analyse effects of biotic factors, three matrices were built: a diversity matrix, a competition matrix and a stability matrix using selected variables at the plot level. Diversity matrix represented the diversity *sensu lato* of the community since it contained species diversity indices such as Simpson diversity index and evenness, but also diversity in terms of functional groups with the proportion of grasses, legumes and forbs in the community. The competition matrix contained community aggregated values of SLA, LDMC and vegetation height. Therefore this matrix represented community properties in terms of competitive effect and nutrient cycling. The last matrix “dynamics” contained turnover within and between years in terms of species and functional composition.

To analyse the effects of management, geocytotype, community diversity, community competition and community dynamics on target survival and growth, generalised linear model (GLM) and linear model (LM) were used respectively. Each of the three matrices was reduced to one dimension vector by using coordinates of plots on the first axis of the principal component analysis (PCA) done on variables of the considered matrix. Separated PCA’s were done for each combination of year and target species. Target growth was also reduced to one variable by taking the coordinates of targets on the first axis of the PCA on target traits, for each year. Effect of management, geocytotype, community diversity, competition and dynamics on survival (binary data) of target plants was analysed each year using GLM fitted with a binomial distribution and a logit link function (Venables and Ripley 1999). To analyse effect of management, geocytotypes, community diversity, competition and dynamics on surviving target growth, a linear model was used.

Lastly, multiple factor analyses (MFA) were done for each combination of target and vegetation season to analyse the correlations between target growth and the different matrices of biotic factors, as well as to visualise the most important variables affecting each trait of surviving target. All the analyses were carried out with R 2.7.2 (R Development Core Team, 2008).

6.3. Results

6.3.1. *Impact of management and target species on resident community*

All the redundancy analyses constraining floristic composition by management type, targets and geocytotypes yielded very low variance (between 4.3 and 5.6%) and were non significant (Table 1). Therefore, management (unselective *vs* selective clipping), transplantation (plants *vs* controls) and geocytotypes did not have significant impacts on floristic composition at the plot scale.

Table 1: Results of the redundancy analysis (RDA) constraining community floristic composition by management (unselective *vs.* selective clipping), target (*C. maculosa* and *S. inaequidens vs.* controls as peat pellets and empty spaces) and geocytotype (native diploid, native tetraploid or introduced tetraploid). P-values were obtained following 999 permutation tests.

Year	Period	% variance explained by the model	df	variance	F-value	P-value
2006	Spring	5.0	6	33.90	1.056	0.3880
			121	647.46		
	Summer	5.4	6	36.07	1.153	0.2650
			121	631.16		
	Autumn	5.3	6	27.19	1.131	0.2500
			121	484.77		
2007	Spring	5.0	6	18.99	1.059	0.3240
			121	361.63		
	Summer	4.3	6	16.61	0.898	0.6800
			121	373.30		
	Autumn	5.6	6	27.59	1.195	0.1550
			121	465.70		

6.3.2. *Impact of community on target performance*

6.3.2.1. Survival

At the end of the first vegetation season, survival was very high since only 12 seedlings out of 96 were dead. By contrast, survival was very low at the end of the second vegetation season, since only 16 seedlings out of 96 were still alive. Primary cause of mortality at the end of the second vegetation season was community vegetative height for both species (Figure 2). The threshold was very similar between species since mortality of *C. maculosa* and *S. inaequidens* occurred when vegetation was higher than 37 cm and 36 cm respectively. Under low vegetation height, cause of mortality was species specific. Mortality of *C. maculosa* was higher under high community evenness (Figure 2a) whereas mortality of *S. inaequidens* was enhanced in communities with aggregated specific leaf area (SLA) higher than 18.9 $m^{-2}\,kg^{-1}$ (Figure 2b).

However, marginal significance of both models (p=0.10 and p=0.08 for *C. maculosa* and *S. inaequidens* respectively) indicates that the second factors of mortality has to be taken into account cautiously.

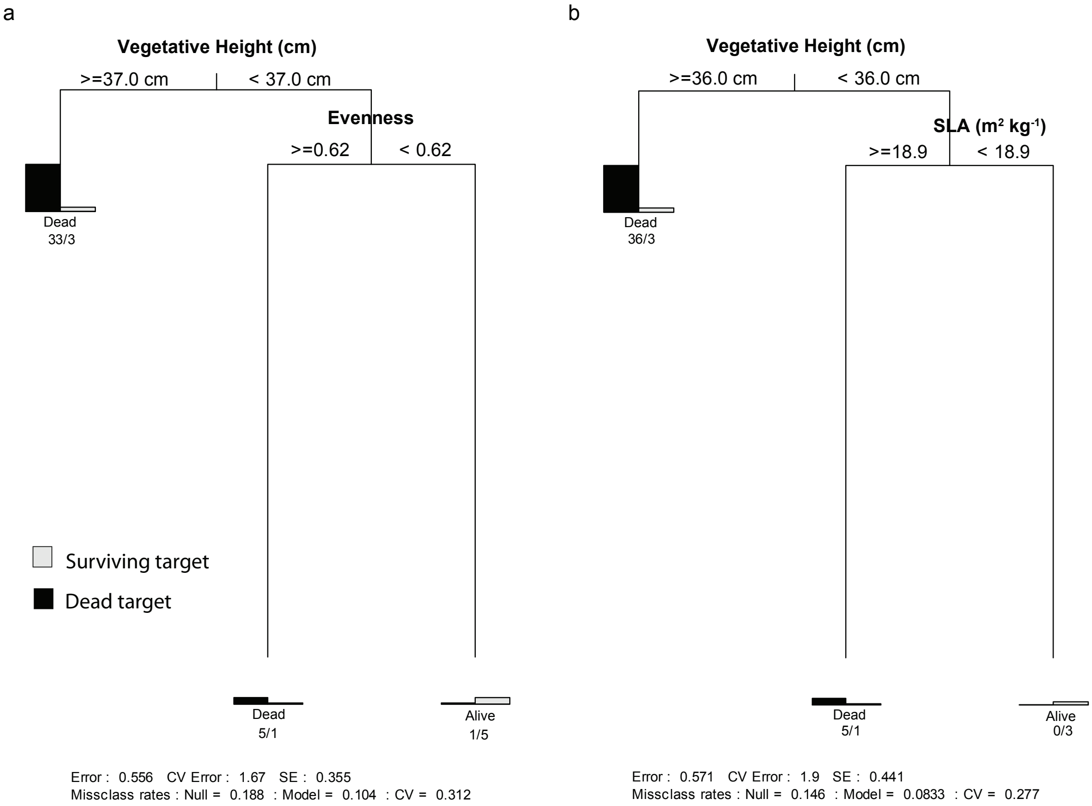

Figure 2: Results of pruned regression trees on survival of target seedlings of *C. maculosa* (2a) and *S. inaequidens* (2b) at the end of the second vegetation season. Models are based on 50 multiple cross validations. Errors of prediction (CV error) are respectively 0.312 and 0.277 for *C. maculosa* and *S. inaequidens*.

Abiotic and biotic factors did not impact target survival during the first vegetation season whereas they had significant effects on *C. maculosa* and *S. inaequidens* survival at the end of the second vegetation season (Table 2). At the end of the second vegetation season, both species were affected by management. Survival under unselective clipping was very low since only one individual of each species survived. Aggregated functional traits of the community, *i.e.* competitive effect, had a significant impact on *C. maculosa*'s survival, whereas both competitive effect and community dynamics affected survival of *S. inaequidens*.

Lastly, there were no differences of survival between geocytotypes of *C. maculosa* regardless of vegetation season. By contrast, survival of *S. inaequidens* differed according to geocytotypes at the end of the second vegetation season since no diploid individuals of *S. inaequidens* survived.

Table 2: Results of generalised linear models testing effects of management, geocytotype, diversity, competition and community dynamics on *C. maculosa* and *S. inaequidens* survival at the end of the first and the second vegetation season.

First vegetation season

		Centaurea maculosa		*Senecio inaequidens*	
	df	*F*-value	*P*-value	*F*-value	*P*-value
Management	1	<0.001	1	0.605	0.437
Geocytotype	2	4.612	0.091	2.363	0.307
Diversity	1	0.577	0.448	0.035	0.852
Competition	1	0.008	0.928	1.598	0.206
Dynamics	1	2.912	0.088	0.321	0.571
Residuals	41				

Second vegetation season

		Centaurea maculosa		*Senecio inaequidens*	
	df	*F*-value	*P*-value	*F*-value	*P*-value
Management	1	7.461	**0.006**	4.55	**0.032**
Geocytotype	2	0	1	6.869	**0.032**
Diversity	1	2.062	0.151	0.295	0.587
Competition	1	4.124	**0.042**	4.308	**0.038**
Dynamics	1	0.683	0.408	5.066	**0.024**
Residuals	41				

6.3.2.2. Growth and reproduction of surviving targets

Management treatment had significant effect on both species' fitness during the first vegetation season but not during the second one (Table 3). At the end of both vegetation seasons, growth of target species was not significantly different according to geocytotype.

Table 3: Result of linear models testing effects of management, geocytotype, diversity, competition and community dynamics on *C. maculosa* and *S. inaequidens* growth at the end of the first and second vegetation season.

First vegetation season

		Centaurea maculosa			*Senecio inaequidens*	
	df	*F*-value	*P*-value	df	*F*-value	*P*-value
Management	1	14.317	**<0.001**	1	11.594	**0.002**
Geocytotype	2	1.778	0.183	2	0.833	0.444
Diversity	1	1.222	0.276	1	0.335	0.567
Competition	1	0.0003	0.987	1	0.065	0.800
Dynamics	1	7.736	**0.008**	1	0.015	0.904
Residuals	37					

Second vegetation season

		Centaurea maculosa			*Senecio inaequidens*	
	df	*F*-value	*P*-value	df	*F*-value	*P*-value
Management	1	15.769	0.058	1	3.066	0.330
Geocytotype	2	2.041	0.329	2	0.527	0.600
Diversity	1	36.248	**0.027**	1	6.005	0.247
Competition	1	0.943	0.434	1	0.043	0.871
Dynamics	1	0.629	0.511	1	1.288	0.460
Residuals	2			1		

At the end of the first vegetation season, *C. maculosa*'s growth was significantly affected by community dynamics (Table 3) and more precisely by functional stability (Figure 3a). Indeed the lateral extension of the canopy of *C. maculosa* was positively correlated to the annual functional turnover of the community. At the end of the second vegetation season, growth of *C. maculosa* was significantly affected by community diversity (Table 3). Indeed, shoot and root biomass, lateral extension of the canopy and capitulum production were positively correlated with the proportion of grasses in the community and with the percentage of soil covered by vegetation. By contrast, root-shoot ratio was positively correlated with the proportion of legumes in the community (Figure 3b). Impact of all biotic factors was higher at the end of the second vegetation season than after the first one as shown by the matrix of RV coefficients (Table 4). Furthermore, the ranking of importance of biotic factors on target growth changed from the first vegetation season to the second one since dynamics was the most correlated matrices at the end of the first vegetation season, whereas diversity was more important at the end of the second vegetation season (Table 4).

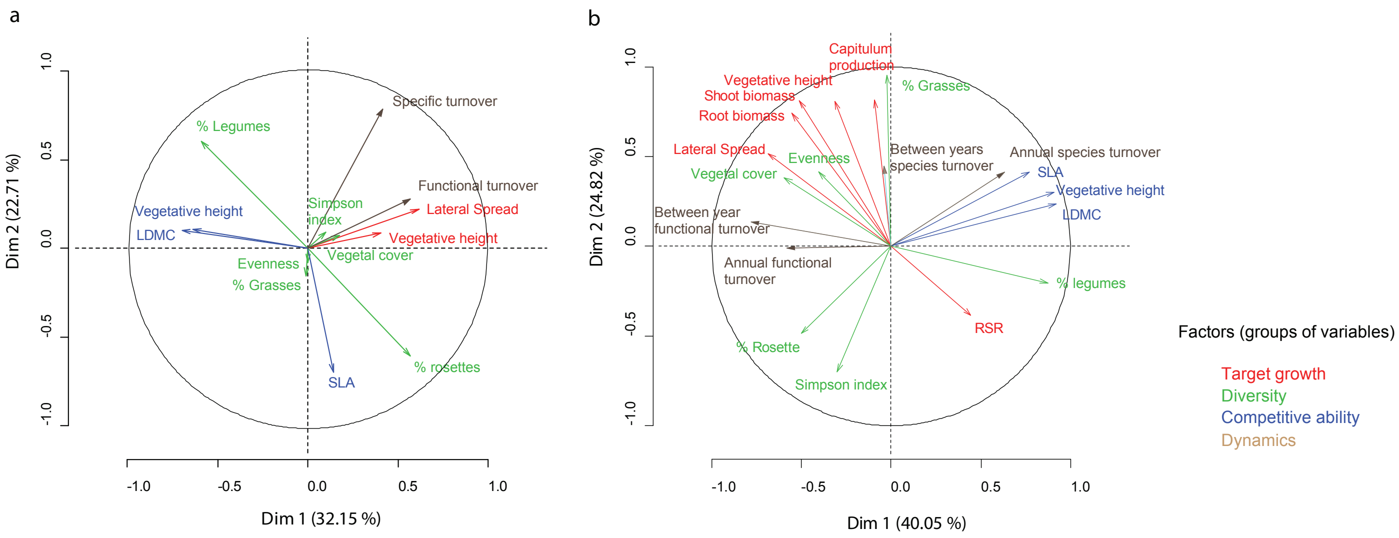

Figure 3: Correlation circles of variables used in MFA on *C. maculosa* at the end of the first (3a) and second (3b) vegetation season. Colours represent the four matrices used in the MFA, *i.e* surviving target growth (red), community diversity (green), community competitive effect (blue) and community dynamics (brown)

First vegetation season

	Target	Diversity	Competition
Diversity	0.033		
Competition	0.015	0.134 **	
Dynamics	0.054	0.056	0.044

Second vegetation season

	Target	Diversity	Competition
Diversity	0.363		
Competition	0.172	0.477 *	
Dynamics	0.141	0.324	0.392

Table 4: RV coefficients showing strength of relationships between matrices used in the MFA on *C. maculosa* growth at the end of the first and second vegetation seasons. RV coefficients range between 0 and 1 (0 signifying no relationship and 1 meaning perfect match between the two matrices). Significance of RV coefficients was tested with Monte Carlo permutation tests and is noted as follow: * $p < 0.05$, ** $p < 0.01$, *** p < 0.001.

Fitness of *S. inaequidens* was not affected by biotic factors, regardless the vegetation season (Table 3). However, at the end of both vegetation seasons, diversity was the factor the most correlated to *S. inaequidens'* fitness (Table 5). Indeed, lateral extension of the canopy was positively correlated with proportion of legumes in the community (Figure 4a), whereas capitulum production and vegetative height were negatively correlated to the proportion of grasses in the community and the evenness. At the end of the second vegetation season, vegetative height of *S. inaequidens* was positively correlated with the proportion of plants with basal rosettes, whereas root-shoot ratio was negatively correlated with this proportion (Figure 4b). Other target traits, *i.e* shoot and root biomass, lateral extension of the canopy and capitulum production were positively correlated with community SLA and vegetative height and negatively correlated with within-year turnover, either in terms of species or in terms of functional traits. As for *C. maculosa*, the correlations between biotic factors and target growth were higher at the end of the second vegetation season (Table 5). However, by contrast to *C. maculosa*, the ranking of importance of biotic factors was constant between the two vegetation seasons, diversity having more impact than respectively dynamics and competition.

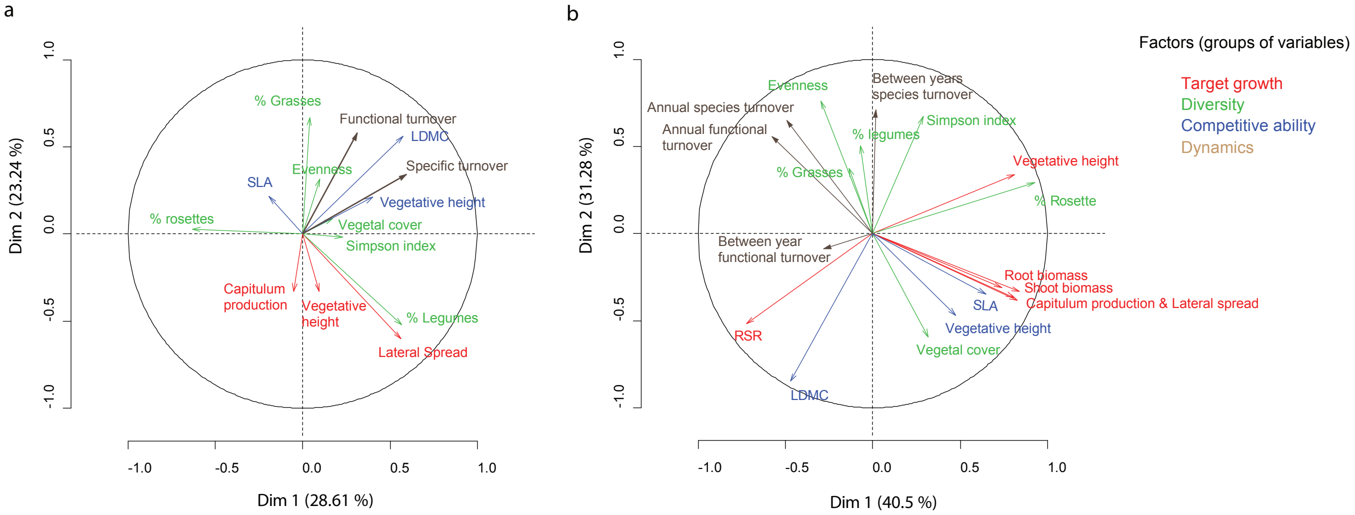

Figure 4: Correlation circles of variables used in MFA on *S. inaequidens* at the end of the first (3a) and second (3b) vegetation season. Colours represent the four matrices used in the MFA, *i.e* surviving target growth (red), community diversity (green), community competitive effect (blue) and community dynamics (brown)

First vegetation season

	Target	Diversity	Competition
Diversity	**0.114***		
Competition	0.003	0.063	
Dynamics	0.015	0.075	0.066

Second vegetation season

	Target	Diversity	Competition
Diversity	**0.428**		
Competition	0.023	0.512	
Dynamics	0.202	0.261	0.127

Table 5: RV coefficients showing strength of relationships between matrices used in the MFA on *S. inaequidens* growth at the end of the first and second vegetation seasons. RV coefficients range between 0 and 1 (0 signifying no relationship and 1 meaning perfect match between the two matrices). Significance of RV coefficients was tested with Monte Carlo permutation tests and is noted as follow: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

6.4. Discussion

The aim of this study was to test whether the different geocytotypes of invasive species performed differently in natural communities and to disentangle the effects of some abiotic and biotic factors in invasibility of the community.

Overall geocytotype had little effect on target survival and growth since only survival at the end of the second vegetation season of *S. inaequidens* was affected by geocytotype. Indeed, no diploid individuals of *S. inaequidens* survived at the end of the second year, regardless of the management treatment applied. This result could be linked to species' life cycle since it has been argued that polyploidisation could cause a shift from an annual or biennial life cycle to a perennial one (Muller 1989). Surviving targets' growth was not significantly different according to geocytotypes, which indicates that all geocytotypes had the same ability to establish in this grassland community. Lastly, survival of both model species at the end of the second vegetation season was highly limited by community vegetative height. A tall vegetation might reduce light availability and may be used as a proxy for community productivity. Negative effect of light reduction and community aboveground biomass on invasive seedlings establishment success has already been demonstrated in previous experimental studies (Emery and Gross 2007; Milbau *et al.* 2005).

Management treatment affected target growth during the first vegetation season and target survival during the second one. Only few individuals survived under the unselective disturbance (*i.e.* mowing). Thus management treatment could be considered as one of the most important factor influencing establishment success. From a management point of view, mowing frequently invaded areas could limit invasion success of *C. maculosa* and *S. inaequidens*.

Importance of biotic factors on invasion success seems to evolve according to invader's life stage. Indeed, in our experiment, biotic factors had more impact on target survival and growth during the second vegetation season than during the first one. Furthermore, it seems that abiotic factors, such as management or resource release, impact the first stages of invasive species growth whereas biotic factors affect the later stages. Moreover, the importance of different biotic factors on invasive fitness can also change with invasive's life stage. Indeed, while *C. maculosa* was mainly affected by community dynamics during its first stages, community diversity became the most important factors affecting growth at the later stages. By contrast, none of the biotic factors affected significantly growth of surviving individuals *S. inaequidens*, which may indicate either that we did not measured the variables that affect S. *inaequidens* growth or that this invasive species is not significantly affected by biotic interactions.

Diversity has often been linked to invasibility despite controversial results. In our experiment, diversity component did affect invasive species growth. However, Simpson diversity index was not the variable that affected species growth. Indeed, *C. maculosa* was mainly affected by the proportion of grasses in the community and to a lesser extent by community evenness whereas *S. inaequidens* was mainly affected by the proportions of legumes and plants with basal rosette. Therefore, functional dominance, and to a lesser extent evenness, may affect invasive species fitness more than species diversity *per se* (Emery and Gross 2007). Evenness was positively correlated with *C. maculosa* growth and flowering, and negatively with root-shoot ratio. Thus, community with high evenness led to investment in aboveground growth and reproduction, whereas community with low evenness led to investment of resources in belowground tissues. Since invasion mechanism of *C. maculosa* is based on plant soil interactions (Callaway *et al.* 2004; Callaway and Vivanco 2007; Perry *et al.* 2005; Weir *et al.* 2003), high evenness, by limiting resource allocation to belowground tissues, may limit invasion success. The negative correlation between evenness and invasibility has already been found in field experiments (Tracy and Sanderson 2004; Wilsey and Polley 2002) and can be explained by efficiency of resource use that limits available resources for invasive species (Emery and Gross 2007).

In our experiment, dominant species had significant impacts on invasibility. Indeed, both species fitness was affected by proportion of grasses, legumes or plant with basal rosette in the community. Since proportion is linked to dominance, both species seemed to be affected by dominant species identity. During the second vegetation season, *C. maculosa'*s resource allocation to aboveground growth and reproduction was enhanced under grass dominance whereas vegetative height of *S. inaequidens* was enhanced by legumes dominance during the first vegetation season and dominance of plants with basal rosette during the second one. Moreover, aboveground growth and space occupation of *C. maculosa* was significantly positively correlated with community functional turnover during the first growing season. Since community aggregated traits were based on dominant species traits and their abundance, growth of *C. maculosa* was enhanced by high rate of changes in dominant species and therefore the consequent change in community aggregated values of traits. We conclude that the first establishment stages of *C. maculosa* were enhanced by high turnover rate. Consequently, its establishment success should be limited by functional stability. The positive effect of community stability on invasibility has already been studied in terms of compositional stability (Foster *et al.* 2002), but to our knowledge, this study is the first one to demonstrate the effect of functional stability on invasibility.

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Chapter 7

Synthesis

General discussion and perspectives

7.1. Introduction

The objective of my thesis was to disentangle invasiveness from invasibility in the invasion process. These two aspects of invasion ecology have hardly been studied together despite the fact that invasion success is recognised to be the result of the interaction between the ability of the species to invade and the lack of resistance of the recipient community. In this thesis, all the experiments were conducted with native and introduced genotypes of two worldwide invasive species, *Centaurea maculosa* and *Senecio inaequidens*, to study genotype-specific effects of environmental factors. The aim of this chapter is to give a general overview of this thesis. I will summarise and synthesise main results of all previous chapters in order to highlight the importance of the interaction between species invasiveness and community invasibility for the invasion success of invasive species. More particularly, I asked two main questions:

- What are the invasion strategies of the model species?
- What are the effects of (i) community diversity, (ii) neighbouring competition, (iii) community spatial pattern and (iv) community dynamics on the establishment success of invasive species' genotypes?

In the first part of this chapter, I will present growth and invasive strategies of the two model species, mainly based on results of chapters 2 and 4. In a second part, I will discuss the interactions between invasiveness and invasibility for each studied species (Chapters 2, 3, 5 and 6). In the third part, I will attempt to define a hierarchy of the different factors influencing invasion success. Fourthly, I will present some implications of my results for management practices and finally I will acknowledge some limits of this thesis and suggest some research perspectives in the continuity of this work.

7.2. Growth and invasion strategies of model species

7.2.1. Different growth strategies leading to two different invasion strategies

Based on the measurement of functional traits relative to competitive ability (Chapter 2), both model species can be considered as competitive and exploitative species. However, higher values of leaf dry matter content and root-shoot ratio for *C. maculosa* as compared to *S. inaequidens* revealed a slow nutrient cycling strategy, based on resource conservation and investment in belowground tissues. By contrast, the growth strategy of *S. inaequidens* is rather based on fast acquisition of nutrients and investment in growth and photosynthesis. The different growth strategies of the model species, as well as their different life forms (basal rosette for *C. maculosa vs.* erect stem for *S. inaequidens*), could be linked to their different invasion strategies.

Growth experiments in pot (Chapter 2), field (Chapter 3) or artificial communities (Chapter 5) showed that introduced tetraploids genotypes of *S. inaequidens* produced more capitula than native ones. Thus, high propagule pressure most likely explains the invasion success of *S. inaequidens*. An additional field observational study in a sheep-grazed meadow recently invaded by *S. inaequidens* (Aosta Valley, Italy) supports this result (Appendix 2). The invasion mechanism of *C. maculosa* seems to be based on plant-soil interactions, as suggested by the measurements of plant root-shoot ratio and the analysis of rhizosphere communities (Chapters 2 and 4). Compared to native genotypes, very few resources are allocated to aboveground growth and reproduction of introduced genotypes. Analyses of rhizosphere microorganisms showed a shift in bacterial community composition from native genotypes to introduced ones. Since few years, allelopathy and more precisely release of (±) catechin has been suggested as a mechanism for invasion success of *C. maculosa* (Bais *et al.* 2002; Callaway and Ridenour 2004; Ridenour and Callaway 2001). More recently, this theory has been disputed since it was argued that the amounts of (±) catechin released by the plants in natural conditions are not high enough to allow inhibition of neighbouring plants (Blair *et al.* 2006). Although the chemical compounds in the soil were not measured, I suggest that the mechanism involved in the invasion process of *C. maculosa* is related to exudation of secondary compounds. As stated by the novel weapon hypothesis (Callaway and Ridenour 2004), these compounds can alter plant-soil interactions since they have never been experienced before by resident species of the invaded communities. However, more detailed studies are needed to investigate which compound(s) affect the bacterial communities of *C. maculosa* and this would represent a good follow-up study to this thesis.

7.2.2. *On the importance of polyploidisation*

Polyploidisation, by changing biotic interaction outcomes, seems to have been an essential evolutionary step in the invasion process of *C. maculosa* and *S. inaequidens*. According to recent studies, both diploid and tetraploid genotypes would have been introduced in the new range but only tetraploid ones managed to establish (Lafuma *et al.* 2003; Treier *et al.* in press). The importance of polyploidisation in invasion success has also been recently demonstrated for *Solidago gigantea* (Schlaepfer 2008).

Polyploidisation in the native range allowed both species to increase their competitive ability through either higher vegetative height (*S. inaequidens* - Figure 1a) or higher growth rate (higher SLA for *C. maculosa* - Figure 1b). This increase in competitive ability for the tetraploid genotypes most likely allowed their successful establishment in the new range. Once established in the new range, a further selection towards either reproduction effort (increase in capitulum production of *S. inaequidens* – Figure 1a) or investment in below ground tissues (increase of root-shoot ratio (RSR) of *C. maculosa*- Figure 1b) further enhanced their spread and invasion success. Therefore, the invasion success of both species can be explained by traits trade-offs:

- between competition and reproduction (*S. inaequidens*, Figure 1a)
- between aboveground competition and allocation of resources to roots (*C. maculosa*, Figure 1b).

The trade-offs that occurred in the new range might be a second essential step for invasion success and can explain the time lag between species first introduction and the beginning of invasion.

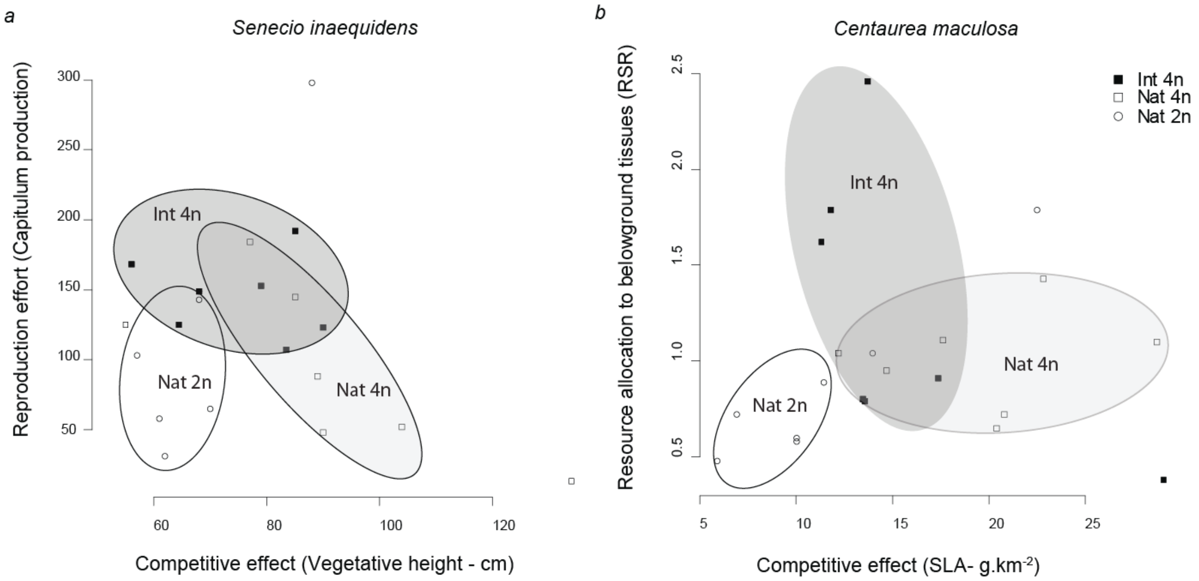

Figure 1: Illustration of trade-offs between competition and reproduction (*S. inaequidens*, 1a) and between competition and resource allocation to belowground parts (*C. maculosa*, 1b), using data from the pot experiment (Chapter 2).

7.3. Invasiveness * invasibility

Since polyploidisation seems to have been an essential step in invasion success of both species, the interaction between invasiveness and invasibility will be focused on the results of tetraploid individuals only.

Invasion success is the result of the interaction between abiotic and biotic factors at the community level and invasive ability of the introduced individual. Therefore, phenotypic plasticity, which is the ability of a genotype to express different phenotypes in changing environments (Bradshaw 1965) may explain invasion success either by the ability of the invader to maintain a high fitness in unfavourable environments, or its ability to take advantage of favourable conditions by increasing fitness. Robustness of fitness under unfavourable conditions is defined as a Jack-of-all-Trades strategy, whereas opportunism under favourable conditions is defined as a Master-of-Some strategy. Since these two strategies are not exclusive, a strategy combining robustness under unfavourable conditions and opportunism under favourable conditions is defined as a Jack-and-Master strategy (Richards *et al.* 2006). Comparing fitness of native and introduced genotypes of invasive species under the same environmental constraints helps understanding whether invasion success can be explained by higher phenotypic plasticity of introduced genotypes than native ones.

In the different experiments of this thesis, I tested the effects of resource release following disturbance (Chapter 3), community spatial pattern (Chapter 5), community diversity (Chapters 5 and 6), biotic competition (Chapter 6), community dynamics (Chapter 6) and management (Chapter 6) on native and introduced tetraploid genotypes of *C. maculosa* and *S. inaequidens*. Their survival was highly affected by community spatial pattern and management (and consequently resource release) and neighbouring competition. Responses of growth and reproductive output of native and introduced genotypes to tested factors were species-specific. Growth and reproductive output of both genotypes of *S. inaequidens* were affected by biotic and abiotic factors, whereas introduced genotypes of *C. maculosa* were less affected than native ones. I will discuss these results in the light of the three invasive strategies (Jack-of-all-Trades, Master-of-Some and Jack-and-Master) defined above.

7.3.1. Centaurea maculosa, a Jack-of-all-Trades invader

Figure 2 shows that resource release, community diversity and community spatial patterns did not affect native and introduced genotypes with the same intensity (Chapters 3 and 5). Indeed, native genotypes performed better in favourable conditions, *i.e* with stochastic resources releases or in aggregated communities with high diversity, whereas introduced genotypes' performance was independent of the stress encountered (Figure 3). The fact that native and introduced genotypes' traits respond consistently to different biotic and abiotic factors provides some evidence on the invasive strategy of *C. maculosa*. Being able to maintain fitness in all kinds of environments, either favourable or stressful, I define introduced genotype of *C. maculosa* as a Jack-of-all-Trades invader (Figure 2).

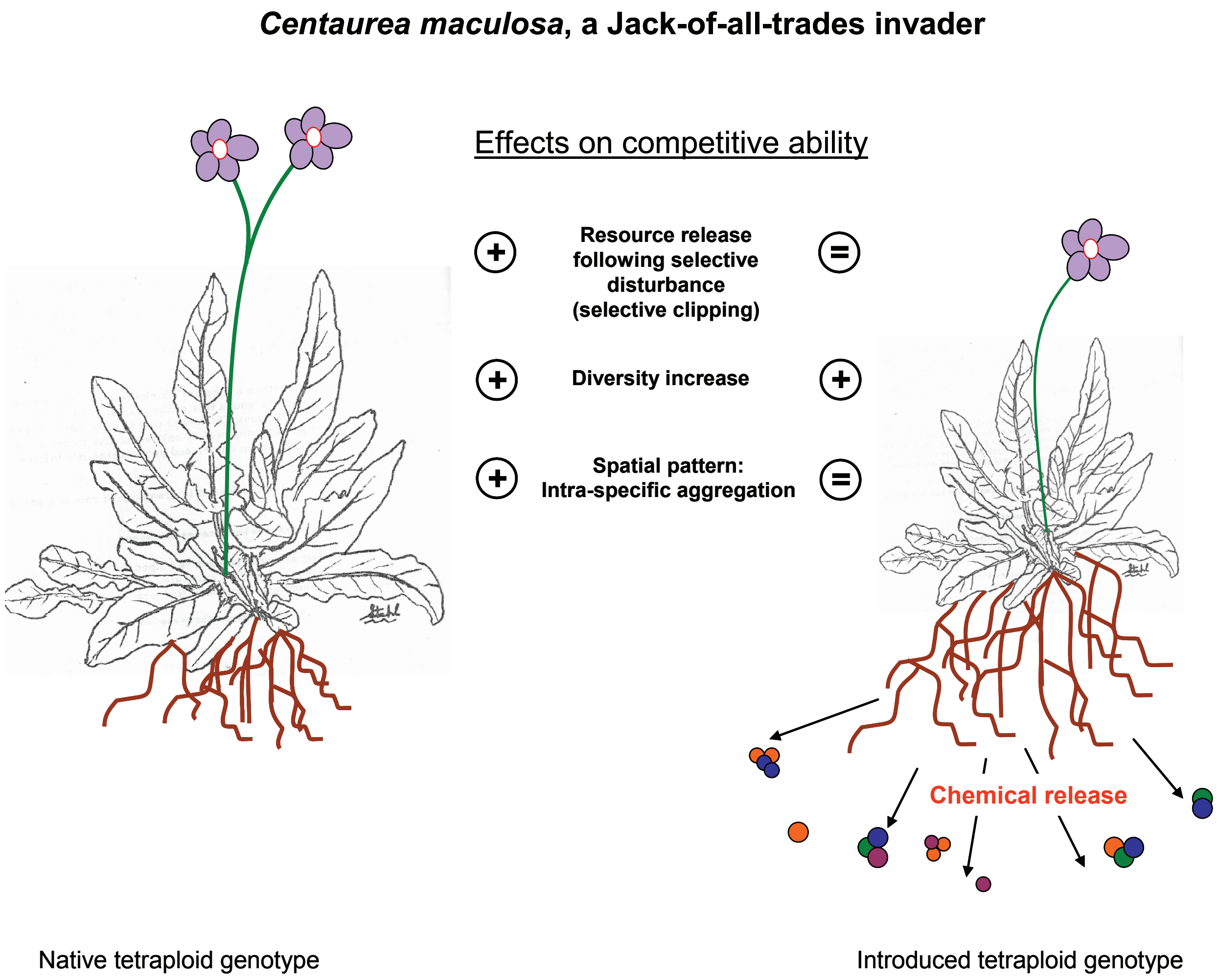

Figure 2: Invasion mechanism based on different traits and genotypes-specific effects of abiotic (stochastic resources release following a disturbance) and biotic (community diversity, and spatial pattern) factors on competitive ability (lateral extension of the basal rosette or aboveground biomass) of native and introduced tetraploid genotypes of *C. maculosa*. Native genotypes are characterised by high allocation of resources to aboveground growth whereas introduced ones invest resources preferentially in belowground tissues.

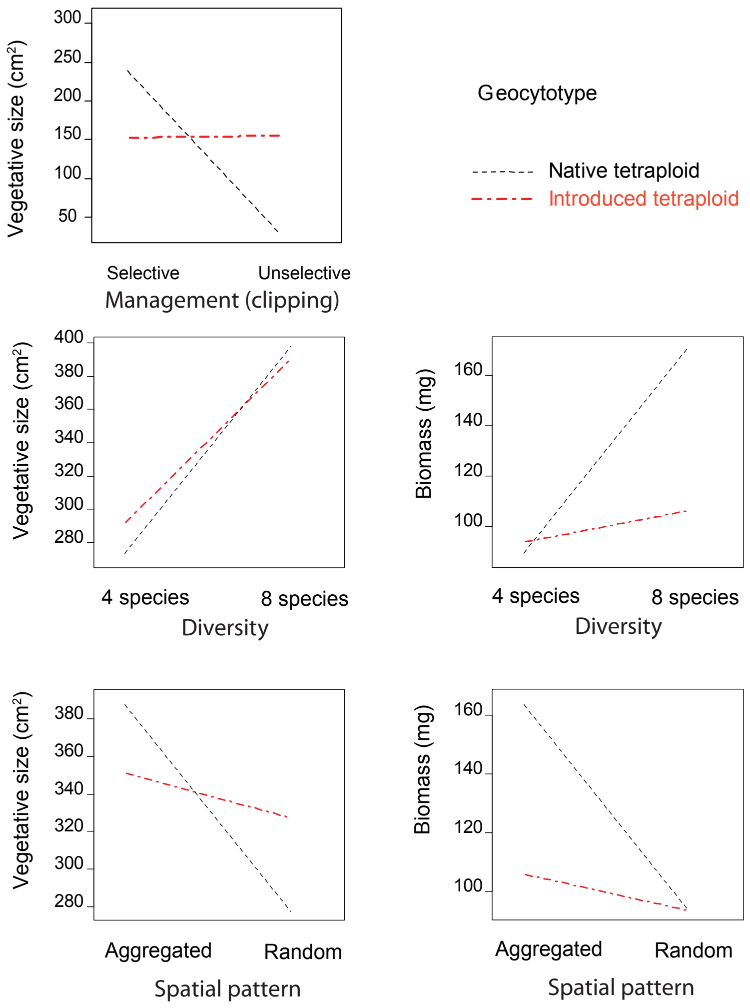

Figure 3: Interaction plots showing the effects of resource release following disturbance (unselective *vs.* selective clipping, Chapter 3), community diversity (4 species *vs.* 8 species) and spatial pattern (aggregated *vs.* random, Chapter 5) on vegetative size (*i.e.* lateral extension of the basal rosette) and shoot biomass of native ("Nat 4n", dashed black lines) and introduced ("Int 4n", dotdashed red lines) genotypes of *C. maculosa*

Biotic and abiotic factors did not markedly affect fitness of introduced genotypes of *C. maculosa.* Therefore, the species might be able to invade many types of environments. If *C. maculosa*'s strategy of invasion is indeed linked to interactions with the soil microorganisms via allelopathic effect of novel compounds, introduced genotypes of *C. maculosa* should be able to invade any area in which soil microorganisms did not co-evolve with allelopathic compounds released by this plant. Therefore, in the context of a potential re-introduction of North-American genotypes in Europe, I would predict that the risk of invasion in Europe is not insignificant as soon as *C. maculosa* enters a community where soil microbial communities did not follow the same co-evolutionary trajectories as itself (Callaway and Vivanco 2007; Hallett 2006).

7.3.2. *Senecio inaequidens, a Jack-and-Master invader*

Both genotypes of *S. inaequidens* responded to resource release, community diversity and spatial pattern (Figure 4) but introduced genotypes were generally less affected than native ones, especially for capitulum production (Figure 5). Since introduced genotypes had almost always a higher fitness than native ones, *S. inaequidens* can be considered as an invasive species with a Jack-and-Master strategy, *i.e* a species able to deal with all kinds of environments and to increase its fitness in favourable conditions.

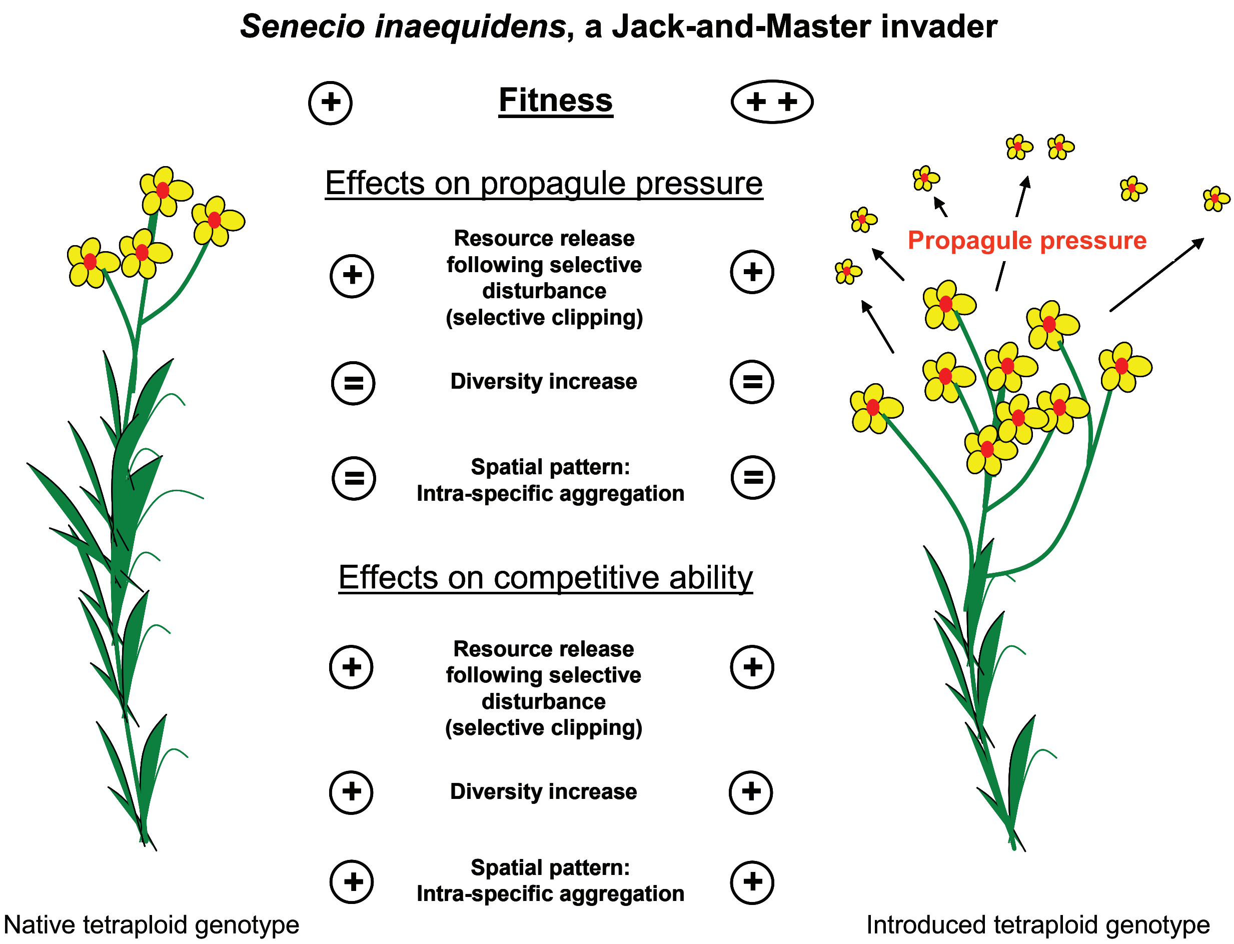

Figure 4: Invasion mechanism based on different traits and genotypes-specific effects of abiotic (stochastic resources release following a disturbance) and biotic (community diversity and spatial pattern) factors on propagule pressure (capitulum production) and competitive ability (vegetative height or aboveground biomass) of native and introduced tetraploid genotypes of *S. inaequidens*. Native genotypes are characterised by a tall vegetative stem whereas introduced genotypes invest resources preferentially in capitulum production.

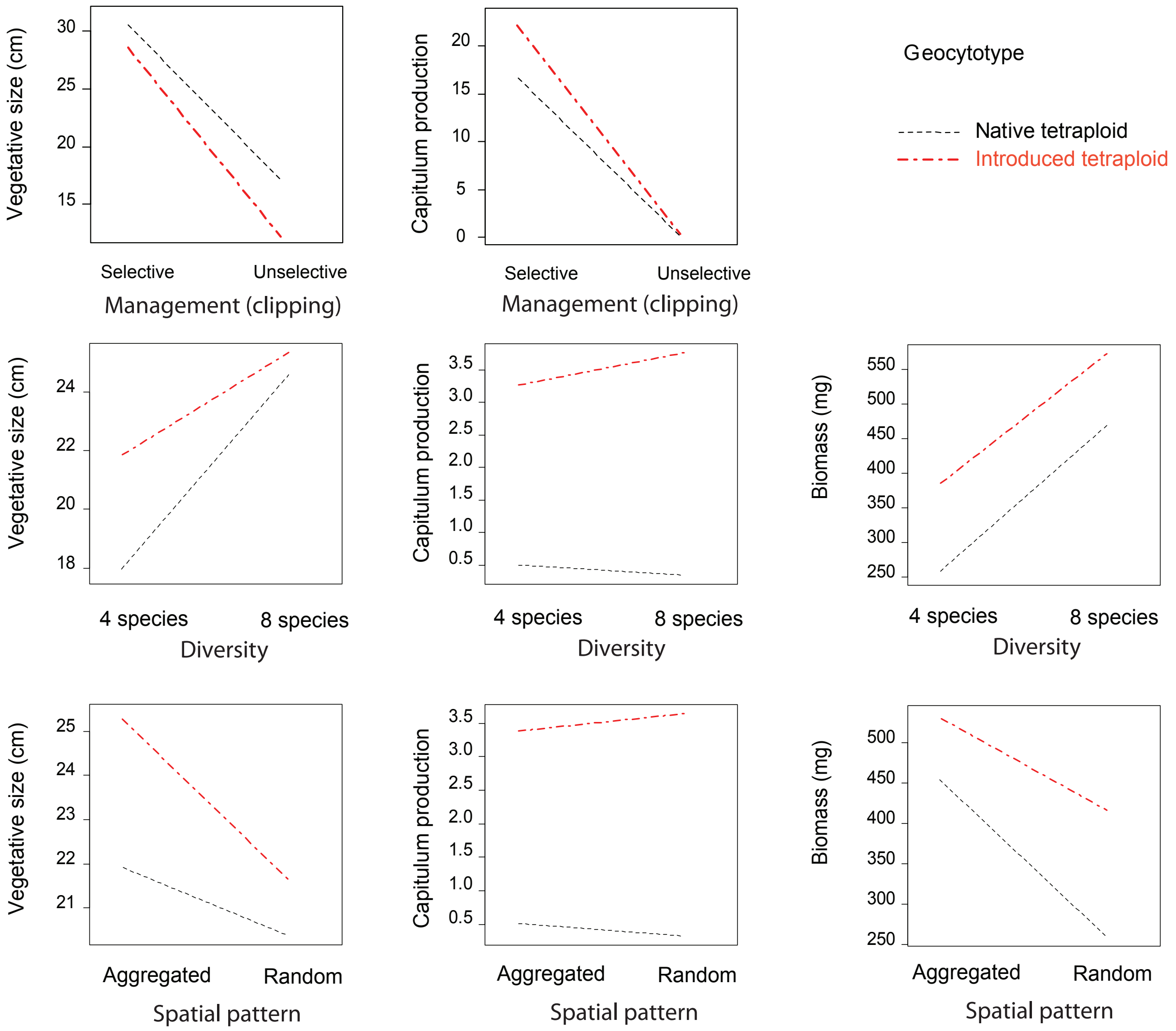

Figure 5: Interaction plots showing effects of resource release following disturbance (unselective *vs.* selective clipping, Chapter 3), community diversity (4 species *vs.* 8 species) and spatial pattern (aggregated *vs.* random, Chapter 5) on vegetative size (*i.e.* vegetative height), shoot biomass and capitulum production of native (Nat 4n, dashed black lines) and introduced (Int 4n, dotdashed red lines) genotypes of *S. inaequidens.*

One could think that finding the appropriate unfavourable conditions might help to manage *S. inaequidens'* invasion. According to the strategy of invasion described in the previous section, studying response of capitulum production to abiotic and biotic factors is essential to understand invasion success. Capitulum production was unaffected by community changes in diversity and spatial pattern (Figure 4). The same was observed in the field experiment, where we did not found any significant effect of community diversity, community dynamics and neighbouring competition on *S. inaequidens*'s fitness in general (Chapter 6), and on capitulum production more particularly. Therefore, despite a decrease in competitive ability due to some biotic conditions, capitulum production of introduced tetraploid was not affected by community changes. According to results of the experiments, only unselective clipping could decrease capitulum production of *S. inaequidens* and therefore limit invasion success.

7.4. Towards a classification of impacts of biotic and abiotic factors

To understand invasion success, factors that limit establishment success of introduced tetraploid genotypes during the different steps of their invasion (survival, competitive interactions leading to growth and reproduction leading to spread - Figure 1 of the Chapter 1) were organised into a hierarchy. I analysed the evolution of the ratio of introduced/native genotypes for survival, vegetative size, biomass and capitulum production of *S. inaequidens* and *C. maculosa* from favourable (selective clipping, diversity 8, aggregated community) to unfavourable conditions (unselective clipping, diversity 4, randomly assembled communities) as defined in the previous section (see also Appendix 3). Table 1 shows the importance of the effects of management treatment, community diversity and community spatial pattern at each step of the invasion process.

Table 1: Importance of the effects of abiotic and biotic factors at the different steps of invasion of *C. maculosa* and *S. inaequidens*. (+ + + :factor with the highest impact on the considered variables, + : factor with the lowest impact on the considered variable).

		Abiotic factor	Biotic factors	
Step of invasion	Measured variables	Management	Diversity	Spatial pattern
Centaurea maculosa				
1- Survival	Survival	+	**+ + +**	+ +
2- Competitive interactions	Vegetative size	+	**+ + +**	+ +
3- Growth	Aboveground biomass		+	**+ +**
4- Reproduction	Capitulum production			
Senecio inaequidens				
1- Survival	Survival	**+ + +**	+	+ +
2- Competitive interactions	Vegetative size	**+ + +**	+	+ +
3- Growth	Aboveground biomass		**+ +**	+
4- Reproduction	Capitulum production	+ +	**+ + +**	+

It is thus possible to define, for both invasive species, two invasion phases, the introduction phase and the establishment phase, which are impacted by different factors (Figure 6). The introduction phase corresponds to the survival of seedlings and their ability to deal with neighbouring competition. If seedlings manage to survive despite neighbouring competition, they grow and reproduced in order to spread, which corresponds to the establishment phase.

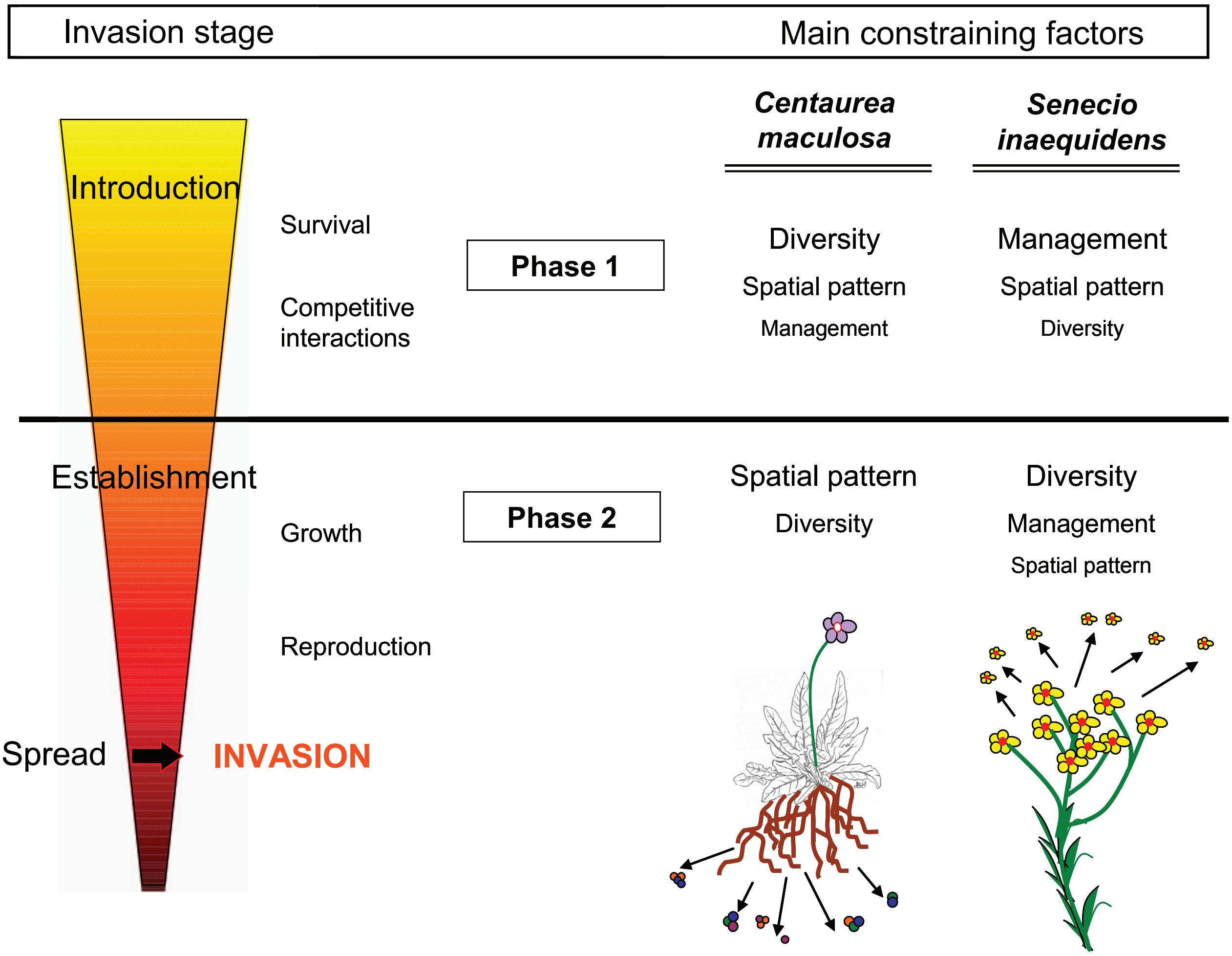

Figure 6: Illustration of the main abiotic and biotic factors impacting introduction and establishment phase of *C. maculosa* and *S. inaequidens*. Size of characters for each factor (abiotic and biotic) reveals the importance of this factor for the considered phase of invasion.

The introduction phase of *C. maculosa* is mainly affected by community diversity whereas the effect of spatial patterns is more important in the establishment phase. These findings are confirmed by results from the field experiment on effects of community diversity, dynamics and neighbouring competition on seedling fitness (Chapter 6), where community diversity (*sensu lato*) affected *C. maculosa*'s seedlings fitness during the first year of the experiment but not in the second one. Since community dynamics had significant impact during the second year of experiment, we could conclude that temporal patterns also affect the establishment phase.

Disturbance has more impact on *S. inaequidens* than on *C. maculosa*. Indeed, management is the most important factor affecting the introduction phase of *S. inaequidens*. It is likely that the erect stem of *S. inaequidens* is more often directly affected by mowing than the basal rosette of *C. maculosa*, decreasing the competitive ability of *S. inaequidens*' seedlings. This result has large impacts in terms of management perspectives. Diversity is the main factor that influences the establishment phase of *S. inaequidens*. This conclusion is confirmed by the results from the field experiment (Chapter 6) where we found a high correlation between *S. inaequidens'* fitness and community diversity (*sensu lato*).

The synthesis of all the experiments conducted in this thesis with *C. maculosa* and *S. inaequidens* highlights (1) the importance of polyploidisation in the invasion process as well as (2) the species-specific invasion strategies and consequently (3) the species-specific response of invasive species to abiotic and biotic factors. It also emphasizes on (4) the temporal evolution of the interaction between invasiveness and invasibility since the community factors that affects invasive species fitness change according to the invasion stage (introduction *vs.* establishment phase) of the invader (Figure 6).

7.5. Management implications

Invasive species are a threat to biodiversity and ecosystem functioning and there is a strong belief that they will increase in number and abundance following global warming (Thuiller *et al.* 2008). It is almost impossible to predict precisely which species may become invasive in the future (Broennimann and Guisan 2008), however being able to deal with them once established so as to limit their spread and therefore their environmental damages is of major importance.

As shown in this thesis, no single solution can be found for all invasive species and management efforts have to be targeted towards each individual case. I showed that *S. inaequidens* invaded through high propagule pressure and dissemination (Chapter 1) and that among the factors, unselective clipping was the only treatment able to decrease propagule production of introduced genotypes (Figure 4). Therefore, as a way of controlling *S. inaequidens'* expansion, I suggest mowing several times per year whenever possible. The mowing treatment should occur during flowering or even just before fructification. In this way, the plant will have spent an important amount of resources in capitulum production without any success of dissemination, leading to a decrease in population fitness in the long term. In traditionally grazed pastures, since *S. inaequidens* is avoided by cattle, it seems necessary to monitor flowering and to cut manually whenever capitula appear.

Management options for *C. maculosa* are less obvious since (1) the way of invasion seems to be based on belowground interactions and (2) introduced genotypes are quite safe regarding changes in environmental conditions (Figures 2 and 3). Mowing could also limit invasion success of *C. maculosa* as resprouting after unselective clipping was low (Chapter 3 and 5). However, cutting only the aboveground parts will not prevent plant-soil interactions and therefore might fail in reducing *C. maculosa*'s invasion success. Moreover, *C. maculosa* seems to have the ability to resprout in extreme conditions (resprouting occurred in dark conditions at 4°C temperature, personal observation). I guess that, giving enough time, *C. maculosa* is able to resprout and therefore to maintain a self-sustainable population. Thus, cutting may not limit *C. maculosa* invasion success at local scale but could have an effect at larger scale since mowing aboveground parts will still limit capitula production and therefore seed dissemination and spread. Limited community diversity appeared to limit the introduction success (Figure 6). However, from an ecological point of view, it would be controversial to orient management options towards lower diversity to control invasive success of *C. maculosa*. The negative effect of low diversity in my experiment could be due to the higher abundance of the clonal species *Trifolium repens* in low diversity plots as compared to high diversity plots. A plant with a basal rosette, like *C. maculosa,* requires space to establish and a high vegetation cover could limit germination and growth success of such plants. Therefore, the use of highly covering species, such as clonal ones, could allow limiting establishment success. However, more precise analyses of the performance of *C. maculosa* in *Trifolium* spp. monocultures as compared to other monocultures (aggregation treatment in Chapter 5) are needed to formerly conclude on the effect of *Trifolium* spp. on establishment success of *C. maculosa.*

7.6. Limits and perspectives

As in many field studies, my main constraint was the short term character of the field experiments. Following growth and reproduction of invasive species for several years will certainly help to get a better understanding of the interaction between species invasiveness and community resistance (invasibility). However, a second major constraint, more ethical than scientific, appears when dealing with invasive species. Working with invasive species involves some particular precautions concerning dissemination. A notification was written in collaboration with Francis Cordillot from the Swiss federal office for the environment (OFEV-BAFU) to define the protection measures to be taken during and after the experiments (Appendix 4). All capitula of all genotypes of *C. maculosa* and *S. inaequidens* had to be regularly cut in the experiments. I agree that this could have influenced the absolute outcome of resource allocation and growth. However, since capitula were cut for all genotypes, we can consider cutting as a treatment applied to all genotypes in all experiments.

Another major limit of this thesis was the low number of populations used for the experiments. The unit of replication was the geocytotype level. Therefore, I tried to take as much populations as possible within geocytotypes to get a “mean response” at the geocytotype level, but I was also limited by the number of available seeds. This choice led to the absence of replication at the population level. I acknowledge that there could be large differences among population responses to experimental factors and that I did not address this question.

Many invasive species have undergone polyploidisation. As we suggested, polyploidisation might be a first essential step to invasion success. However, the effects of polyploidisation on biotic interactions have hardly been studied. We showed that polyploidisation affected rhizosphere communities (Chapter 4). Since polyploidisation have already been shown to increase secondary metabolites production (De Jesus-Gonzalez and Weathers 2003; Dhawan and Lavania 1996; Kim *et al.* 2004), more precise studies on the changes in type and amount of metabolites released to the soil following polyploidisation are required to understand the role of plant-soil interactions in invasion success. I think that changes in chemical composition of aboveground tissues should also be analysed since it could also affect plant-soil interactions *via* litter decomposition.

Despite the growing evidence of the importance of plant-soil interactions in ecological processes, belowground mechanisms have hardly been studied so far in the ecology of invasion. Studying belowground competition mechanisms in response to community diversity or spatial pattern could provide critical novel knowledge on the interrelations between aboveground, belowground processes and invasion success. The "compartimentation" of topics in ecology tends to disfavour our understanding of general processes. Integrating aboveground and belowground aspects of community resistance as well as ecological and evolutionary process might give new insights in ecology of invasions.

The diversity-invasibility relationship has often been studied in the last decades and the nature of this relation has been proven to depend on the scale considered (Fridley *et al.* 2007). At small scale, diversity should limit invasive success. Our unexpected results of manipulation of diversity at small scale (Chapter 5) raise the question of co-varying factors (Fridley *et al.* 2007; Wardle 2001). Diversity and productivity often co-vary in natural areas. Studies on the effect of productivity *per se* at the same small scale could increase our insights into interactions between community factors at small scale affecting invasibility.

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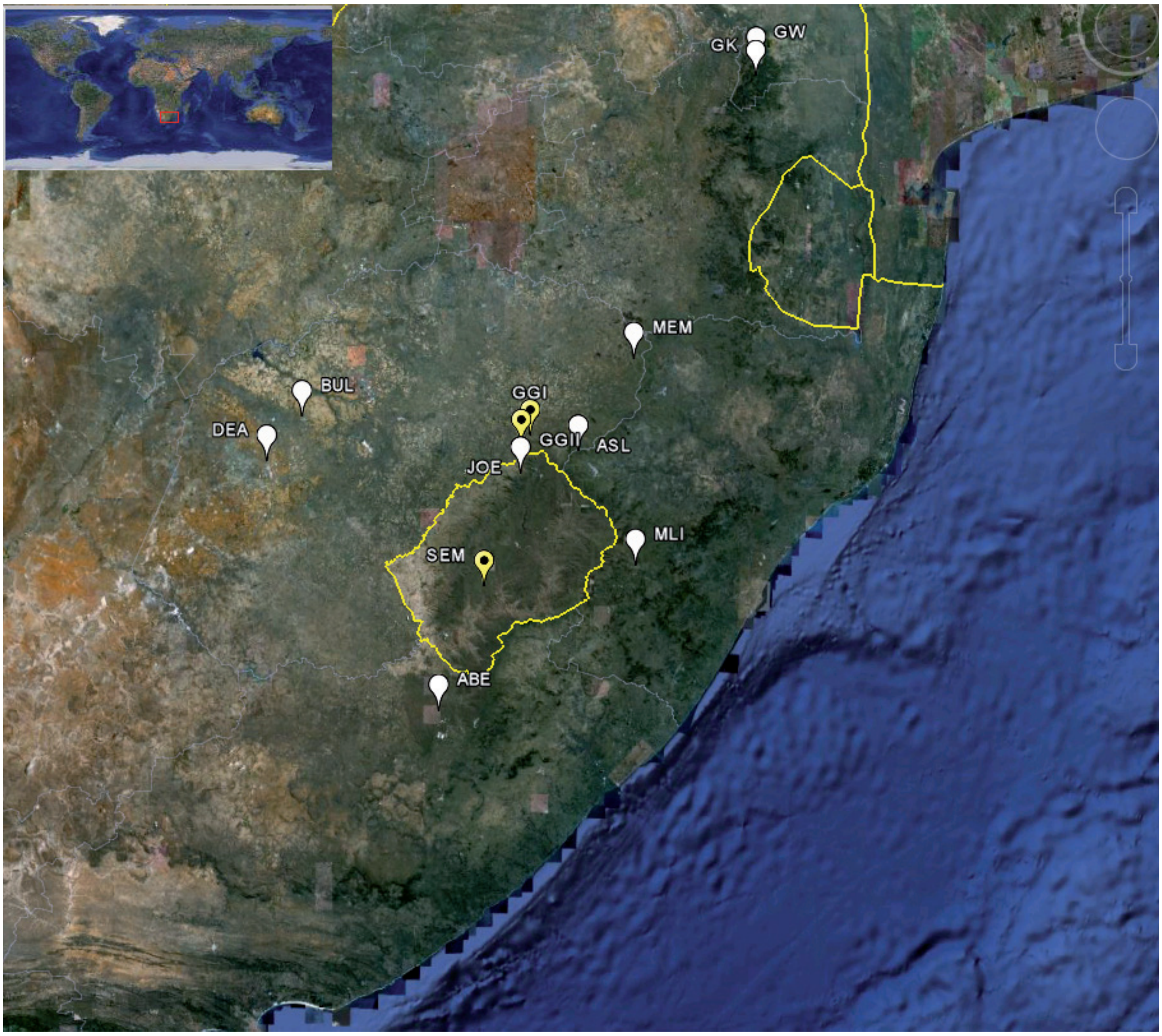
Appendices

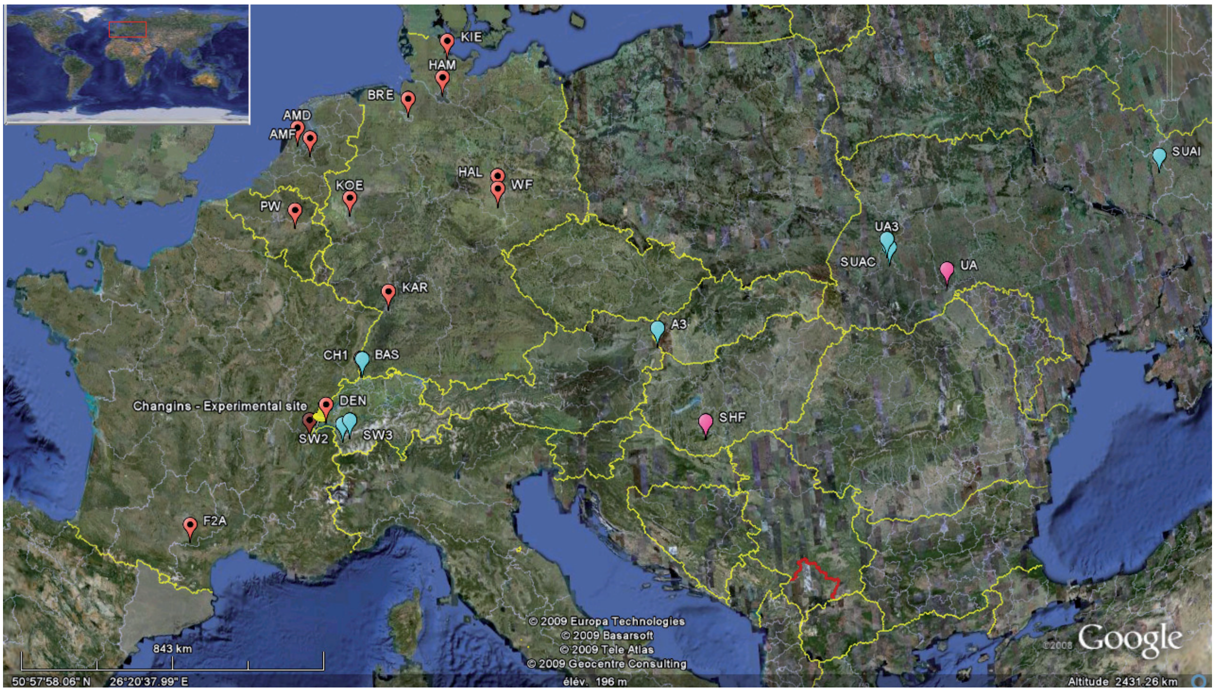
List of appendices

APPENDIX I

Maps of populations of *Centaurea maculosa* and *Senecio inaequidens* used in the experiments



Picture 1: Map of diploid (white) and tetraploid (yellow) populations of *S. inaequidens* sampled in its native range, *i.e.* South-Africa and Lesotho. (Google Earth Website: http://earth.google.com)



Picture 2: Map of introduced tetraploid populations of *S.inaequidens* (red) and native diploid (green) and native tetraploid (pink) populations of *C. maculosa*, sampled throughout Europe. (Google Earth Website: http://earth.google.com)

Picture 3: Map of introduced tetraploid populations of *C. maculosa* sampled in the new range, *i.e* North-America. (Google Earth Website: http://earth.google.com)

APPENDIX II

Study of a recent invasion process in Aosta Valley (Italy)

Site description

In Autumn 2006, the regional agronomic institute of Aosta was told that S. *inaequidens* appeared in the Lys valley and raised some agricultural issues. In the “Monte Rosa” valley, above Pont-Saint-Martin, Montcervier (45° 37’ 49” N, 7° 49’ 50” E) was one of the most invaded area of the Lys valley. According to stakeholders, the invasion of Montcervier sheep-grazed meadow by *S. inaequidens* began in 2001 after a fire that destructed much of the vegetation. This terraced grassland is composed of grazed terraces and walls (Pictures 1 and 2).

The aim of this observational study was to understand invasion process of *S. inaequidens* by studying a real case of invasion, at its beginning stage.

Picture 1 : Terraced grassland of Montcervier, Liliannes, Aosta Valley.

Methods

During July 2006, we measured traits of adult individuals and seedlings of *S. inaequidens* growing on the terraces, in the walls, at the bottom and the top of walls. We defined adult plants as plants presenting traces of buds, and seedlings as individuals that had never flowered until the first visit, in June 2006. In July 2006, we ended with measures of vegetative height, lateral extension of the canopy and number of capitula of 95 adult plants and 90 seedlings (table 1)

	Adults	Seedlings
Top walls	15	15
Middle walls	15	15
Bottom walls	15	15
Total walls	45	45
Terraces	50	45

Table 1: Number of individuals (adults and seedlings) measured (vegetative height, lateral spread and number of capitula) on walls and terraces during summer 2006

Picture 2: Panoramic view of the study site of MontCervier, Italy

Differences of vegetative height and lateral extension of the canopy between place of growth (walls *vs.* terraces) and individual life stage (adult *vs.* seedlings) were analysed using linear models. Vegetative height and lateral extension of the canopy (*i.e.* lateral spread) were log transformed prior to the analysis. Difference in number of capitula between walls and terraces was tested using generalised linear model fitted with a quasipoisson distribution to reduced over-dispersion.

Main results

Adults growing on walls were voluminous as shown by their mean vegetative height (Figure 1a) and lateral spread (Figure 1b). However, they did not have different vegetative height or lateral spread than individuals growing on terraces (Table 1), whereas they produced significantly more capitula (Table 1, figure 1c). Seedlings showed the same trends as indicated by the absence of significant interaction between place of growth and individual life stage (Table 1).

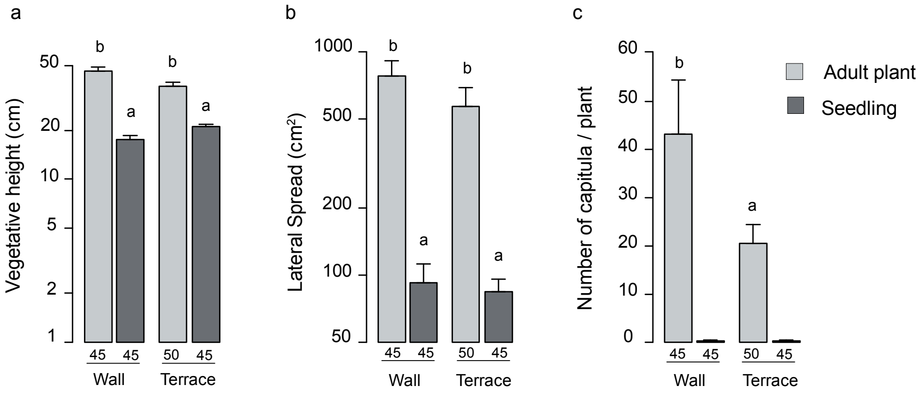


Figure 1: Vegetative height (cm), lateral extension of the canopy (cm^2) and capitulum production (number of capitula per flowering plants) according to *S. inaequidens'* place of growth (wall *vs*. terrace) and life stage (adult plant *vs.* seedling). Numbers on the x-axis indicate the number of observations. Bars indicate the standard error of the mean. Letters indicate significant differences ($p < 0.05$) according to Tukey post-Hoc tests. Vegetative height and lateral spread are represented on a logarithmic scale.

Table 1: Result of linear models (¶) and generalised linear model (§) testing effect of place of growth and target life stage on vegetative height, lateral spread and capitulum production of individuals of *S. inaequidens*.

		Vegetative height (¶)		Lateral Spread (¶)		Capitulum production (§)	
	df	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Place of growth	3	0.751	0.523	0.234	0.873	5.254	**0.002**
Lige stage	1	49.625	**< 0.001**	94.864	**< 0.001**	104.022	**< 0.001**
Place * Stage	3	1.308	0.273	0.703	0.552	0.181	0.91
Residuals	177						

Discussion

Vegetative height and lateral extension of the canopy are indicators of competitive effect of the individual plant (Goldberg and Landa 1991). Individuals growing on terraces did not show higher competitive effect than individuals growing on terraces. However, in this terraced grassland, biotic competition on walls was less intense than competition in terraces (personal observation). With low competition on walls, *S. inaequidens* can invest more resources in capitulum production. In terraces, it seems that the major part of resources are invested in competitive effect, as shown by the lower number of capitula produced in terraces compared to walls (Figure 1). With high capitulum production on walls, propagule pressure could be the invasion mechanism of *S. inaequidens* in this area (Lockwood et al. 2005).

In this sheep-grazed meadow, *S. inaequidens* settled first in low stressful areas (*i.e.* walls) which allowed the plant to allocate resources to capitulum production. Once established in these areas, a high propagule pressure allows invading more competitive areas (*i.e.* terraces). When dealing with high competition, allocation of resources to reproduction is limited. Nevertheless, in the case of Montcervier grassland, the propagule pressure from walls is now so high that every future single disturbance might allow *S. inaequidens'* germination and establishment, as stated by the fluctuating resource hypothesis (Davis et al. 2000).

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APPENDIX III

Evolution of introduced genotypes' advantage over native ones from favourable to unfavourable conditions

Objective

The objective is to organise into a hierarchy the factors that limit establishment success of introduced tetraploid genotypes during the three main steps of the invasion process: survival, competitive interactions leading to growth and capitulum production leading to spread.

Methods

Based on data from chapters 4 and 5, I calculated for each growing condition the ratio between introduced tetraploid genotypes value for a traits and native tetraploid genotype value for the same traits (Table 1). Growing conditions were separated into 3 main factors: (i) management (Chapter 4), (ii) community spatial pattern and (iii) diversity (Chapter 5). Each factor had two treatments to which a level (1,2) was associated according the "favourability" of growing conditions. Therefore, growing conditions of level 1 are more favourable than growing conditions of level 2.

Table 1: Values of ratio between introduced tetraploid and native tetraploid genotypes' survival, vegetative size, shoot biomass and capitulum production of *C. maculosa* and *S. inaequidens* according to management type, community spatial pattern and diversity level.

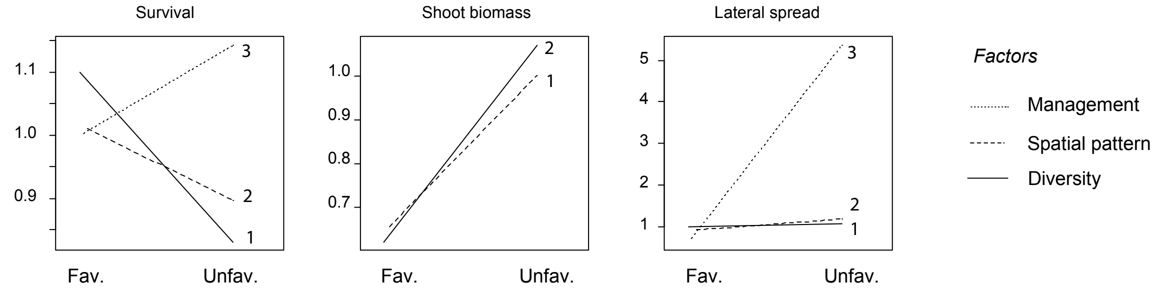
				Introduced genotype trait/ Native genotype trait			
Thesis chapter	Factor	Treatment	Level	Survival	Vegetative size	Shoot biomass	Capitulum production
Centaurea maculosa							
4	Management	Grazing	1	1.000	0.611		
		Mowing	2	1.143	5.377		
5	Spatial pattern	Aggregated	1	1.016	0.900	0.642	
		Random	2	0.897	1.180	1.002	
	Diversity	High : 8 species	1	1.099	0.981	0.619	
		Low : 4 species	2	0.830	1.069	1.072	
Senecio inaequidens							
4	Management	Grazing	1	1	0.939		1.331
		Mowing	2	0.625	0.716		3.226
5	Spatial pattern	Aggregated	1	1.065	1.266	1.163	6.413
		Random	2	1.015	1.074	1.604	11.218
	Diversity	High : 8 species	1	0.961	1.031	1.22	11.148
		Low : 4 species	2	1.139	1.222	1.514	6.341

Results

Evolution of ratio according to change in growing conditions are plotted for each combination of factor, trait and species (Figure 1). For each point, value of the ratio gives information about the "superiority" of the introduced genotypes over native ones.

The sign of the slope of the evolution of the ratio according to the change in growing condition gives indication about the relative effect of the factor on native and introduced genotypes respectively. A negative slope shows that introduced genotypes are more affected by the change in growing conditions than native ones. Such a factor might be therefore important to limit invasive success of introduced genotypes. A positive slope indicates that introduced genotypes are less affected than native ones by the change in growing conditions. Lastly, the steep of the slope gives indication on the importance of the difference between introduced and native genotypes response to changing growing conditions. To summarise, the steepest positive slope indicates the factor that affects the least introduced genotypes compared to the native one. The steepest negative slope shows the factor which has the highest impact on introduced genotypes' trait as compared to native genotypes. This factor might therefore limit invasion success. Classification of potential limiting factors according to effect on species traits is therefore possible (Figure 1).

Centaurea maculosa



Senecio inaequidens

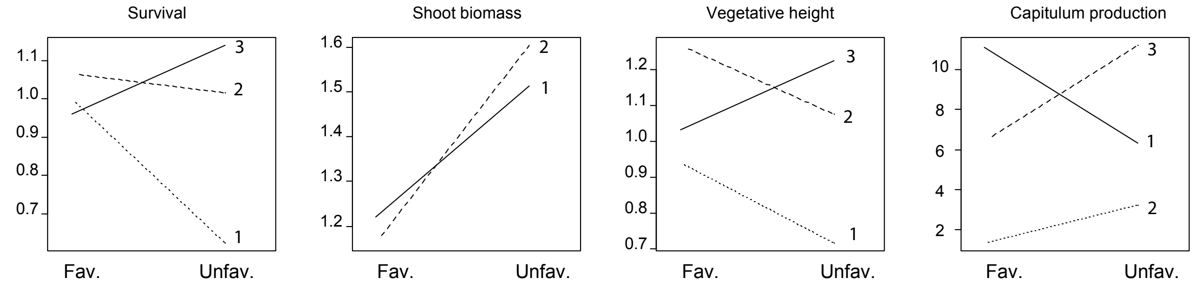


Figure 1: Evolution of the ratio "introduced genotype/native genotype" for survival, shoot biomass, vegetative size (respectively lateral spread for *C. maculosa* and vegetative height for *S. inaequidens*) and capitulum production according to favourable (Fav.) or unfavourable (Unfav.) growing conditions. Numbers on each graphs shows the most limiting factor (number 1) to the least one (number 2 or 3 according to the number of factors studied) for introduced genotypes' evolution of trait.

APPENDIX IV

Analyse de risque liée aux expériences de la thèse d'Aurélie Thébault

Thèse sur la résistance des communautés végétales à l'introduction d'espèces invasives

Espèces invasives modèles :
Centaurea maculosa
Senecio inaequidens

Thèse financée par
le Fond National de la Recherche
le Pôle National de Recherche « Survie des Plantes » de Neuchâtel

Projet dirigé par Dr. Antoine Guisan,
professeur-assistant à l'université de Lausanne

Thèse encadrée par :

Prof. Alexandre Buttler,
directeur du laboratoire des systèmes écologiques,
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Désignation et caractérisation des organismes concernés

I. Centaurea maculosa

(d'après les informations du Cabi Crop Protection Compendium
http://www.cabicompendium.org/cpc/home.asp)

1.1. Désignation scientifique et autres noms

Centaurea maculosa
(=Centaurea stoebe subsp. micranthos, Centaurea biebersteinii, Centaurea maculosa subsp. micranthos)

Nom communs **:**

Spotted knapweed
Centaurée maculée, Centaurée tachetée
Gefleckte Flockenblume, Kleinköpfige Rispen-Flockenblume
Rispen- Flockenblume

1.2. Caractéristiques génétiques et phénotypiques

Au sein de cette espèce, il existe des individus diploïdes (2n=18) et tétraploïdes (4n=36). Il semble que la forme diploïde soit monocarpique et bisannuelle alors que la forme tétraploïde est polycarpique et pérenne.

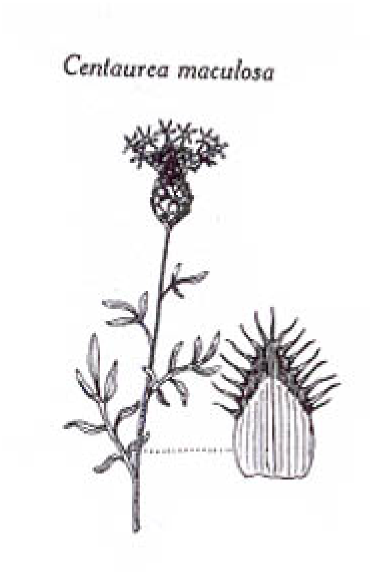
Identification :

Germinations : les cotylédons sont ovoïdes Les premières vraies feuilles ont une forme de cuillère et présentent un pétiole. Plus tard, ces feuilles subissent une fine division, se dotent de poils et formeront une rosette.

Jeune plantules : Les feuilles forment une rosette et sont ovoïdes. Les feuilles les plus basses tendent à être très divisées par rapport aux feuilles supérieures qui apparaissent plus linéaires. Toutes les feuilles sont alternes.

Individus matures: Les plantes matures sont érigées, ramifiées et peuvent attendre 1m de haut. Les feuilles sont linéaires, alternes, poilues et finement divisées (feuilles pinnatifides, à segments linéaires).. Les tiges apparaissent vert-grisâtre et peuvent présenter des poils.

Fleurs : Les capitules sont petits. Les fleurs sont roses à violettes et parfois blanches. Elles sont entourées de bractées présentant une frange noire marquée très caractéristique.



1.3. Méthodes de culture des organismes

Les organismes seront mis à germer en serre et/ou en chambre climatisée. Des individus de quelques semaines (ayant germé en serre) seront transplantés dans des communautés naturelles *via* des rondelles de tourbe.

1.4. Source et description des souches

Les souches utilisées proviennent de l'aire de répartition naturelle (Europe) mais également de l'aire d'introduction (Amérique du Nord : Montana et Oregon).

Le tableau ci-dessous recense les provenances des graines :

Pays	Continent	native/non native	Nombre de sites
Europe			
Autriche	Europe	native	4
Suisse	Europe	native	4
Allemagne	Europe	native	4
France	Europe	native	13
Hongrie	Europe	native	9
Roumanie	Europe	native	2
Ukraine	Europe	native	8
Amérique du Nord			
Oregon	US	non native	3
Montana	US	non native	8

Aussi bien les graines européennes que les graines américaines seront utilisées dans les expériences en milieu naturel.

1.5. Régions de dissémination involontaire

A l'échelle mondiale, l'espèce est présente sur 4 continents : l'Europe, l'Océanie et les continents Asiatique et Américain.
C. maculosa est considérée comme invasive dans les aires d'introduction (Amériques, Asie et Océanie) :

Asia				
Iran	present	introduced	**invasive**	Rechinger, 1980
Europe				
Austria	Established	native	**Not invasive**	The IUCN/SSC Invasive Species Specialist Group (ISSG) (http://www.issg.org) - USDA-ARS, undated
Belarus	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Bulgaria	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Czech Republic	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Former Yugoslavia	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
France	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Germany	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Greece	Established		**Not invasive**	ISSG 2006; USDA-ARS, undated
Hungary	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Italy	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Latvia	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Lithuania	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Macedonia	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Moldova	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Poland	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Romania	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated

Russian Federation	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Slovakia	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Slovenia	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
Switzerland	**Established**	**native**	**Not invasive**	**ISSG 2006; USDA-ARS, undated**
Ukraine	Established	native	**Not invasive**	ISSG 2006; USDA-ARS, undated
North America				
Canada	Established	introduced	**invasive**	Rice, 2003 ; Duncan, 2001 ; Watson & Renney, 1974
USA	Established	introduced	**invasive**	USDA-ARS, 2003 ; Duncan, 2001 ; Rice, 2003 ; Sheley et al., 1999b
South America				
Argentina	Established	introduced	**invasive**	ISSG 2006; USDA-ARS, undated
Oceania				
New Zealand	Established	introduced	**invasive**	Holm et al., 1979

1.6. Biologie et Ecologie

1.6.1. Type de pathogénicité, organisme hôtes

C. maculosa n’est pas pathogène.

1.6.2. Résistance ou sensibilité aux antibiotiques et autres agents spécifiques

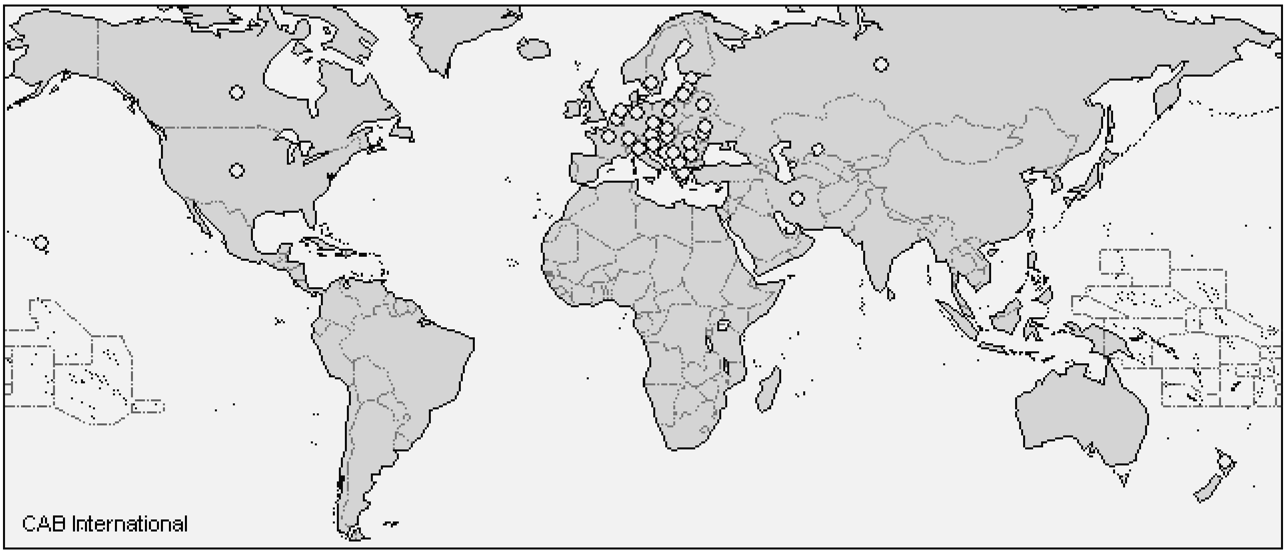
Néant

1.6.3. Ennemis naturels

Agapeta zoegana (attaque les racines au Montana)
Bangasternus fausti (attaque les graines)
Chaetorellia acrolophi (attaque les graines)
Cyphocleonus achates (attaque les racines)
Larinus minutus (attaque les graines)
Larinus obtusus (attaque les graines)
Megalonotus chiragrus sabulicolus
Metzneria paucipunctella (attaque les graines au Montana)
Pelochrista medullana (attaque les racines)
Pterolonche inspersa (attaque les racines)
Sphenoptera jugoslavica (attaque les racines)
Terellia virens (attaque les graines)
Urophora affinis (attaque les graines au Montana)
Urophora quadrifasciata (attaque les graines au Montana)

1.6.4. Répartition géographique actuelle et biotope naturel

A l’échelle mondiale, l’espèce est présente sur 4 continents : l’Europe, l’Océanie et les continents Asiatique et Américain :



Biotope naturel :

Amplitudes climatiques :
Altitude : 0 -3040m
Pluviométrie annuelle moyenne : 200 - 2000 mm
Durée de la saison sèche: 0 - 8 mois
Températures moyennes annuelles : 1 - 18ºC
Température maximale du mois le plus chaud (moyenne): 22 à 34ºC
Température minimale du mois le plus froid (moyenne): -23 à - 7ºC

Sol :
Texture : Légère, moyenne
Irrigation : insensible
pH : neutre

1.6.5. Capacité de survie dans les conditions suisses, temps de génération, type de reproduction et modes de propagation

C. maculosa est une herbacée pérenne ou bisannuelle selon les individus. Si l'espèce se reproduit principalement par graines, la reproduction végétative semble possible. L'espèces est entomogame. Les graines, produites en grandes quantités (de 65 à 2000 graines par plantes suivant les références et localisations), sont disséminées par le vent.

Cette espèce est reconnue pour la longévité et la durabilité de ses graines. Si la plupart des graines germent immédiatement, certaines peuvent entrer en dormance. Il est reconnu que les graines peuvent rester viables mais en dormance pendant au moins 8 ans. Le pourcentage de germination est relativement élevé, que ce soit dans un couvert fermé ou ouvert. Par contre, ce pourcentage de germination diminue avec la profondeur du sol, devenant quasi-nul à 5cm de profondeur.
C. maculosa passe l'hiver sous forme de graines ou rosette. Les graines germent en automne ou au début du printemps. Après la germination, les graines développent une racine pivotante et une rosette basale. Certains individus peuvent développer une tige florale la première

année, d’autres la deuxième année seulement. Les bourgeons floraux sont formés au début du mois de juin et la floraison s’étale entre Juillet et Septembre. Les graines deviennent mâtures courant Août et les têtes florales s’ouvrent 2 à 3 semaines après cette maturation, généralement à la fin de l’été. Très peu de graines passent l’hiver dans les boutons floraux.

Selon Ochsmann (2001), *C. maculosa* a été introduite en Suisse et y est invasive… mais elle est également inscrite sur la liste rouge des espèces menacées en Suisse (statut IUCN « Endangered » ou « Vulnerable » selon la taxonomie utilisée).

1.6.6. Participation à des processus environnementaux

Néant

1.6.7. Risques et impacts

Invasive dans son aire native de répartition	✓*
A été démontré invasive en dehors de son aire native de répartition	✓
Fortement adaptable à différents environnements	✓
Fort potentiel reproductif	✓
Fortement mobile localement	✓
Ses propagules restent viables pour plus d’un an	✓
Tolère les pratiques culturales, le pâturage, la mutilation, le feu …	**No***
Compétitive dans les cultures et les prairies	✓
Affecte les écosystèmes	✓
Affecte négativement les communautés naturelles	✓
Affecte négativement la structure des communautés	✓
Affecte négativement la santé humaine	✓
A des impacts sociologiques sur les activités de récréation, la valeur esthétique…	✓
Nocif pour les animaux	**No**
Produit des épines	✓
Vecteur ou hôte de maladies	**No**
Susceptible d’être accidentellement transporté à l’échelle mondiale	✓
Susceptible d’être intentionnellement transporté à l’échelle mondiale	**No**
Difficile à identifier ou détecter *in situ*	**No**
Contrôle difficile ou coûteux	✓

* : Désigne les points sur lesquels des avis controversés existent.

II. Senecio inaequidens

(d’après les informations du Cabi Crop Protection Compendium
http://www.cabicompendium.org/cpc/home.asp)

2.1. Designation scientifique et autres noms

Senecio inaequidens
(= Senecio burchellii)

Noms communs :

narrow-leaved ragwort, South African ragwort
séneçon du Cap
schmalblättriges Greiskraut, schmalblättriges Kreuzkraut, südafrikankisches Greiskraut,
senecione sudafricano

2.2. Caractéristiques génétiques et phénotypiques

Comme pour *Centaurea maculosa*, il apparaît que *Senecio inaequidens* présente des individus diploïdes et tétraploïdes. En Europe, seuls des individus tétraploïdes sont présents.

Identification :

S. inaequidens est une herbacée pérenne touffue, ligneuse à la base, atteignant 40 cm à plus de 1.10m de haut.

Plantule : Les cotylédons sont allongés en forme de cuillère, tronqués au sommet. Les feuilles simples, lancéolées, sont plus ou moins dentées.

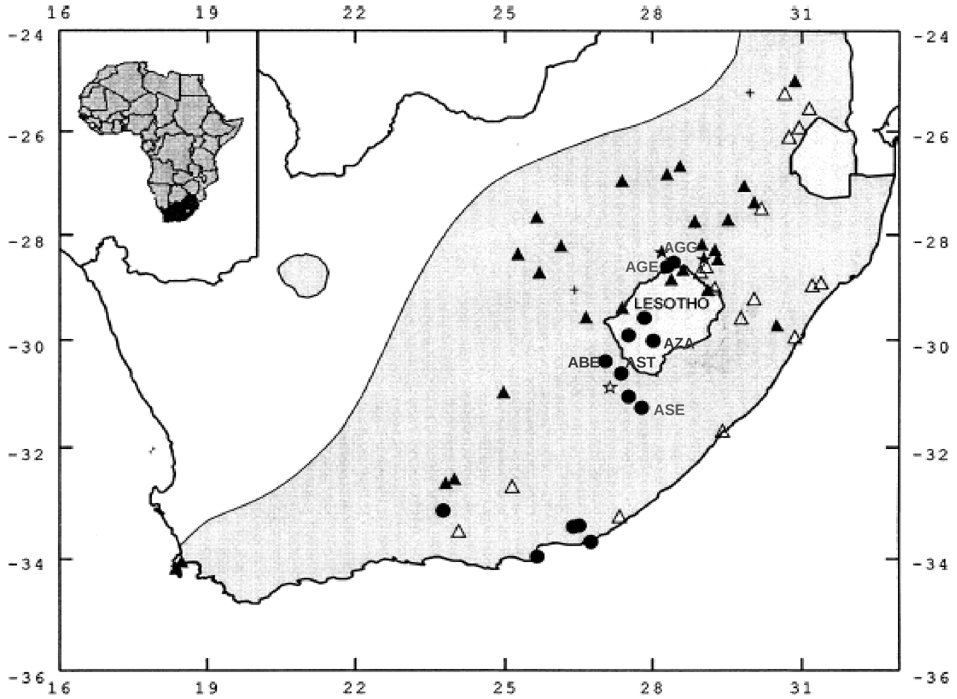
Plante adulte : Elle présente une tige ligneuse, érigée plus ou moins glabres et présentant généralement des ramifications dès la base. Les feuilles, vertes clair, sont alternes, glabres et étroitement linéaires, de 4 à 10 cm de long sur 3 à 4 mm de large. Elles sont parfois irrégulièrement munies de dents courtes, obtuses et espacées. La nervure principale est saillante. Entre la base et le sommet de la tige, les feuilles présentent un polymorphisme prononcé. Les inflorescences terminales, présentes sur les nombreuses ramifications présentent des boutons floraux en capitules jaunes de 15 à 25 mm de diamètre, groupés en corymbes irréguliers et portant entre 10 et 15 fleurs ligulées externes. Les fleurs internes sont en tube. Le calicule des fleurs est composé de nombreuses bractéoles étroites et acuminées et l’involucre présente environ une vingtaine de bractées étroites et acuminées. Les fruits sont des akènes blanchâtre-argentés, soyeux, généralement surmontés d’un pappus.

2.3. Méthodes de culture des organismes

Les organismes seront mis à germer en serre et/ou en chambre climatisée. Des individus de quelques semaines (ayant germé en serre) seront transplantés dans des communautés naturelles *via* des rondelles de tourbe.

2.4. Source et description des souches

Les souches utilisées proviennent de l'aire de répartition naturelle (Afrique du Sud et Lesotho) mais également de l'aire d'introduction (Europe).
La carte ci-dessous présente les provenances géographiques des graines Africaines



D'après Lafuma et al., 2003

Fig. 2. Cytotype distribution of sampled populations of the *S. inaequidens* species complex in South Africa. ● Indicates tetraploid populations, ▲ and △ refer to diploid populations where ▲ indicates diploid populations with more than 1.26 pg of DNA and △ designates diploid populations with less than 1.26 pg of DNA. ★ and ☆ refer to populations with both cytotypes where ★ indicates that the diploid cytotype contain more than 1.26 pg of DNA and ☆ that the diploids contain less than 1.26 pg of DNA. + denotes populations for which ploidy level was uncertain. Grey area refers to the distribution range of the *S. inaequidens* complex in South Africa

Le tableau ci-dessous recense les provenances des graines :

Provenance	Pays	Continent	native/non native	Nombre de sites
Liège	Belgique	Europe	non native	1
Mazamet	France	Europe	non native	1
Bremen	Germany	Europe	non native	14
Budapest	Hongaria	Europe	non native	2
Amsterdam	Netherlands	Europe	non native	4
Basel	Switzerland	Europe	non native	4
God's Window	Mpumlanga	South Africa	native	4
Golden Gate II	Free State	South Africa	native	10

Semonkong	Lesotho	South Africa	native	4
South Africa	South Africa	South Africa	native	13
Mont Lebanon I	KwaZula-Natal	South Africa	native	2

Aussi bien les graines européennes que les graines sud-africaines seront utilisées dans les expériences en milieu naturel.

2.5. Régions de dissémination volontaire

A l’échelle mondiale, l’espèce a été introduite sur 2 continents : l’Europe, l’Amérique. Cependant, *S. inaequidens* n’est pas toujours considérée invasive dans ces aires d’introduction:

Europe				
Andorra	present	introduced	not invasive	Montserrat Recoder & Benito Alonso, 2000; EPPO, 2005
Belgium	present	introduced (1922)	**invasive**	Mosseray, 1936; D'Hose & de Langhe, 1989; EPPO, 2005
Czech Republic	present	introduced	not invasive	Pysek et al., 2002; EPPO, 2005
Denmark	present	introduced	not invasive	Skovgaard, 1993; EPPO, 2005
Finland	present	introduced	not invasive	Bornkamm, 2002; EPPO, 2005
France	localized	introduced (1935)	**invasive**	Jovet & Bosserdet, 1962; Chater & Walters, 1976; EPPO, 2005
Germany	widespread	introduced (1889)	**invasive**	Stieglitz, 1977; Kuhbier, 1977, 1996; Brennenstuhl, 1995; EPPO, 2005
Italy	widespread	introduced (1947)	**invasive**	Pignatti, 1982; Martini & Zappa, 1993; Tammaro & Giglio, 1994; Brandes, 1999; EPPO, 2005
Netherlands	widespread	introduced (1939)	**invasive**	Adema & Mennema, 1978; Ernst, 1998; EPPO, 2005
Norway	present	introduced	not invasive	Often, 1997; EPPO, 2005
Poland	present	introduced	not invasive	Ernst, 1998; EPPO, 2005
Spain	present	introduced	not invasive	Guillerm et al., 1990; EPPO, 2005
Sweden	present	introduced	not invasive	Bornkamm, 2002; EPPO, 2005
Switzerland	**present**	**introduced**	**Black list**	**Mayor, 1996; EPPO, 2005**
United Kingdom	present	introduced (1908)	not invasive	Hayward & Druce, 1919; Lousley, 1961; MacPherson, 1997; EPPO, 2005
Africa				
Botswana	present	native		Hilliard, 1977; Werner et al., 1991; EPPO, 2005
Lesotho	present	native		Werner et al., 1991; EPPO, 2005
Mozambique	present	native		Werner et al., 1991; EPPO, 2005
Namibia	present	native		Hilliard, 1977; Werner et al., 1991; EPPO, 2005
South Africa	widespread	native	not invasive	Hilliard, 1977; EPPO, 2005
Swaziland	present	native		USDA-ARS, 2003; EPPO, 2005
North America				
Mexico	present			EPPO, 2005

South America				
Argentina	present			EPPO, 2005
Colombia	absent, unreliable record	introduced		Najar et al., 2001; EPPO, 2005

2.6. Biologie et Ecologie

2.6.1. Type de pathogénicité, organisme hôtes

Néant

2.6.2. Résistance ou sensibilité aux antibiotiques et autres agents spécifiques

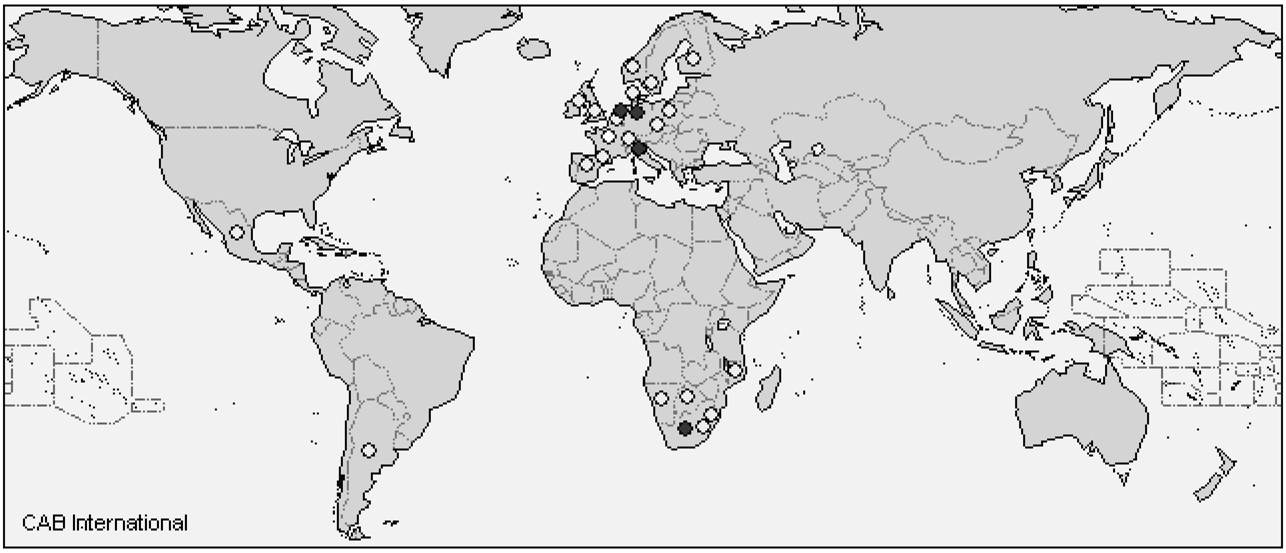
Néant

2.6.3. Ennemis naturels

Il n’est fait aucune mention d’ennemi naturel de *S. inaequidens*. Des larves de *Tyria jacobaeae,* un Lépidoptère souvent trouvé sur *Senecio jacobaea,* a été trouvé sur *S. inaequidens* aux Pays-Bas (Ernst, 1998). *Puccinia lagenophorae* apparaît comme étant specifique au genre *Senecio* et est actuellement testé comme mycoherbicide sur *S. vulgaris* en Europe.

2.6.4. Répartition géographique actuelle et biotope naturel

A l’échelle mondiale, l’espèce est présente sur 3 continents : l’Europe, l’Amérique et le continent Africain :



2.6.5. Capacité de survie dans les conditions suisses, temps de génération, type de reproduction et modes de propagation

S. inaequidens est une herbacée lignifiée, pérenne. La reproduction est principalement sexuée mais une propagation végétative est possible par le développement d’un système racinaire sur les tiges lorsque celles-ci touchent le sol.

Le nombre d’achènes (dispersées par le vent) produit par individu semble très variable et peu d’informations sont disponibles sur la longévité des achènes (certains disent supérieur à 10 ans). Stockées au sec, les achènes restent viable au moins 2 ans.

Biotope naturel :

Amplitudes climatiques :
Pluviométrie annuelle moyenne : 500 - 1500 mm
Températures moyennes annuelles : 10 - 20ºC
Température maximale du mois le plus chaud (moyenne): 30 à 35ºC
Température minimale du mois le plus froid (moyenne): -5 à 0ºC

Sol :
Texture : Légère, moyenne
Irrigation : insensible
pH : neutre à basique
Tolérances spéciales : infertile

2.6.6. Participation à des processus environnementaux

Néant

2.6.7. Risques et impacts

Invasive dans son aire native de répartition	**No**
A été démontré invasive en dehors de son aire native de répartition	✓
Fortement adaptable à différents environnements	✓
Fort potentiel reproductif	✓
Fortement mobile localement	✓
Ses propagules restent viables pour plus d’un an	✓
Tolère les pratiques culturales, le pâturage, la mutilation, le feu …	✓
Compétitive dans les cultures et les prairies	**No***
Affecte les écosystèmes	**No***
Affecte négativement les communautés naturelles	**No***
Affecte négativement la structure des communautés	**No***
Affecte négativement la santé humaine	**?**
A des impacts sociologiques sur les activités de récréation, la valeur esthétique…	✓
Nocif pour les animaux	✓
Produit des épines	**No**
Vecteur ou hôte de maladies	**No**
Susceptible d’être accidentellement transporté à l’échelle mondiale	✓
Susceptible d’être intentionnellement transporté à l’échelle mondiale	**No***
Difficile à identifier ou détecter *in situ*	**No**
Contrôle difficile ou coûteux	✓

* : Désigne les points sur lesquels des avis controversés existent.

Evaluation du risque

I. *Détermination du préjudice potentiel*

Compte tenu du caractère invasif des deux espèces dans leur région d'introduction, le risque de propagation à grande échelle est envisageable si aucune mesure de protection n'est prise. Les espèces utilisées ont toute deux une très forte capacité de dissémination par le vent notamment (nombreuses graines produites, graines légères). Par ailleurs, *Senecio inaequidens* présente la capacité de se reproduire végétativement.
Malgré le fait que la durée de vie des graines dans le sol n'a pas été déterminée précisément, nous savons que les graines peuvent germer même après une période de 5 ans. Les graines sont extrêmement résistantes au froid et au feu.

II. *Détermination de l'ampleur des dommages*

2.1. Rappel des impacts reconnus des organismes

Impact	***C. maculosa***	***S. inaequidens***
Invasive dans son aire native de répartition	✓*	**No**
A été démontré invasive en dehors de son aire native de répartition	✓	✓
Fortement adaptable à différents environnements	✓	✓
Fort potentiel reproductif	✓	✓
Fortement mobile localement	✓	✓
Ses propagules restent viables pour plus d'un an	✓	✓
Tolère les pratiques culturales, le pâturage, la mutilation, le feu …	**No***	✓
Compétitive dans les cultures et les prairies	✓	**No***
Affecte les écosystèmes	✓	**No***
Affecte négativement les communautés naturelles	✓	**No***
Affecte négativement la structure des communautés	✓	**No***
Affecte négativement la santé humaine	✓	**?**
A des impacts sociologiques sur les activités de récréation, la valeur esthétique…	✓	✓
Nocif pour les animaux	**No**	✓
Produit des épines	✓	**No**
Vecteur ou hôte de maladies	**No**	**No**
Susceptible d'être accidentellement transporté à l'échelle mondiale	✓	✓
Susceptible d'être intentionnellement transporté à l'échelle mondiale	**No**	**No***
Difficile à identifier ou détecter *in situ*	**No**	**No**
Contrôle difficile ou coûteux	✓	✓

* : Désigne les points sur lesquels des avis controversés existent.

2.2. Ampleur des dommages compte tenu du préjudice potentiel

Centaurea maculosa :
Centaurea maculosa ne présente aucun signe de pathogénicité ou de toxicité pour les animaux. Elle est reconnue pour sa libération d'exsudats racinaires lui permettant de devenir extrêmement compétitive dans les milieux qu'elle colonise. Compte tenu de l'importance de l'invasion dans la zone d'introduction, il semble que les dommages sur la diversité floristique sont irréversibles.
En Europe, *C. maculosa* ne présente aucun caractère invasif et apparaît même comme rare et en danger.
Le risque lié à l'introduction de *Centaurea maculosa* se situe donc notamment dans le fort potentiel reproducteur de l'espèce et dans ses impacts sur le système souterrain (et donc les modifications des relations sol-plantes via les micro-organismes du sol que cela pourrait entraîner).

Senecio inaequidens :
Une propagation importante du Séneçon du Cap, *Senecio inaequidens* pourrait avoir des conséquences importantes sur la biodiversité et l'économie agricole. En particulier, une invasion des pâturages mènerait à une baisse de la diversité floristique. De plus, étant toxique, l'espèce est refusée par le bétail ce qui rend les pâturages envahis inexploitables.
Concernant les effets sur le sol, peu de choses sont connues pour le moment. Jusqu'à présent, aucune étude scientifique n'a permis de démontrer un impact quelconque sur les microorganismes du sol. Il apparaît que l'espèce a un impact relativement réduit tant sur les écosystèmes que sur les communautés végétales qu'elle envahi (structure et fonctionnement).
Dans l'immédiat, compte tenu du caractère récent de l'invasion par *S. inaequidens* en Europe, nous ne savons pas si les dommages sont réversibles ou non.

Si des dommages sont reportés sur la parcelle d'étude, le risque de propagation des dommages aux parcelles avoisinantes reste cependant très limité compte tenu de la courte durée des expériences et du faible nombre d'individus étudiés.

III. Détermination de la probabilité d'occurrence de dommages

Dans leur aire d'introduction, les deux espèces se sont caractérisées par un délai assez important entre l'introduction des graines et le début de l'invasion. Les expérimentations en question ne durant que deux à trois années et l'introduction de plantes dans le milieu ne se faisant que deux années consécutives, les risques d'invasion apparaissent relativement limités. Le risque principal réside dans la forte potentialité de reproduction, tant sexuée que végétative, des deux espèces. L'effort de contrôle doit donc se faire sur les moyens de reproduction des deux espèces.

IV. Détermination des mesures de sécurité nécessaires

Compte tenu du risque de propagation des graines et du risque d'hybridation avec les espèces indigènes, l'ensemble des têtes florales sera coupé avant pollinisation.

La durée de vie des graines dans le sol étant relativement inconnue, la résistance des graines au feu et au froid étant reconnue, nous veillerons à ne mettre aucune graine dans le sol. Les graines germeront donc en chambre de culture. Les germinations seront ensuite repiquées dans des rondelles de tourbe qui seront elles-mêmes transplantées dans la communauté naturelle. Les têtes florales étant coupées avant la fructification, nous nous assurons donc de ne laisser aucune graine dans la banque de graine du sol.

Afin de limiter les risques de dispersion par les animaux, nous clôturerons les zones de travail.

Par ailleurs, à la fin du travail de doctorat d'Aurélie Thébault, tous les individus introduits dans le milieu naturel seront arrachés, séchés et pesés (fin 2007 au plus tard). Tous les individus ayant été marqués lors de leur introduction dans le milieu, nous nous assurons donc ainsi de ne laisser aucun individu susceptible de fleurir et de produire des graines les années suivantes. Nous limitons également le risque de multiplication végétative à grande échelle. L'impact sur les communautés microbiennes étant peu connu, notamment pour le Séneçon du Cap, nous l'analyserons au cours de ce travail de doctorat.

En cas de doute sur l'éventualité de la présence de graines dans le sol, nous pourrons envisager, à la fin du travail de doctorat, une stérilisation complète du sol de la zone d'étude à la vapeur. Un prélèvement de la partie supérieure du sol afin de la brûler pourra également être envisagée si nécessaire.
Par ailleurs, un suivi des repousses potentielles sera effectué après l'essai.

Si malgré toutes les précautions mentionnées ci-dessus, le contrôle de la propagation des deux espèces s'avérait plus difficile que prévu, l'essai sera interrompu.

V. Evaluation du risque

Compte tenu de l'ampleur et de la probabilité d'occurrence des dommages, compte tenu des mesures de sécurité envisagées, nous classons l'activité concernée dans la catégorie de **risque 1**, soit la plus faible possible.

Curriculum vitae

Aurélie Thébault

Born : May 11th, 1981, Rennes, France
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Research interest:

Plant invasions, community ecology, biotic interactions, plant-soil interactions, ecosystem dynamics and biodiversity

Education

2005-2009	Ecole Polytechnique Fédérale de Lausanne (Switzerland) PhD research in Ecology
2003-2004	Ecole Nationale supérieure agronomique of Rennes (France) M.Sc in Environmental sciences, ecology and conservation
2003	McGill University (Montreal, Quebec, Canada) Exchange scolarship (1 semester)
2002	Ecole Nationale supérieure agronomique of Rennes (France) B.Sc in Agronomy
1999 – 2001	Chateaubriand High School, Rennes (France) National competition for schools of agronomy entrance
1996 – 1999	Bréquigny High School, Rennes (France) Scientific Baccalauréat with Honour – Option Mathematics

Research experience

2005-2009	Resistance of plant communities to invasive species – disentangling invasiveness from invasibility PhD research, Laboratory of Ecological Systems (ECOS), EPFL (Lausanne) *Supervisor*: Prof. Alexandre Buttler
2004-2005	Response of subalpine grasslands to land use changes in the French Alps: linking ecological process with ecosystem services in subalpine meadows Master thesis and post graduate research project**,** Laboratoire d'Ecologie Alpine, CNRS - UMR 5553 (Grenoble, France) *Supervisors*: Sandra Lavorel (Research director), Fabien Quétier (PhD)
2003	Response of *Cryptosporiopsis sp*., a pathogen of chestnut trees, to chemical control : an assessment of pest management practices M.Sc. project, Laboratoire régional de la protection des végétaux (Villenave d'Ornon, France) – 2 months *Supervisor*: Alain Baudry
2003	Assessment of the possibility to reintroduce *Salmo salar* L., in the Moselle river M.Sc. project, Union Régionale du grand Est des fédérations de pêche (Epinal, France) – 2 months *Supervisor*: Fabien Millot

Teaching experience

2006 -2008	Graduate teaching assistant in Landscape Ecology (1 year) and Numerical Ecology (3 years) at the Laboratory of Ecological Systems, Ecole Polytechnique Fédérale de Lausanne, Switzerland
2005-2008	Supervisor of Master students at the Laboratory of Ecological Systems, Ecole Polytechnique Fédérale de Lausanne, Switzerland

Technical skills

Languages:

English: High spoken and written level.
German: School level in writing, basic level in speaking.
Spanish: Basic communicative level
Italian: Basic communicative level

Software**:**

Microsoft Office, Adobe
Geographical Information: MapInfo, ArcView, Idrisi Kilimandjaro
Statistics: R, SPSS, JUMP, SPAD, ADE 4, Matlab
Programming: Turbo-Pascal, C language

Papers in peer reviewed journals

1. Fortunel C., Garnier E., Joffre R., Kazakou E., Quested H., Grigulis K., Lavorel S., Ansquer P., Castro H., Cruz P., Dolezal J., Eriksson O., Freitas H., Golodets C., Jouany C., Kigel J., Kleyer M., Lehsten V., Lepš J., Meier T., Pakeman R., Papadimitriou M., Papanastasis V.P., Quétier F., Robson M., Sternberg M., Theau J.P., **Thébault A**. and Zavorali M.P. (2009). Leaf traits capture the effects of land use changes and climate on litter decomposability of grasslands across Europe. Ecology 90 (3):598-611.
2. Pakeman R.J., Garnier E., Lavorel S., Ansquer P., Castro H., Cruz P., Doležal J., Eriksson O., Golodets C., Kigel J., Kleyer M., Lepš J., Meier T., Papadimitriou M., Papanastasis V.P., Quested H., Quetier F., Rusch G., Sternberg M., Theau J.P., **Thébault A**. and Vile D. (2008). Impact of abundance weighting on the response of seed traits to climate and land use. *Journal of ecology* 96: 355-366
3. Lavorel, S., Grigulis, K., McIntyre, S., Williams, N.S.G., Garden, D., Dorrough, J., Berman, S., Quétier, F., **Thébault, A**. and Bonis, A. (2008). Assessing functional diversity in the field: methodology matters! *Functional Ecology* 22: 134-147
4. Quétier F., **Thébault A**., Lavorel S. (2007). Plant traits in a state and transition framework as markers of ecosystem response to land-use change. *Ecological Monographs* 77(1): 33-52.
5. Garnier E., Lavorel S., Ansquer P., Castro H., Cruz P., Dolezal J.,Eriksson O., Fortunel C., Freitas H., Golodets C., Grigulis K., Jouany C., Kazakou E., Kigel J., Kleyer M., Lehsten V., Lepš J., Meier T., Pakeman R., Papadimitriou M., Papanastasis V.P., Quested H., Quétier F., Robson M., Roumet C., Rusch G., Skarpe C., Sternberg M., Theau J.P., **Thébault A**., Vile D. and Zarovali M.P. (2007). Assessing the effects of land use change on plant traits, communities and ecosystem functioning in grasslands: a standardized methodology and lessons from an application to 11 European sites. *Annals of Botany* 99(5):967-985.

SUBMITTED

1. **Thébault A.**, Frey B., Mitchell E.A.D. & Buttler A. Species-specific effects of polyploidisation, range and plant traits of Centaurea maculosa and Senecio inaequidens on rhizosphere microorganisms. *Submitted to Oecologia*.
2. **Thébault A.**, Gillet F., Müller Schärer H. & Buttler A. Polyploidisation may determine invasion success through trade-offs among plant traits. *Submitted to Biological Invasions.*
3. Quétier F., Liancourt P., **Thébault A.**, D. Davies I. and Lavorel S. Predicting past and present management effects on subalpine grasslands using a plant trait based generic modelling framework. *Submitted to Journal of Applied Ecology*
4. Lavorel S., Lepš J., de Bello F., Grigulis K., Garnier E., Castro H., Dolezal J., Godolets C., Quétier F., Sebastià M.T., **Thébault A**. Response of functional divergence to decreasing land use along aridity gradients in Europe. *Submitted to Oecologia*

IN PREPARATION

1. **Thébault A.** & Buttler A. What makes Centaurea maculosa and Senecio inaequidens successful invaders: Inherent superiority, disturbance benefit or evolutionary changes? *In preparation*.
2. **Thébault A.**, Stoll P. & Buttler A. Effects of spatial patterns and community diversity on invasive success. *In preparation.*
3. **Thébault A.**, Lavorel S., Gillet F., Delabays N., Jeangros B. & Buttler A. Dominant species identity and turnover may limit invasive success. *In preparation*.
4. **Thébault A.**, Gomis Bataller P. & Buttler A. Community productivity *per se* limits establishment success of invasive species. *In preparation*.

Papers in books or non peer-reviewed journals

1. **Thébault A**. (2008). Plantes invasives dans le contexte agricole: le cas du Seneçon du Cap. *La Forêt* (3), pages 24-25.
2. **Thébault A**. (2006). Plantes invasives : la résistance des communautés végétales. *La Forêt* (5), page 13.
3. Lavorel, S., Quétier, F., **Thébault, A.,** Daigney, S., Davies, I.D., De Chazal, J. and VISTA consortium (2006). Vulnerability to land use change of services provided by alpine landscapes. In: Price, M.F. (Ed). Global Change in Mountain Regions, pp. 215-216. Sapiens Publishing, Perth, Scotland

Reviewer for peer-reviewed journals

1. Ecological Applications
2. Landscape Ecology

Seminars and presentation/posters at meetings

INTERNATIONALES COMMUNICATIONS

1. **Thébault A.,** Gillet F., Lavorel S. & Buttler A. (2008). Invasibility of a grassland community by native and introduced genotypes of two invasive plant species (*Centaurea maculosa* Lam. and *Senecio inaequidens* DC.). *93rd Ecological Society of America Annual Meeting*, Milwaukee, WI, USA, 3-8 August 2008.
2. **Thébault A.,** Gillet F., Buttler A. (2007). Plantes envahissantes: Interaction entre les traits des espèces et la résistance de la communauté végétale. *2èmes rencontres francophones sur les invasions biologiques: adaptation, plasticité et traits d'histoire de vie*, Rennes, France, 14-16 November 2007.
3. **Thébault A.**, Gillet F., Buttler A. (2007). Colonization and invasion in European grasslands: Interaction between species traits and community resistance. *Workshop Colonization* vs. *Invasion: do the same traits matter ?*, Ascona, Switzerland, 25 February-2 March 2007
4. **Thébault A.**, Pasche D, Gillet F., Müller-Schärer H. and Buttler A.(2006). Comparison of the life strategy of two invasive species (Centaurea maculosa Lam. and Senecio inaequidens DC.). *An evolutionary perspective of biological invasions Symposium*. Fribourg, Switzerland, 2-3 October 2006 (Poster and Abstract)

OTHER COMMUNICATIONS

1. **Thébault A.** (2008). Resistance of plant communities to invasive species: Effects of disturbance and community structure and dynamics. *Seminaires du Laboratoire d'Ecologie Alpine*, CNRS - UMR 5553, University Joseph Fourier, Grenoble, France, 24 April 2008
2. **Thébault A.** (2008). Resistance of plant communities to invasive species: toward an experimental approach to improve management. *Cycle de Séminaires "Conservation Biology"*, University of Berne, Berne, Switzerland, 27 February 2008 (invited speaker)
3. **Thébault A.** (2006). Etude de la résistance des communautés végétales aux espèces invasives : le cas du Séneçon du Cap (*Senecio inaequidens* DC.). *Cours «Lutte contre les Néophytes : moyens et priorités», Chambre Romande des Ingénieurs Forestiers (CRIFOR)*, Fribourg, Switzerland, 29 August 2006 (invited speaker)
4. **Thébault A.**, Pasche D, Gillet F. and Buttler A.(2006). Plant invasions: invasiveness *vs.* invasibility. *GDRE "Dynamique des écosystèmes méditerranéens et montagnards dans un monde changeant"*, Lautaret Pass, France, 11-12 May 2006
5. **Thébault A.**, Pasche D, Gillet F. and Buttler A.(2006). Assessing invasiveness and invasibility in European grasslands. *NCCR "Plant Survival" Annual Meeting*, Neuchâtel, Switzerland, 21 April 2006