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Sources de variation phénotypique des traits d'histoire de vie d'une espèce invasive, *Senecio inaequidens* DC. (Asteraceae).

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Essai présenté en vue de l'obtention du grade de docteur en sciences agronomiques et ingénierie biologique

Promoteur : Pr. Grégory Mahy
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SOURCES DE VARIATION PHÉNOTYPIQUE DES TRAITS D'HISTOIRE DE VIE D'UNE ESPÈCE INVASIVE, *SENECIO INAEQUIDENS* DC. (ASTERACEAE).

Arnaud MONTY

ESSAI PRÉSENTÉ EN VUE DE L'OBTENTION DU GRADE DE DOCTEUR EN SCIENCES AGRONOMIQUES ET INGÉNIERIE BIOLOGIQUE

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Résumé

L'importance des différentes sources de variation phénotypique que sont l'adaptation, la plasticité phénotypique, les effets maternels environnementaux, le niveau de ploïdie et la dérive génétique, a été analysée pour différents traits d'histoire de vie, au sein de l'aire colonisée par une espèce végétale invasive. A cette fin, plusieurs expériences en jardins communs ont été installées, dont deux en transplantations réciproques. Les populations considérées étaient localisées en Belgique, en France, ainsi que dans l'aire d'indigénat de l'espèce. *Senecio inaequidens* DC. (Asteraceae), l'espèce étudiée dans ce travail, est une plante d'origine africaine accidentellement introduite en Europe par le commerce lainier, vers la fin du $19^{ème}$ siècle. Dans son aire d'indigénat, elle présente deux niveaux de ploïdie, diploïde et tétraploïde. Seuls des plants tétraploïdes sont recensés en Europe. L'espèce présente un historique de colonisation particulier, bien documenté, qui en fait un modèle idéal pour les études évolutives. Les traits d'histoire de vie considérés ont été scindés en deux groupes. Les premiers concernaient la germination, la croissance et la reproduction sexuée. Les sources de variation phénotypique dans ces traits ont été principalement analysées en relation avec les variations climatiques dans l'aire d'invasion, le long de gradients d'altitude, ainsi qu'en relation avec le niveau de ploïdie et l'aire d'origine (aire d'indigénat *vs* aire d'invasion). Le deuxième groupe de traits considérés concernaient les capacités de dispersion de l'espèce. Celles-ci ont été modélisées. La variabilité phénotypique dans ces traits a ensuite été analysée, parmi les populations françaises, en relation avec l'éloignement depuis le site de première introduction.

Les résultats ont montré des différences entre les cytotypes de l'espèce, principalement dans les capacités de survie hivernale. Le long des gradients d'altitude, les populations de *S. inaequidens* présentaient des différenciations phénotypiques de type clinal, dans les traits de croissance. Ces différenciations étaient d'origine génétique, même si les effets maternels environnementaux sont apparus comme des sources non-négligeables de variation phénotypique dans les zones à climat rigoureux. Parmi les traits liés à la dispersion, le *plume loading* était le mieux corrélé aux capacités de dispersion par le vent. Des différenciations clinales ont été détectées dans les traits de dispersion, en jardin commun, mais n'ont pas été vérifiées en populations naturelles.

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Summary

The importance of different sources of phenotypic variation, namely adaptation, phenotypic plasticity, environmental maternal effects, ploidy level and genetic drift, were estimated in several life history traits among populations of an invasive plant species within its invasion range. Several common garden experiments were set out, two of which in a reciprocal transplants experiment. The populations considered in this study were located in Belgium, in France and in the native range of the model species. *Senecio inaequidens* DC. (Asteraceae), the model plant species, is native to Africa. It was accidentally introduced in Europe via wool trade in the late 19th century. In the native range, the species occurs at two co-existing cytotypes: diploid and tetraploid. Only tetraploid individuals are reported in Europe. The particular and well-documented invasion history of *S. inaequidens* makes it an excellent plant model for evolutionary studies. Two groups of life history traits were measured. The first group of traits is related to germination, growth and sexual reproduction. The sources of phenotypic variation in those traits were analysed with respect to climatic variation along altitudinal gradients in the invaded areas. The influence of the ploidy level and the range (native *vs* introduced) on those traits was also analysed. The other group of traits were related to dispersal. First, dispersal capacity was modelled. Second, phenotypic variability in dispersal traits was analysed, among French populations, with respect to the distance between populations and the primary introduction French site. Results showed that diploid and tetraploid populations differed, mainly in winter survival capacity. Along altitudinal gradients, clinal phenotypic differentiations with a genetic basis were observed among populations. However, environmental maternal effects were found to significantly influence phenotypic variation in areas with harsh climatic conditions. Among dispersal-related traits, the plume loading showed the highest correlations with wind-dispersal capacity. Clinal variations were found among populations for dispersal traits, in controlled conditions. In the field, however, such patterns were not confirmed.

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Sources de variation phénotypique des traits d'histoire de vie d'une espèce invasive, *Senecio inaequidens* DC. (Asteraceae).

Essai présenté par Arnaud Monty, en vue de l'obtention du grade de docteur en sciences agronomiques et ingénierie biologique.

Introduction

Depuis les débuts de l'agriculture, l'Homme s'est déplacé en emportant avec lui diverses espèces végétales. Il a ainsi introduit des espèces de culture, ainsi que toute une flore associée, dans diverses régions du globe. Néanmoins, à partir du 17ème siècle, de profondes modifications ont eu lieu dans les patrons de distribution des espèces végétales, induites par la colonisation européenne, le développement du commerce international, l'expansion des voies de communication, la création des jardins botaniques ainsi que l'avancement de l'horticulture et de la sylviculture. De plus en plus rapidement, les activités humaines ont ainsi donné une nouvelle dimension aux capacités de dispersion des espèces végétales (Cronk et al., 1995 ; Prinzing et al., 2002 ; Reichard et al., 2001). Ce bouleversement dans les patrons de répartition des végétaux est au cœur du phénomène d'invasion biologique, qui est actuellement reconnu comme l'une des causes principales de perte de biodiversité dans le monde (Cronk et al., 1995 ; Parker et al., 1999). Il est directement impliqué dans la disparition d'espèces autochtones (Vitousek et al., 1996), mais aussi dans des préjudices aux activités humaines ou dans l'émergence de problèmes sanitaires (Mack et al., 2000). Dans le cadre de ce travail, nous entendrons par « espèce invasive » une espèce non-indigène, naturalisée, introduite relativement récemment (après 1500 C.E.) et montrant une dynamique d'expansion rapide dans la zone d'introduction (Pysek et al., 2004).

Malgré les problèmes qu'elles engendrent, les invasions biologiques offrent de nouvelles perspectives de recherche fondamentale, notamment concernant l'évolution des espèces (Callaway et al., 2006 ; Lee, 2002 ; Sakai et al., 2001). En effet, lorsqu'un processus d'invasion est documenté, il représente une opportunité rare d'observer les différenciations évolutives en réponse à différents paramètres environnementaux, et de relier celles-ci à une période temporelle connue, en général de l'ordre du siècle. Ceci permet de comprendre les mécanismes évolutifs présents dans les populations naturelles, de connaître les pressions de sélection agissant sur les espèces, de déterminer les traits importants pour le maintien des espèces ou encore d'appréhender le potentiel évolutif de ces traits. Or, dans un monde en perpétuel changement, connaître les mécanismes et la rapidité de réponse des populations végétales aux changements environnementaux est crucial pour anticiper le devenir de la flore, tant invasive qu'indigène. Il est donc de premier intérêt de déterminer l'importance de l'évolution rapide (Thompson, 1998), celle de la plasticité phénotypique ou encore l'ampleur de la dérive génétique dans les processus d'invasion, afin d'appréhender quels pourront être les effets à moyen ou long terme du changement global.

Le processus d'invasion d'une espèce introduite, lorsqu'il a lieu, est divisé en trois phases : l'établissement, la naturalisation, et l'expansion proprement dite (Kolar et al., 2001 ; Sakai et al., 2001). A chacune de ces phases, des événements évolutifs peuvent se produire. Ainsi, de nombreuses études se sont concentrées sur les comparaisons génétiques et phénotypiques entre populations au sein de l'aire d'indigénat et au sein de l'aire d'invasion (Bossdorf et al., 2005). Par la suite, les recherches se sont penchées sur les différenciations pouvant avoir lieu au sein même de l'aire d'invasion, en réponse à divers facteurs biotiques ou abiotiques

(Dietz et al., 2006 ; Mihulka et al., 2001 ; Siemann et al., 2001). Parmi ces derniers, le climat est l'un des facteurs influençant le plus la distribution des espèces. L'expansion d'une espèce introduite dépendra donc fortement de la façon dont cette espèce pourra faire face à l'hétérogénéité climatique au sein de la nouvelle aire de répartition (Montague et al., 2008). Plusieurs études (tableau 1) ont mis en évidence des différenciations évolutives de traits de vie, chez des plantes introduites, le long de gradients environnementaux traduisant des variations climatiques. Ces évolutions se traduisent généralement par des variations clinales de traits de vie, étant donné que les facteurs climatiques varient ordinairement de façon graduelle (Endler, 1977). Outre le climat et les facteurs abiotiques de l'environnement, de nombreux facteurs biotiques peuvent influencer les réponses évolutives des espèces introduites (e.g. Blossey et al., 1995 ; D'Antonio, 1993). Enfin, des facteurs intrinsèques à la dynamique de colonisation, tels que l'introduction d'un nombre limité d'individus ou l'expansion rapide de l'espèce, peuvent avoir des implications évolutives (Allendorf et al., 2003). Par exemple, les capacités de dispersion des organismes introduits peuvent se différencier génétiquement au cours de l'invasion, sous l'influence de facteurs intrinsèques à la dynamique de colonisation (Travis et al., 2002). Or, les capacités de dispersion sont des caractères-clés dans l'invasion, qui vont largement influencer la cinétique de colonisation et la structure génétique des espèces au sein de l'aire envahie (Kot et al., 1996).

L'objectif du présent travail est d'estimer l'importance des différentes sources de variation phénotypique que sont l'adaptation, la plasticité phénotypique, les effets maternels environnementaux, le niveau de ploïdie et la dérive génétique, pour différents traits d'histoire de vie, au sein de l'aire colonisée par une espèce végétale invasive. Plus spécifiquement, nous analyserons :

- Les sources de variation phénotypique en réponse aux variations de climat, pour des traits concernant la germination, la croissance et la floraison.

- Les sources de variation phénotypique agissant sur les capacités de dispersion, en relation avec des facteurs intrinsèques à la dynamique de colonisation.

Modèle d'étude

Un problème récurrent dans les études évolutives d'organismes introduits est la multiplicité des introductions, ajoutant une source de variation inopportune à l'interprétation des différences observées. Ceci est notamment typique pour les espèces ornementales (Ellstrand et al., 2006). L'espèce prise comme modèle de recherche dans la présente étude, *Senecio inaequidens* De Candolle (Asteraceae), offre l'avantage de n'avoir été introduite que dans un nombre très limité de sites en Europe. De plus, l'expansion de l'espèce depuis ces sites fut bien documentée par les botanistes du 20ème siècle et les différentes zones d'invasion, chacune relative à un site d'introduction, sont encore relativement indépendantes. Le Séneçon du Cap, *S. inaequidens*, fut introduit en Europe via le commerce de laine en provenance d'Afrique du Sud, vers 1900 (Ernst, 1998). A l'état natif, il se retrouve en milieu ouvert, à des altitudes variées, au Lesotho et en Afrique du Sud (Hilliard, 1977 ; Lafuma et al., 2003). Deux cytotypes y sont présents, diploïde et tétraploïde, alors que seuls des individus tétraploïdes sont recensés en Europe (Lafuma et al., 2003). *S. inaequidens* est une herbacée arbustive vivace, pouvant atteindre ca. 1 m de haut, et produisant de nombreux capitules de couleur jaune or. La pollinisation se fait par des insectes variés, et les fruits sont des akènes munis d'un pappus permettant la dispersion par le vent.

Dans ce travail, nous nous focaliserons principalement sur deux zones européennes d'introduction et d'invasion. L'une se situe en Belgique, l'autre en France. En Belgique, l'espèce fut introduite dans les usines lainières de la région de Verviers, vers 1892 (Verloove, 2006). Pendant près de 40 ans limitée à cette région précise (Mosseray, 1936), l'espèce s'est répandue de plus en plus rapidement : elle atteignit Namur, Charleroi et Gand dans les années 1970 (Lebeau et al., 1978) et Amsterdam vers 1985 (Ernst, 1998). En France, les premiers recensements de l'espèce datent de 1936, dans la petite ville lainière de Mazamet (Tarn, Midi-Pyrénées) (Guillerm et al., 1990). Là aussi, l'espèce ne fut observée, pendant plusieurs années, qu'à proximité du lieu d'introduction. Ce n'est que vers 1950 que l'expansion commença. Dans les années 1970, l'espèce fût considérée comme un adventice des cultures dans la région d'introduction (Jovet et al., 1975). Vers 1980, l'espèce atteignit la côte méditerranéenne (Guillerm et al., 1990). Actuellement, elle est présente dans les Pyrénées-Orientales jusqu'à environ 1700 m d'altitude (Cottrel et al., 1998).

Tableau 1. Etudes empiriques portant sur les différenciations génétiques d'espèces végétales introduites au sein de l'aire d'invasion, en relation avec un gradient environnemental d'ordre climatique.

Espèces	Familles	Type de gradient environnemental	Méthodes				Traits étudiés et différenciations génétique des populations *						Références
			Etude en populations naturelles	Jardins communs	Transplantations réciproques	Analyses moléculaires	Germination	Croissance	Phénologie de la reproduction	Quantification de la reproduction	Morphologie foliaire	Résistance au froid	
Solidago altissima, S. gigantea	Asteraceae	Latitude	+	+				+	+	+	+/-		Weber et al., 1998
Tamarix ramosissima	Tamaricaceae	Latitude (2 zones latitudinales)		+				+			-		Sexton et al., 2002
Verbascum thapsus	Scrophulariaceae	Altitude	+	+			-	-			+		Parker et al., 2003
Hypericum perforatum	Clusiaceae	Latitude		+	+	+		+		+	+/-		Maron et al., 2004
Impatiens glandulifera	Balsaminaceae	Latitude	+	+				+	+	-	-		Kollmann et al., 2004
Eschscholzia californica	Papaveraceae	Latitude, précipitations, température		+			+	+	+	+			Leger et al., 2007
Hypericum perforatum	Clusiaceae	Latitude		+							+		Maron et al., 2007
Solidago altissima	Asteraceae	Latitude		+				+			+		Etterson et al., 2007
Lythrum salicaria	Lythraceae	Latitude	+	+				+	+	+			Montague et al., 2007
Tamarix ramosissima, T. chinensis, and hybrids	Tamaricaceae	Latitude		+		+						+	Friedman et al., 2008
Hypericum canariense	Clusiaceae	Latitude	+	+		+		-	+				Dlugosch et al., 2008
Conyza canadensis, Erigeron annuus, Matricaria discoidea, Solidago canadensis	Asteraceae	Altitude		+	+		-						Poll et al., 2009
Eupatorium adenophorum	Asteraceae	Altitude, disponibilité en eau, température	+	+			+						Li et al., 2009

* + indique une différenciation d'ordre génétique pour au moins un des traits mesurés dans cette catégorie
- indique l'absence de différenciation pour tous les traits mesurés dans cette catégorie

L'évolution des plantes invasives dans leur aire d'introduction : état de la littérature

Le tableau 1 rend compte des études empiriques portant sur les différenciations génétiques d'espèces végétales introduites, au sein des aires d'invasion, en relation avec des gradients climatiques. Ceci devrait permettre au lecteur de replacer le présent travail dans le cadre plus général des travaux actuellement publiés. Ce tableau, exhaustif, est le résultat d'une recherche à l'aide des moteurs de recherches « Web of Science ® » et « Cab Abstract ® ».

L'analyse du tableau 1 amène à plusieurs constatations :

- Diverses familles ont été étudiées, mais la famille des Asteraceae est la plus représentée.
- Les gradients latitudinaux ont été largement plus étudiés que les gradients altitudinaux. Ces derniers ont fait l'objet d'études parmi les plus récentes.
- Les jardins communs peuvent êtres considérés comme la base de ce genre d'études empiriques. Ils ont été effectués dans toutes les études présentées, mais peu y ont été couplés à des mesures en populations naturelles. De façon encore plus nette, très peu d'expériences en transplantations réciproques ont été menées, malgré que de telles expérimentations soient théoriquement nécessaires pour mettre en évidence une adaptation locale.
- Récemment, certaines études en jardins communs ont été couplées à des analyses génétiques moléculaires. Néanmoins, aucune étude n'intègre les quatre méthodes considérées dans le tableau.
- Les traits de croissance ont été les plus étudiés. Dans de nombreux cas, des différenciations d'ordre génétique ont été trouvées pour ces traits. Ce type de trait, au-delà de son coté évident dans l'histoire de vie des végétaux, présente donc un intérêt dans le cadre de la comparaison d'études et des méta-analyses.
- La germination, par contre, fut très peu étudiée. En effet, la plupart des méthodes utilisées dans les études en jardins communs comportaient une phase de production de plantules en conditions contrôlées (*i.e.* serre), préalable à l'expérience proprement dite. Il en résulte, par ailleurs, que les effets maternels environnementaux, comme source de variabilité phénotypique, ont été très peu étudiés.
- Dans les études incluant des traits liés à la reproduction, ces traits ont très généralement montré des différenciations génétiques le long des gradients considérés.
- D'autres traits ont été étudiés, comme la morphologie foliaire et la résistance au froid, sans que l'on puisse, à ce stade, faire de généralisation.

Dans ce contexte, le présent travail vise à développer les aspects peu étudiés dans les mécanismes évolutifs liés aux invasions, en relation avec les variations de climat : importance des effets maternels environnementaux, utilité des gradients altitudinaux comme gradients climatiques, mise en évidence d'adaptation locale par l'usage de transplantations réciproques, etc.

Comparaisons envisagées et articulation des différents articles

L'historique d'invasion du Séneçon du Cap en fait un excellent modèle d'étude pour la biologie évolutive. Dans cette thèse de doctorat, nous envisagerons plusieurs comparaisons de traits de vie. Chacune de ces comparaisons s'inscrit à une échelle géographique donnée, et permet de répondre à des questions précises quant aux sources de la variation phénotypique au sein de l'espèce. Cette variation phénotypique sera généralement appréhendée à l'échelle de la population. Ainsi, nous comparerons des populations de cytotypes différents, des populations africaines et européennes, ainsi que des populations situées le long de gradients environnementaux et de gradients d'éloignement par rapport à un site d'introduction. La figure 1 présente le cadre géographique relatif aux différentes comparaisons envisagées. Les comparaisons le long de transects d'altitudes, principalement en France, constitueront la partie la plus détaillée de l'étude.

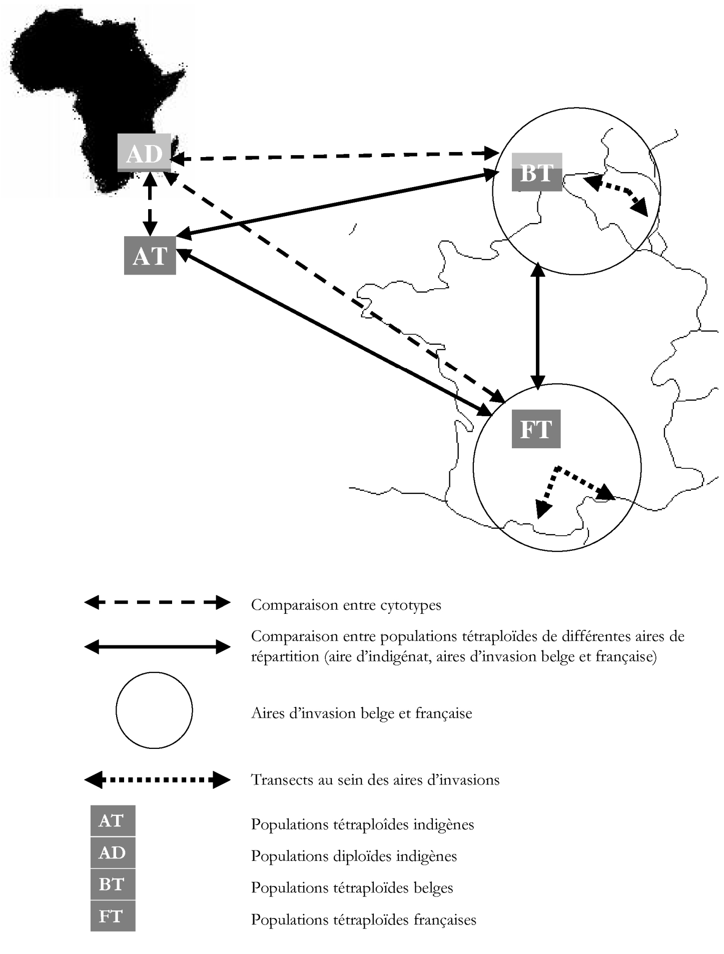

Figure 1. Représentation schématique des comparaisons envisagées dans le présent travail, en termes géographiques. Les comparaisons le long des transects altitudinaux au sein de l'aire d'invasion française constituent la partie la plus développée de l'étude.

Comme nous l'avons présenté plus haut, les traits de vie étudiés seront divisés en deux groupes : les traits phénologiques, concernant la germination, la floraison et la croissance d'une part, et les traits directement liés à la dispersion des akènes, d'autre part. Nous verrons que la masse des graines sera considérée dans les deux groupes. Les sources de variation phénotypique considérées seront le niveau de ploïdie, la plasticité phénotypique, les effets maternels environnementaux, les différenciations génétiques en réponse à la sélection naturelle et la dérive génétique. Nous ne considérerons pas explicitement l'influence des mutations.

Afin d'analyser les sources de variation phénotypique au sein de l'espèce, des akènes ont été prélevés en populations naturelles, mesurés et utilisés dans plusieurs expériences. Au total, 25 populations ont été considérées. Quatre, dont deux diploïdes et deux tétraploïdes, sont situées en Afrique du Sud. En Belgique, huit populations se situent le long d'un gradient d'altitude allant de la côte jusqu'à l'Ardenne, en passant par la zone d'introduction de Verviers. Deux autres populations ont été considérées pour les mesures de capacités de dispersion. En France, 13 populations ont été échantillonnées le long d'un transect suivant un gradient d'altitude, allant de la côte méditerranéenne aux Pyrénées-Orientales, en passant par le site d'introduction de Mazamet. En plus des collectes de diaspores, différentes mesures de terrain ont été réalisées dans ces populations.

Par ailleurs, plusieurs expérimentations ont été menées au cours de trois années. Ainsi, quatre expériences en jardins communs ont été installées, à Gembloux (Belgique), dont une en chambre conditionnée à des fins de croisements contrôlés. Deux autres, en transplantations réciproques, ont été mises en place à Montpellier (France, Hérault) et à La Cabanasse (Pyrénées-Orientales). Ces différentes expériences peuvent être mises en relation avec les comparaisons de la figure 1. Deux expériences en jardins communs, installées en 2006 et 2007 à Gembloux, avaient pour but les comparaisons entre cytotypes, les comparaisons entre populations tétraploïdes de différentes aires de répartition, ainsi que les comparaisons le long des deux transects d'altitude, belge et français. En 2007, l'expérience en chambre contrôlée, suivie d'une expérience en jardin commun également installée à Gembloux, concernaient uniquement les comparaisons de populations le long du transect français. Enfin, les transplantations réciproques n'incluaient que des populations françaises, originaires des parties centrales (Mazamet) et extrêmes (côte méditerranéenne et zone d'altitude dans les Pyrénées) du gradient.

Le travail de thèse est composé de sept articles, en plus du présent essai. Les articles sont classés comme suit :

Article 1 : Monty A., Mahy G. Evolution des traits d'histoire de vie lors des invasions végétales. Sous presse dans la revue *BASE*.

> Cette synthèse bibliographique décrit le contexte théorique dans lequel les recherches se déroulent. Il reprend les différents concepts nécessaires à la compréhension des travaux effectués, tels que ceux d'expériences en jardins communs et en transplantations réciproques.

Article 2: Monty, A. Maurice S., Mahy G. Life history traits variation among native diploid, native tetraploid and invasive tetraploid forms of *Senecio inaequidens* (Asteraceae).. En préparation pour la revue *Plant Species Biology*.

> Premier article résultant des jardins communs réalisés en 2006 et 2007, cet article correspond aux comparaisons les plus larges, géographiquement. Il concerne en effet la comparaison entre populations africaines et européennes. Par ailleurs, il met l'accent sur les comparaisons entre cytotypes diploïde et tétraploïde, afin de comprendre l'influence du niveau de ploïdie sur les traits d'histoire de vie.

Article 3: Monty A., Mahy G. Clinal differentiation during invasion: *Senecio inaequidens* (Asteraceae) along altitudinal gradients in Europe. *Oecologia* 159:305–315.

> Deuxième article relatif aux expériences en jardins communs menées en 2006 et 2007, cette publication concerne les comparaisons réalisées au sein des aires d'invasion belge et française, le long des gradients d'altitudes. Il montre des patrons de variations clinaux dans les traits d'histoire de vie phénologiques, et met ceux-ci en relation avec le climat.

Article 4: Monty A., Lebeau J., Meerts P., Mahy G. An explicit test for the contribution of environmental maternal effects to rapid clinal differentiation in an invasive plant. *Journal of Evolutionary Biology (*Early view – doi: 10.1111/j.1420-9101.2009.01728.x).

> Cet article présente les résultats d'un troisième jardin commun, réalisé en 2007 à Gembloux. Comparant les traits phénotypiques de plants issus des populations françaises, à partir de diaspores collectées *in situ* et de diaspores produites en conditions contrôlées (jardin commun annexe, en chambre de culture), il présente un test explicite quant à l'importance des effets maternels environnementaux sur les variations clinales observées antérieurement.

Article 5: Monty A., Escarré, J., Mahy G. Environmental maternal effects influence *Senecio inaequidens* (Asteraceae) phenotypes under harsh climatic conditions. Note préliminaire, en préparation pour la revue *Ecology letters.*

> Poursuivant le raisonnement de l'article précédent, cette note présente les résultats des transplantations réciproques menées en France en 2008. De nouveaux éléments, ayant traits à l'importance des effets maternels dans les variations phénotypiques, sont apportés. Par ailleurs, d'autres sources de variations, d'ordre génétique, sont discutées.

Article 6: Monty A., Stainier C., Lebeau F., Pieret N., Mahy G. (2008) Seed rain pattern of the invasive weed *Senecio inaequidens* (Asteraceae). *Belgian Journal of Botany* 141 (1): 51-63.

> Cette partie de l'étude concerne les traits liés à la dispersion de l'espèce. Dans un premier temps, il permet l'identification d'un trait proxy de la dispersion, utilisé ultérieurement. Par ailleurs, il présente l'application d'un modèle gaussien à la dispersion du Séneçon.

Article 7: Monty A., Mahy G. Evolution of dispersal traits along an invasion route in the wind-dispersed Senecio inaequidens (Asteraceae). Soumis à la revue *Oikos* le 17/02/2009.

> Grâce à l'identification préalable d'un trait proxy de la dispersion, ainsi qu'à la récolte et la mesure de diaspores dans un jardin commun et dans des populations naturelles, cet article présente l'analyse de la variabilité phénotypique des traits de dispersion au sein de l'aire d'invasion française. Cette variabilité est mise en relation avec la distance séparant les populations du site d'introduction de Mazamet.

Résultats et discussion

Au terme de cette étude, plusieurs patrons de variation ont été dégagés au sein de la variabilité phénotypique de l'espèce. Premièrement, une différence d'histoire de vie a été mise en évidence entre plants diploïdes et plants tétraploïdes (article 2). Les premiers ont une production de biomasse soutenue et une production de capitule plus importante que les seconds, pendant la première année de croissance en jardin commun. Mais ils présentent un taux de survie hivernal nul, sous nos latitudes. Autrement dit, les populations diploïdes tendent vers un cycle de vie annuel sous les conditions climatiques rencontrées dans le centre de la Belgique. Ceci affecte très probablement la valeur adaptative moyenne de ces populations: aucun plant diploïde ne semble d'ailleurs s'être installé dans les alentours du site d'expérience. L'expérience menée n'a pas permis de mettre en évidence de différences entre cytotypes pour d'autres traits, concernant la germination et la floraison. La tétraploïdie semble néanmoins favoriser le caractère invasif de *S. inaequidens*, principalement via la résistance aux conditions hivernales rigoureuses. Ceci est en accord avec la littérature soulignant la plus grande résistance des polyploïdes aux stress environnementaux (Bretagnolle et al., 1998 ; Levin, 1983). Entre populations tétraploïdes de continents différents, peu de différences ont été observées. De nouveau, c'est le suivi de la survie hivernale qui a permis de montrer des différences entre les plants africains et européens. Avec un taux de survie de 30 à 40 %, les populations européennes se sont révélées supérieures aux africaines, dont le taux de survie est de l'ordre de 15 %. Une explication plausible de cette différence, soutenue par une étude récente menée en Allemagne, est que seuls les génotypes pré-adaptés aient pu s'établir en Europe (Bossdorf et al., 2008).

A coté de ces comparaisons à grande échelle géographique, l'étude de la variabilité phénotypique et de ses causes le long des gradients altitudinaux s'est elle aussi révélée informative. Les transects d'étude suivaient des gradients climatiques différents (article 3). Ainsi, le gradient belge depuis la côte (altitude : 4 m) jusqu'aux altitudes plus élevées (480 m) correspondait à la fois à une diminution des températures moyennes mensuelles de toute l'année et une augmentation des précipitations, sur toute l'année également. Le gradient échantillonné en France, dont les altitudes étaient plus contrastées (2 à 1695 m) correspondait plutôt à un gradient de sécheresse estivale et de températures moyennes mensuelles sur toute l'année. En altitude, les températures sont plus basses mais la sécheresse estivale s'estompe. Notons qu'en altitude, d'autres paramètres abiotiques varient : par exemple, les radiations ultraviolettes s'intensifient.

En conditions homogènes comme en conditions naturelles, les populations du transect français ont montré une différenciation relativement nette des traits phénologiques, comme la hauteur à la première floraison, la hauteur finale et la biomasse aérienne (article 3). Plus les plants étaient originaires de populations d'altitude élevée, plus ils présentaient un phénotype de petite taille. Un patron similaire, quoique moins net, fut observé le long du transect belge (article 3). Ces résultats ont montré que la plasticité phénotypique chez l'espèce étudiée ne pouvait, à elle seule, expliquer la variabilité phénotypique. Néanmoins, plusieurs explications sont possibles pour rendre compte des patrons observés (article 3). L'adaptation en réponse aux conditions climatiques apparaît comme une explication plausible. En effet, des patrons similaires furent observés au sein des deux aires d'études

supposées indépendantes, la réduction de la taille au sein des populations de montagne est une observation courante chez diverses espèces (Körner, 2003), et ceci fut observé à la fois dans les populations naturelles et en conditions contrôlées. Rappelons que dans ce dernier cas, les variations phénotypiques observées sont principalement attribuables aux variations génétiques (Clausen et al., 1940).
Néanmoins, à ce stade de l'étude, l'influence des effets maternels environnementaux (EME) n'était qu'en partie appréhendée (à travers l'influence de la masse des akènes). Or, ceux-ci peuvent se marquer, même en conditions contrôlées (Roach et al., 1987). Ils pouvaient donc, du moins en partie, expliquer le patron de variation observé. Parallèlement, même si la différenciation génétique est acquise, une expérience en jardin commun ne peut pas, par essence, démontrer le caractère adaptatif de cette différenciation. Des différenciations génétiques non-adaptatives, induites par la dérive génétique et/ou des effets de fondation répétés, peuvent aussi résulter en des patrons clinaux (Endler, 1977). Au terme des expériences en jardin commun de 2006 et 2007 (article 3), plusieurs sources de variations phénotypiques pouvaient donc encore être avancées pour expliquer les résultats obtenus. Afin de mieux cerner ces sources de variations, nous avons recentrés nos recherches sur le gradient environnemental français, plus contrasté.

Les expériences suivantes ont montré l'intérêt de scinder, le long du transect français, l'invasion vers la montagne et celle vers la plaine. Les mécanismes évolutifs accompagnant les deux phénomènes d'invasion ne sont en effet pas tout à fait identiques, même si certains mécanismes sont comparables et qu'il y a, bien sûr, eu des connections entre les deux invasions.

L'une des différences principales concerne l'importance des effets maternels environnementaux dans la variation phénotypique. Ainsi, en plaine, où les conditions climatiques sont plutôt favorables lors de la germination printanière (vers avril), la rapidité de la germination fait que les effets maternels sont négligeables (article 5). Ceci fut observé à Gembloux (article 4) et à Montpellier (article 5). De plus, à Gembloux, nous avons pu mettre en évidence qu'il n'y avait pratiquement pas d'effets maternels induits, indépendamment de la masse des graines, par les conditions environnementales de production des diaspores. En montagne, par contre, les effets maternels environnementaux jouent un rôle non-négligeable dans la variabilité phénotypique. En conditions climatiques printanières plus rudes (températures plus basses et plus fluctuantes), la masse des graines influence significativement le délai de germination, les graines les plus grosses germant plus rapidement (article 5). Dès lors, les plants issus de ces graines ont une avance de croissance par rapport aux autres, qui se traduit par une plus grande taille et une production de capitules plus importante au terme de la saison de végétation. En montagne, donc, les effets maternels environnementaux apparaissent comme une source essentielle de variation phénotypique, modifiant la valeur adaptative (*fitness*) des individus (article 5). Comme il a par ailleurs (articles 7 et 4) été observé qu'une réponse plastique semblait induire la production de graines plus grosses en montagne, il apparaît qu'une plasticité trans-générationnelle adaptative (Mousseau et al., 1998) pourrait expliquer, du moins en partie, le succès de *S. inaequidens* dans les régions montagnardes.

Une autre différence fondamentale entre l'invasion vers la plaine et vers la montagne concerne la différenciation génétique des populations. Cette différence est avérée à travers l'ensemble du gradient altitudinal français, puisqu'elle fut observée plusieurs fois (articles 3, 4 et 5), notamment en l'absence d'effets maternels environnementaux. Néanmoins, bien que les causes exactes de cette différenciation ne soient pas définitivement déterminées, celles-ci semblent varier selon la zone climatique envahie.

En plaine méditerranéenne, l'invasion s'est faite progressivement depuis Mazamet. Au cours de cette progression, les plants à croissance rapide, produisant beaucoup de biomasse et de capitules dès la première année, semblent avoir été sélectionnés. En effet, quand on cultive en plaine méditerranéenne (Montpellier) des plants de 1) Mazamet et 2) la plaine méditerranéenne, les derniers présentent globalement des phénotypes plus grands et plus féconds (en fait, la plus grande fécondité n'est pas statistiquement significative entre les deux zones, mais elle est avérée pour au moins une population) (article 5). D'après la théorie, ceci rend compte d'un cas d'adaptation locale (Kawecki et al., 2004). Néanmoins, il est étonnant de constater que même s'ils sont cultivés en montagne, les plants méditerranéens sont aussi les plus grands et les plus féconds. Il s'agirait donc de génotypes investissant plus de ressources dans la croissance, la première année du moins. Ceci devrait leur conférer un avantage adaptatif, au moins en méditerranée.

Inversement, les plants montagnards étaient plus petits que ceux de Mazamet, ou de méditerranée, dans les deux jardins en transplantations réciproques (article 5). Par ailleurs, ils n'étaient pas particulièrement plus féconds. Ceci pourrait être typique d'un cas de dérive génétique, induisant une perte de variabilité génétique, et/ou de dépression de consanguinité augmentant l'homozygotie et la fixation d'allèles délétères. On peut en effet imaginer que la colonisation montagnarde, plus aléatoire que celle de la plaine à cause de nombreuses barrières géographiques, se soit caractérisée par des effets de fondation répétés et des flux de gènes extrêmement faibles entre populations. Néanmoins, il se pourrait aussi que les plants montagnards soient adaptés aux conditions climatiques plus rigoureuses. En effet, s'il existe un compromis entre la croissance et la production de capitules pendant la saison de végétation, d'une part, et d'autre part la capacité de survie hivernale en conditions rudes, alors la petite taille des plants montagnards pourrait simplement traduire une adaptation locale.

A ce stade, nous ne pouvons toujours pas établir laquelle des deux explications est valide. Cela va dépendre du patron observé au niveau de la survie hivernale, dans les deux jardins en transplantations réciproques (toujours en cours):

- Si les plants montagnards montrent un taux de survie plus élevé dans le jardin d'altitude, alors un patron de type adaptatif sera évident. En effet, la plus petite taille et la plus faible production de capitules seraient alors des adaptations à un climat plus rude et des hivers plus rigoureux, permettant d'assurer la reprise végétative l'an suivant. Les plants de plus basses altitudes, qui croissent et fleurissent abondamment la première année, seraient au final contre-sélectionnés si cette croissance rapide se faisait au détriment de la survie hivernale. *A contrario*, si la survie est importante à Montpellier grâce à la douceur relative de l'hiver, ces plants de plaine produisant beaucoup de capitules chaque année seraient sélectionnés favorablement, alors que la « prudence » des plants montagnards ne serait pas récompensée. Il y aurait adaptation locale à la fois en plaine et en montagne.

- S'il n'y pas de patron significatif ou si le patron ne montre pas un avantage des plants montagnards en terme de survie hivernale en montagne, alors les variations phénotypiques observées ne seront pas attribuables à de l'adaptation locale. Nous avons vu qu'elles étaient, du moins en montagne, partiellement attribuable aux effets maternels. Mais nous avons aussi mis en évidence qu'au-delà de ces effets maternels, il existait une différenciation génétique entre les zones altitudinales. Si ces différences ne sont pas liées à de l'adaptation, elles seront attribuables à la dérive génétique et/ou la consanguinité des populations de montagne.

Un autre aspect de l'histoire de vie, important dans le processus d'invasion, concerne les capacités de dispersion des diaspores. Là aussi, une variabilité phénotypique existe au sein de l'espèce, qui a pu être reliée à des phénomènes évolutifs. Ainsi, nous avons testé l'hypothèse qu'au cours de l'invasion progressive du sud de la France depuis Mazamet, il y avait eu différenciation génétique dans les capacités de dispersion en réponse à la sélection des meilleurs disperseurs au front d'invasion. Selon la théorie, en effet, un tel scénario doit avoir lieu, du moins si les plants du front d'invasion (émanant des meilleurs disperseurs) ne subissent pas d'effet Allee (Travis et al., 2002). Il faut également qu'il y ait une héritabilité suffisante des traits de dispersion.

Nous avons d'abord mis en évidence un trait facilement mesurable, rendant compte de manière précise des capacités de dispersion par le vent. Ce proxy de la dispersion est la racine carrée du *plume loading*, c'est-à-dire la racine carrée du rapport entre la masse de la diaspore et sa surface projetée horizontalement (dans notre cas, la surface du pappus). Cette recherche d'un proxy suffisamment corrélé à la vélocité terminale, elle-même considérée comme l'une des meilleures mesures prédictives des capacités de dispersion chez les plantes anémochores (Augspurger et al., 1987 ; Platt et al., 1977), fait l'objet de l'article 6. Dans l'article 7, nous avons explicitement testé l'hypothèse précitée à partir d'achènes récoltés dans les populations naturelles et d'achènes produits dans les expériences en jardin commun. Il existait bel et bien une tendance d'ordre génétique dans les traits mesurés, quoique subtile. Celle-ci se caractérise par la production d'achènes aux meilleures capacités de dispersion par les plants originaires de populations plus éloignées de Mazamet. Néanmoins, cette tendance ne s'est pas vérifiée dans les populations naturelles, ce qui souligne l'effet de la plasticité phénotypique dans ces traits. Etant donnée l'importance de la masse des graines dans le succès des descendants en montagne (article 5), et comme le front d'invasion étudié était situé dans les Pyrénées, ces résultats font le lien entre les deux types de traits de vie mesurés : une meilleure capacité de dispersion est favorable à la progression de l'invasion, mais les plants issus de graines mieux dotées, plus grosses et moins bien dispersées, auront de meilleures chances d'avoir une descendance importante en montagne. Une réponse plastique aux conditions climatiques, et une sélection génotypique sur les traits de dispersion semblent finalement, dans les conditions montagnardes d'étude, agir de manières opposées.

Conclusions

Finalement, les sources de variation phénotypique sont nombreuses et complexes au sein de l'aire d'invasion européenne de *S. inaequidens*. La tétraploïdie confère certains attributs (article 2), mais comme seuls des individus tétraploïdes sont présents en Europe (Lafuma et al., 2003), le niveau de ploïdie n'est pas, en soi, une source de variation phénotypique au sein de l'aire d'invasion. L'espèce présente de la plasticité phénotypique, notamment en réponse au climat, comme l'ont révélé les différences évidentes entre les jardins en transplantations réciproques (article 5). La plasticité de l'espèce avait par ailleurs été observée, moins formellement, lors des nombreuses expériences menées (par exemple, la taille bien moins importante des plants quand ils étaient cultivés en plus petits pots). Mais cette plasticité, à elle seule, est loin d'expliquer toute la variabilité phénotypique au sein de l'espèce, et le Séneçon du Cap n'est pas un exemple d'espèce se caractérisant par des *general purpose genotypes*, au sens de Baker (Baker et al., 1965). En effet, les populations de *S. inaequidens* se sont différenciées génétiquement au sein de l'aire d'invasion (articles 3, 4, 5 et 7). Nos résultats contrastent donc avec ceux de Parker et al. (2003), qui furent parmi les premiers à étudier la variabilité phénotypique chez une plante invasive le long d'un gradient d'altitude. De même, les récents travaux de Poll et al. (2009) sur la germination de plusieurs espèces d'Asteraceae n'ont pas indiqué de différentiation génétique en relation avec l'altitude. Li et al. (2009), par contre, ont mis en évidence une différenciation génétique altitudinale dans les traits de germination et la morphologie des graines d'Eupatoire (tableau 1). Même si dans notre cas, la part des différentes sources de variabilité génotypique n'est pas exactement connue, il semble probable que la sélection naturelle ait agit sur des traits relatifs, notamment, à la croissance et la dispersion. Enfin, les effets maternels environnementaux, à travers la masse des graines, sont une source de variabilité phénotypique qui n'est exprimée qu'en conditions difficiles (article 5). Il est à noter que la majeure partie des résultats obtenus (articles 4 et 5) sont relatifs à l'aire d'invasion française. Il semble (article 3) que certains aspects soient comparables en Belgique, même si les conditions climatiques sont moins contrastées et le flux de gènes potentiellement plus important (plus de voies de communications importantes).

Les différentes sources de variabilité étudiées au cours de la thèse peuvent être mises en relation avec le tableau 1. Cette approche permet de replacer le présent travail dans le cadre des connaissances actuelles:

- Notre modèle d'étude étant de la famille des Asteraceae, le présent travail étoffe les connaissances évolutives au niveau de la famille déjà la plus documentée, en ce qui concerne l'évolution dans les aires d'invasion. Ceci devrait aider, à l'avenir, à l'émergence de patrons généraux concernant l'évolution de cette famille sous des régimes de sélection nouveaux.
- Les différenciations pour les traits de croissance, observées ici de façon nette, sont aussi les plus évidentes dans les autres études.
- Les études le long de gradients altitudinaux étant largement moins nombreuses que celles le long de gradients latitudinaux, la prise en compte de deux gradients d'altitudes dans le présent travail a permis de tester la pertinence de tels gradients comme modèles de variations climatiques.
- L'analyse poussée de la germination et des effets maternels liés à la germination a souligné l'importance de commencer les expériences en jardins communs à partir de graines, et non de plantules, dans l'étude des sources de variabilité phénotypique agissant en conditions naturelles.

L'apport de l'écologie évolutive des invasions végétales dans un contexte de changement global

Avant de clore cet essai par les critiques et perspectives, la présente section a pour but de replacer les travaux présentés dans un cadre plus général, afin de mettre en évidence l'intérêt des études se situant à l'intersection de la biologie des invasions, de l'étude de l'évolution et du contexte actuel de pression anthropique sur l'environnement.

Le *changement global*, ensemble des modifications induites actuellement par l'Homme sur la planète, incluant les changements d'utilisation des sols et les changements climatiques, est une menace majeure pour la biodiversité. Dans un environnement en perpétuelle évolution, identifier les mécanismes de réponse des espèces aux changements environnementaux est donc de première importance pour anticiper le devenir de la biosphère. L'évolution peut jouer un rôle non-négligeable dans ces mécanismes de réponse, étant donné que les changements induits par l'Homme constituent souvent des pressions de sélection drastiques et nouvelles. Dès lors, il y a lieu de connaître les facteurs de l'environnement les plus susceptibles d'induire l'adaptation (ou d'autres processus évolutifs), et d'appréhender leurs effets en termes de vitesse et direction des différenciations génétiques, ainsi qu'en termes de traits influencés chez les espèces.
Ceci est important pour l'ensemble de la flore, représentant la base trophique de nos écosystèmes. L'évolution de cette flore en réponse au changement global peut avoir des implications différentes, à la fois écologiques, sociales et économiques, selon les (groupes d') espèces concerné(e)s. Ainsi des études ont montré que les changements évolutifs pouvaient être un moyen pour des espèces patrimoniales menacées d'éviter l'extinction (Rice et al., 2003), soulignant par là l'importance d'intégrer ces aspects dans les programme de conservation et de restauration écologiques. Au niveau agronomique également, la prise en compte des changements évolutifs peut aider à anticiper les problèmes futurs. Les plantes adventices des cultures, par leurs cycles de vie rapides et les pressions anthropiques qu'elles subissent, sont susceptibles d'évoluer rapidement (Baker, 1974). Or les programmes de lutte contre ces espèces ne disposent encore que de peu de données concernant le potentiel évolutif en réponse aux variations des conditions abiotiques, comme le climat ou la fertilité des sols. En ce qui concerne les espèces cultivées, dont la génétique est cependant mieux contrôlée, il y a également lieu de s'interroger sur les effets à long termes du changement global, notamment au niveau climatique (De Wet et al., 1975).

Même quand les réponses évolutives sont observables au sein de la flore native, il est difficile de les relier à une échelle temporelle (Reznick et al., 2001). La dimension temporelle des changements environnementaux, et celle des modifications qu'ils engendrent au sein des espèces, est pourtant fondamentale (Reznick et al., 2001; Endler, 1986). Une solution consiste à étudier les sources de variation phénotypique le long de gradients de colonisation dont l'historique est documenté (Reznick et al., 2001; Weber et al., 1998; Till-Bottraud et al., 1990), comme cela est souvent le cas dans le cadre d'invasions végétales. Les plantes invasives représentent donc des modèles d'études écologiques et évolutives concernant les réponses de la flore au changement global, puisqu'elles rencontrent au cours de l'invasion des fluctuations environnementales comparables à celles que subissent les espèces natives actuellement.

Mais les invasions biologiques sont, par ailleurs, l'une des composantes négatives majeures de ce changement global, reconnues comme l'une des principales atteintes à la biodiversité (Pimentel et al., 2000 ; Vitousek et al., 1997). Ceci représente une autre raison de les étudier. Plusieurs solutions ont été avancées pour enrayer les invasions, tant préventives que curatives. Mais toutes se heurtent au manque de compréhension du phénomène, et au manque de généralisation quant aux mécanismes et raisons intrinsèques de l'invasion. De plus, les différentes composantes du changement global peuvent interagir, une composante influençant la dynamique d'une autre composante. Dans ce contexte où les différents élément d'un changement général sont eux-mêmes en perpétuelle modification, la prise en compte de la biologie évolutive dans l'étude des invasions biologiques s'est imposée comme nécessaire (Callaway et al., 2006). Jusqu'ici, les études empiriques ont largement validé l'importance des changements évolutifs dans les invasions (*e.g.* tableau 1).

Les études évolutives, y compris le présent travail, soulignent donc l'importance de considérer les espèces invasives comme des entités variables, et non pas comme immuables dans le temps. Plus que jamais, l'évolution des populations devra se considérer sur des laps de temps relativement courts, de l'ordre de la décennie ou du siècle. Ceci devra se répercuter sur les planifications de gestions, et les développements d'aspects légaux sur les invasions.

La prise en compte d'une vision dynamique de la notion d'espèce invasive peut également avoir diverses répercussions en recherche scientifique. A titre d'exemple, certains efforts de recherche ont actuellement lieu pour modéliser, sur base de l'autécologie mesurée des espèces, les aires potentielles d'invasions d'organismes récemment introduits. S'il s'avère que l'évolution modifie cette autécologie au cours même de l'invasion, de telles modélisations ne sauraient être valables sans en tenir compte, de manière chiffrée. Des données empiriques quantitatives sur la réponse évolutive des organismes exotiques aux variations environnementales seront dès lors nécessaires à l'anticipation, et la modélisation, des invasions. A leur tour, les données empiriques pourront ouvrir de nouvelles pistes de développements théoriques, et les résultats obtenus permettront d'appréhender le devenir de la flore locale.

Plus concrètement, en ce qui concerne la gestion de l'espèce *S. inaequidens* au sein de son aire d'invasion, plusieurs lignes directrices peuvent être avancées au vu des résultats du présent travail :

- Dans les zones où l'espèce est encore peu présente, situées entre des zones plus envahies (comme le centre de la France, par exemple), la gestion doit se faire efficacement et rapidement. En effet, si l'espèce n'y est que faiblement représentée, il y a tout lieu de penser que ce n'est qu'une situation temporaire. Comme des populations ont réussi à se maintenir dans des zones très contrastées, il est vraisemblable qu'elles se maintiendront en zones intermédiaires.
- Si malgré tout les différentes zones d'invasions se rejoignent, la zone de rencontre des fronts d'invasions risque de se caractériser par une forte vigueur des individus, par une recombinaison génétique entre plusieurs *pools* différenciés (*hétérosis*).
- Une attention particulière devra être portée à ne pas laisser l'espèce s'installer en zone montagneuses non-encore envahie, comme les Alpes françaises ou les Pyrénées, à des altitudes supérieures à 1800 m: son absence n'y est vraisemblablement pas liée à une limitation autécologique, ou du moins, cette limitation ne se maintiendrait pas en cas

d’introductions répétées. Ceci pourra être rendu possible par une détection et une éradication précoce de l’espèce dans ces zones, qui représentent souvent des milieux de grand intérêt biologique.

- Dans les zones déjà envahies, il y a également lieu d’essayer, dans la mesure du possible, d’éviter de transporter des graines d’une zone à l’autre (de la plaine à la montagne, ou de Belgique en France, par exemple). Ceci afin d’éviter d’induire de nouvelles combinaisons génétiques, potentiellement favorables à l’espèce et pouvant contrer les effets de la dérive dans les populations isolées.
- Même si le transport lainier depuis l’Afrique du Sud est actuellement interrompu, il est toujours possible que des graines diploïdes soient introduites en Europe (via la recherche scientifique, via des importations autres ou en provenance de zones envahies par le cytotype diploïde, comme le Mexique ou l’Australie) : ceci peut représenter un danger dans la mesure où ce cytotype réussit à effectuer son cycle de reproduction sous nos latitudes.
- Pour les actions de recensement et de gestion de l’espèce, il y a lieu de commencer plus tôt en zone climatique plus clémente (le décalage entre les extrêmes étudiés étant de l’ordre d’un mois).
- Il y a, enfin, lieu de garder à l’esprit que le potentiel évolutif de *S. inaequidens* induit que des techniques de gestion efficace actuellement ne le seront pas nécessairement indéfiniment, ni partout. Par exemple, les méthodes de contrôles biologiques peuvent être rendues inefficaces par des changements évolutifs au niveau de la plante cible (Müller-Schärer et al., 2004).

Comme indiqué plus haut, le présent travail devrait favoriser la compréhension du potentiel de différenciation dont disposent les espèces végétales. Néanmoins, actuellement, le nombre d’études empiriques ne permet pas encore de véritables généralisations. Nous encourageons les recherches futures qui permettront, sur base d’études comparables, utilisant *S. inaequidens* ou d’autres modèles végétaux, de généraliser les connaissances apportées par le présent travail et de mieux appréhender le devenir des plantes face au changement global.

Critiques et perspectives

Au terme de cet essai, plusieurs critiques peuvent être émises quant aux expérimentations menées et leurs interprétations. Ainsi par exemple, il eut été intéressant de présenter, dans les résultats des transplantations réciproques, davantage de traits de vie (dont les mesures sont toujours en cours, pour certains). Considérer d’autres sources de variations que celles, abiotiques, considérées ici, aurait également présenté un intérêt certain. L’influence du type de sol, mais aussi celle des herbivores ou de la compétition exercée par la flore native, sont des sources de variabilité phénotypique qui auraient pu être analysées dans une perspective évolutive. Enfin, une analyse moléculaire de la structure génétique des populations étudiées aurait été précieuse dans les interprétations : elle est prévue dans un avenir proche.

Malheureusement, les temps impartis et les coûts de mise en place des expérimentations sont souvent limitant en recherche fondamentale, et il a été choisi de se focaliser principalement sur les sources de variation phénotypique en réponses aux facteurs climatiques. Que le lecteur intéressé se rassure néanmoins : l’intérêt de *S. inaequidens*

comme modèle d'étude est maintenant largement reconnu et plusieurs équipes de recherches développent certains aspects que nous n'avons pu développer.
Notre restriction dans le champ des recherches a par ailleurs permis une étude relativement exhaustive du domaine en question. Peu d'études, à ce jour, ont mis en relation les différentes sources de variations que sont la polyploïdie, les effets maternels, la différenciation génétique et la plasticité chez une plante exotique envahissante.

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Article 1

Evolution des traits d'histoire de vie lors des invasions végétales

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Résumé : Les invasions par les plantes, malgré les problèmes qu'elles engendrent, sont des opportunités d'étude des phénomènes évolutifs rapides. Dans cet article, sont présentés les principaux changements évolutifs d'histoire de vie accompagnant les invasions végétales. Ceux-ci peuvent avoir lieu lors des différentes phases de l'invasion que sont l'introduction, la naturalisation et l'expansion proprement dite. Leur étude s'articule généralement en deux approches: les comparaisons entre populations de l'aire d'indigénat et de l'aire d'invasion d'une part, et, d'autre part, les études de la différenciation des populations au sein de l'aire d'invasion.

Mots-clés: *Histoire de vie, jardin commun, invasion biologique, hypothèse EICA, sélection naturelle, aire d'indigénat, aire d'introduction, effet de fondation, dérive génétique.*

Abstract: Despite the problems they cause, plant invasions can be seen as opportunities for the study of rapid evolution. In this paper are presented the main evolutionary changes in life history that can occur during the three main plant invasion phases: introduction, naturalization and expansion in the new range. The study of those evolutionary changes is traditionally split in 1) the comparison between native and introduced populations and 2) the study of life history differentiation within the invaded area.

Keywords: *Life history, common garden, biological invasion, EICA hypothesis, natural selection, native range, introduced range, founder effect, genetic drift.*

1. Introduction

Avec l'intensification des échanges inter-continentaux, les activités humaines ont donné une dimension nouvelle aux capacités de dispersion des espèces végétales (Cronck et al., 2001 ; Prinzing et al., 2002 ; Reichard et al., 2001 ; Weber, 1997). Le bouleversement qui en découle dans les patrons de répartition des végétaux est au cœur du phénomène d'invasion par les plantes, et plus globalement du phénomène d'invasion biologique. Ce dernier est actuellement reconnu comme l'une des causes principales de perte de biodiversité dans le monde (Cronck et al., 2001 ; Parker et al., 1999 ; Sala et al., 2000). Il est directement impliqué dans la disparition d'espèces autochtones (Vitousek et al., 1996), mais aussi dans des préjudices aux activités humaines ou dans l'émergence de problèmes sanitaires (Mack et al., 2000 ; Perrings et al., 2000 ; Pimentel et al., 2000). La prise de conscience du phénomène connaît un véritable essor depuis les années 1980, de sorte que l'étude des invasions biologiques est l'une des disciplines de l'écologie à s'être développée le plus rapidement (Pysek et al., 2004). Celle-ci a conduit à des politiques de sensibilisation et de gestion des plantes exotiques invasives (Booth et al., 2003), mais aussi à de nouvelles perspectives de recherche (Callaway et al., 2006). En effet, parallèlement aux impacts négatifs qu'elles engendrent, les invasions biologiques peuvent être considérées comme des expériences, non-voulues, à grande échelle spatiale (*i.e.* de l'ordre du continent ou d'une île) et temporelle (de l'ordre du siècle), sans rapport avec les expériences planifiées. A ce titre, les plantes invasives comptent actuellement parmi les meilleurs modèles d'étude en biologie évolutive (Thompson, 1998). L'étude des changements évolutifs rapides chez les végétaux offrent de multiples intérêts et donne lieu à nombre grandissant d'études, soulignant l'importance de considérer les espèces comme potentiellement fluctuantes, et non comme des entités homogènes et immuables (Lee, 2002). Utiliser des plantes invasives comme modèles pour étudier les changements évolutifs rapides offre plusieurs avantages (Hänfling et al., 2002 ; Lee, 2002 ; Reznick et al., 2001 ; Thompson, 1998): l'historique de l'introduction et de la colonisation est souvent documenté, ce qui place les différenciations observées dans un laps de temps connu, il y a généralement du matériel végétal en suffisance pour toute expérimentation, et celui-ci est de culture facile. De plus, l'étude du potentiel évolutif des espèces introduites est crucial pour comprendre et anticiper les processus d'invasions (Dietz et al., 2006 ; Lee, 2002). En effet, l'approche « écologique » traditionnelle a montré ses limites et révélé un manque de compréhension de la dynamique des populations: à ce jour, elle n'a permis aucune généralisation sur les raisons de l'invasion et ne permet pas de prédire les organismes potentiellement invasifs (Dietz et al., 2006 ; Lambrinos, 2004). Ceci souligne l'intérêt d'introduire une explication potentielle, complémentaire, de l'invasion: les changements évolutifs rapides.

Selon de nombreux auteurs, il existe des caractères-clés, typiques des plantes envahissant avec succès des zones où elles ont été introduites (Baker, 1974 ; Ellstrand et al., 2006 ; Grotkopp et al., 2002 ; Kolar et al., 2001 ; Rejmanek, 1995 ; Rejmanek et al., 1996). Ces caractères expliqueraient la capacité d'invasion d'une espèce. L'identification de ces caractères pourrait orienter les mesures de prévention, et cette approche a été fortement développée (Kolar et al., 2001) sans toutefois faire l'unanimité quant à son pouvoir prédictif (Crawley, 1987 ; Perrins et al., 1992). L'identification de ces caractères permettrait, par ailleurs, de connaître les traits dont il est important de suivre l'évolution rapide face aux changements de conditions environnementales.

L' *histoire de vie* est la distribution des événements importants au cours de la vie d'un individu qui contribuent directement à la production et à la survie des descendants. Elle est donc une notion centrale en biologie évolutive. Un *trait d'histoire de vie* est tout caractère mesurable relatif à cette histoire de vie tel que l'âge de la maturité sexuelle, la taille de l'individu à un âge donné ou le nombre de diaspores produites en une saison.

Dans cet article, nous nous focaliserons sur les évolutions de traits d'histoire de vie observées lors des différentes phases d'invasions chez des espèces végétales. Nous rappellerons d'abord les différentes phases de l'invasion, en présentant les processus évolutifs potentiellement associés. Nous présenterons ensuite les deux approches complémentaires généralement utilisées dans cette discipline: les comparaisons entre aires d'indigénat et d'introduction, et les études menées au sein de l'aire d'invasion.

2. Phases de l'invasion et processus évolutifs associés

Richardson et al. (2000) décrivent le processus d'invasion selon une séquence introduction-naturalisation-invasion. Le passage d'une étape à l'autre nécessite le franchissement d'une ou de plusieurs barrière(s) naturelle(s), et peut s'accompagner de changements évolutifs (figure 1).

L'introduction implique l'apport de propagules ou d'individus dans un site nouveau, situé hors de l'aire de répartition naturelle. L'homme joue un rôle fondamental, voulu ou accidentel, dans cette dispersion à longue distance. Il aide les espèces à passer outre la barrière géographique. Ces propagules doivent ensuite se développer jusqu'au stade de la population adulte en passant la barrière des conditions environnementales locales. La phase d'introduction peut induire un effet de fondation, ou effet fondateur, c'est-à-dire une réduction de la diversité génétique au sein de la zone introduite (Barrett et al., 1990 ; Brown et al., 1981). En effet, le pool génétique introduit peut ne pas être représentatif de toute la variabilité génétique de l'aire d'indigénat.

La naturalisation nécessite que les individus de la (des) population(s) introduite(s) donnent lieu à une nouvelle génération pour atteindre le stade de population viable sans nouvel apport de propagule. Pour cela, l'espèce doit passer outre les barrières empêchant la reproduction végétative à long terme ou la production de descendance. A ce stade, les événements évolutifs possibles sont: la sélection de génotypes pré-adaptés au sein du pool génétique introduit; l'effet de fondation ; la formation de nouvelles lignées génétiques par croisement entre génotypes jusque là isolés dans l'aire native.

L'expansion, menant à l'invasion, nécessite que la population initiale génère de nouvelles populations viables en passant outre la barrière de la dispersion. Une fois l'expansion en cours, les barrières à franchir sont liées aux conditions environnementales dans les habitats progressivement envahis. Elles sont diverses, et peuvent être plus ou moins graduelles. En général, elles s'opposent aux plantes passant d'environnements favorables, anthropisés, riches en ressources, à des milieux plus résistants quant aux facteurs biotiques et abiotiques (Dietz et al., 2006). Les évènements évolutifs liés à l'expansion dans la zone envahie peuvent être: la dérive génétique ; le filtrage, par sélection, des génotypes pré-adaptés ; les recombinaisons génétiques par croisement suivis de la sélection graduelle des génotypes adaptés (adaptation) ; l'hybridation (Ellstrand et al., 2006 ; Tiébré et al., 2007b) et/ou l'introgression (Suehs et al., 2004); la polyploïdisation (Baumel et al., 2001). Ces différents processus ne sont pas exclusifs et peuvent se combiner au cours de l'invasion.

La phase d'expansion est fréquemment précédée d'une phase de latence de quelques dizaines voire quelques centaines d'années (Kowarik, 1995 ; Williamson, 1996). Cette latence peut s'expliquer de différentes façons: le temps inhérent à la croissance des populations, le temps nécessaire aux organismes pour surmonter les contraintes écologiques, le temps nécessaire à l'acquisition de nouvelles capacités liées aux facteurs génétiques améliorant la fitness des individus, *etc.* (Kowarik, 1995).

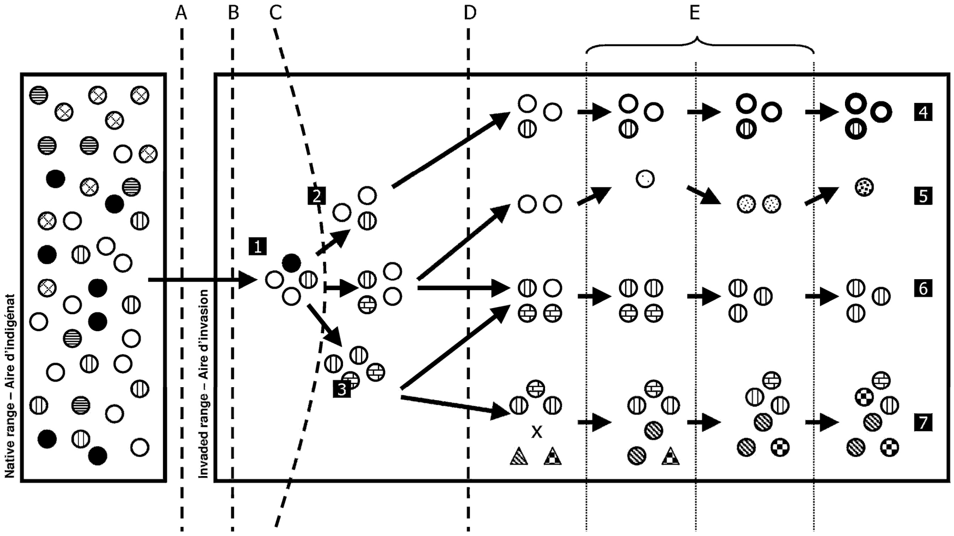

Figure 1 : Représentation schématique des principales barrières limitant l'expansion des espèces introduites, et différents processus évolutifs associés. Les ronds représentent des lignées génétiques de l'espèce invasive. Les triangles représentent une espèce native congénérique. Les lignes pointillées sont les barrières à franchir pour devenir une espèce invasive. (A) barrières géographiques inter- et/ou intracontinentales ; (B) barrières environnementales biotiques et abiotiques au site d'introduction ; (C) barrières reproductives empêchant la reproduction végétative à long terme ou la production de descendance ; (D) barrières aux dispersions locales et régionales ; (E) barrières environnementales dans les habitats colonisés, allant de milieux facilement colonisés aux milieux plus résistants à l'invasion. Processus évolutifs : (1) effet de fondation ; (2) sélection de génotypes pré-adaptés lors de la naturalisation ; (3) formation de nouvelles lignées génétiques par croisement entre génotypes jusque là isolés dans l'aire native ; (4) recombinaisons génétiques et sélection graduelle des certains traits d'histoire de vie ; (5) dérive génétique liée à de faibles effectifs et/ou des effets de fondation répétés ; (6) sélection de génotypes pré-adaptés ; (7) hybridation puis introgression. Modifié d'après Richardson et al., (2000).

3. Etude de l'évolution rapide des traits d'histoire de vie

La base théorique présentée ci-dessus fait l'objet d'un nombre grandissant de validations empiriques. Le rôle des évolutions rapides, ayant lieu sur des laps de temps de l'ordre du siècle, est de plus en plus souvent souligné dans les processus écologiques et principalement dans ceux liés aux invasions (Lee, 2002 ; Maron et al., 2004b ; Sakai et al., 2001 ; Thompson, 1998 ; Weber et al., 1998). Certaines espèces dont les aires d'invasion sont déjà importantes peuvent montrer des adaptations locales, et ce d'autant plus qu'un niveau élevé de variation génétique permet une réponse rapide à la sélection naturelle. Dès lors, les conditions favorisant les adaptations incluent notamment: un taux de reproduction croisée élevé, un nombre assez important d'individus fondateurs, la polyploïdie et la création de génotypes nouveaux suite aux croisements entre individus provenant de différentes zones d'introduction indépendantes. Par ailleurs, la rapidité de l'adaptation dépend de l'intensité de la pression de sélection, et les différenciations évolutives nécessitent que le flux de gènes entre populations soit relativement faible en comparaison des forces évolutives évoquées plus haut (sélection naturelle, dérive, *etc.*) (Lenormand, 2002)

Différentes études ont déjà mis en évidence des adaptations locales chez les espèces végétales (Cordell et al., 1998 ; Hiesey et al., 1965 ; Monson et al., 1992 ; Rice et al., 1991 ; Turesson, 1992). Ces adaptations ont trait à la morphologie, à la physiologie ainsi qu'à l'histoire de vie en général de ces espèces. La différentiation adaptative au niveau de populations introduites peut avoir lieu pour n'importe quel trait de vie, pour autant qu'il induise un avantage dans le nouvel environnement et qu'il y ait, à la base, une variation génétique pour ce trait. Les expérimentations en jardin commun (*common garden experiment*) déterminent dans quelle mesure des différences observées entre populations *in situ* ont une base génétique (e.g. Clausen et al., 1940 ; Tureson, 1922). Ces expériences consistent à cultiver des plants issus de différentes populations en conditions parfaitement homogènes, en serre, en laboratoire ou en plein air. Dès lors, les conditions environnementales étant communes à tous les plants, les différences phénotypiques entre ceux-ci révèlent des différences génétiques. *A priori*, les expériences en jardins communs ne permettent pas de mettre en évidence l'intérêt évolutif des différences observées, puisque les résultats obtenus sont tributaires des conditions de cultures (Thompson et al., 1991). Dans certains cas, toutes les conditions sont homogènes, sauf certains facteurs environnementaux qui sont volontairement modulés (tel que l'arrosage ou la fertilisation). Cela permet de quantifier la norme de réaction des différentes lignées génétiques à ces facteurs environnementaux (Dechamps et al., 2007 ; Schlichting, 1986 ; Whitton et al., 1995). Les transplantations réciproques (*reciprocal transplants*) peuvent, elles, montrer que les organismes sont mieux adaptés à leur région d'invasion/d'indigénat qu'à une autre (e.g. Lacey, 1988). Il s'agit d'expérimentation dans lesquelles plusieurs jardins communs sont installés, dans des conditions (*e.g.* géographiques ou édaphiques) représentatives de chaque (groupe de) population(s). Dans le cas d'une meilleure performance des populations locales (*e.g.* de la même zone géographique ou du même type de sol) dans les conditions correspondantes, l'adaptation de ces populations est mise en évidence et quantifiée (Kawecki et al., 2004). Les transplantations réciproques sont cependant plus difficiles à mettre en place, au niveau logistique, que les jardins communs uniques. Dans les deux cas, l'origine génétique des différences observées peut être remise en question si les effets maternels environnementaux n'ont pas été éliminés ou contrôlés (Roach et al., 1987). Ces effets sont dus au fait que les graines semées dans les expériences ont été produites dans des conditions différentes, selon leur origine. La façon dont chaque graine a été dotée par la plante-mère diffère donc, et peut induire des différences de trait de vie, dans la descendance, qui n'ont pas de base génétique. Pour limiter ces effets maternels, une première génération de plants peut être cultivée afin de réaliser des croisements contrôlés, en conditions homogènes, au sein des populations (Roach et al., 1987). Enfin, la mesure de la masse des graines préalablement à l'expérience permet de tenir compte des effets maternels dans l'analyse des résultats (e.g. Monty et al., 2009).

Une autre méthode d'étude de l'évolution des populations introduites est l'analyse de la variation génétique neutre à l'aide de marqueurs moléculaires (Avise, 2004). Cette méthode renseigne efficacement sur les voies de colonisation suivies, l'identité des populations fondatrice au sein de l'aire d'invasion, ou encore la localisation précise de la zone d'origine des populations introduites. De plus, les analyses moléculaires permettent de mesurer l'importance de la dérive génétique au sein des populations (via des paramètres tels que l'index de fixation, F_{ST}), et l'ampleur de la variation génétique introduite. Ceci est d'autant plus intéressant que cette dernière influe directement sur les évènements évolutifs ultérieurs (Barrett et al., 1990 ; Brown et al., 1981). Les analyses moléculaires sont donc complémentaires aux études sur l'évolution des traits d'histoire de vie, et facilitent leur interprétation (Bossdorf et al., 2005).

Toutes les plantes invasives ne doivent cependant pas nécessairement leur succès à leurs possibilités d'adaptation rapide. Baker (1965) propose le terme anglais de *general purpose genotype* pour décrire des espèces qui prospèrent dans une large gamme d'environnements grâce à une grande plasticité phénotypique. Contrairement aux plantes devenant invasives par adaptation, celles possédant un *general purpose genotype* ne sont pas nécessairement favorisées par de nouvelles recombinaisons génétiques. Elles peuvent s'installer durablement à partir d'un faible nombre d'individus fondateurs, et avoir des systèmes de reproduction de type autogame qui promeuvent avant tout l'assurance de la reproduction, plutôt que les échanges génétiques (Parker et al., 2003). Par ailleurs, de nombreuses plantes invasives ont des capacités de propagation végétative importante, et peuvent étendre leur aire d'invasion sans subir de changements génétiques (*e.g.* Tiébré et al., 2007a). L'étude de l'évolution rapide des traits d'histoire de vie chez les espèces exotiques envahissantes se scinde traditionnellement en deux parties. Il y a d'une part les comparaisons entre les individus/populations de l'aire native et de l'aire d'invasion. D'autre part, l'étude peut se focaliser sur les évènements évolutifs ayant lieu au sein même de l'aire d'invasion, pendant l'expansion de l'espèce.

4. Comparaison entre aire native et aire d'invasion

Nous répartirons les comparaisons entre aires natives et envahies en deux catégories: les comparaisons phénotypiques en populations naturelles et les comparaisons de traits d'histoire de vie en conditions homogènes (Bossdorf et al., 2005).

4.1. Comparaisons en populations naturelles

Chez certaines espèces végétales, il a été observé que les populations naturelles étaient plus grandes au sein de l'aire d'invasion que dans la zone d'indigénat (Eckert et al., 1996 ; Jakobs et al., 2004 ; Prati et al., 2004), et même si ce résultat n'est pas général, aucune étude n'a pour l'instant montré la tendance inverse (Erfmeier et al., 2004 ; Vilà et al., 2005 ; Wolfe, 2002). Au niveau individuel, il a souvent été observé que les plantes exotiques étaient plus grandes et/ou plus fécondes dans l'aire d'introduction que dans l'aire native (Buckley et al., 2003 ; Crawley, 1987 ; Elton, 1958 ; Prati et al., 2004). Même si les mécanismes ne sont encore que mal compris, plusieurs hypothèses ont été formulées et testées (Hierro et al., 2005). Une espèce introduite peut, notamment, rencontrer des conditions environnementales plus clémentes dans la zone d'introduction: réduction des herbivores, des compétiteurs efficaces ou des pathogènes adaptés (Darwin, 1859 ; DeWalt et al., 2004 ; Elton, 1958 ; Fenner et al., 2001 ; Memmott et al., 2000 ; Vilà et al., 2005 ; Wolfe, 2002). S'il s'agit d'une espèce envahissant grâce à une grande plasticité, la réponse se traduira par une croissance plus importante et/ou une descendance plus nombreuse, sans (ou presque) événement(s) évolutif(s). Dans le cas précis de la diminution des ennemis naturels (pathogènes, herbivores), on parlera alors d'*Enemy Release hypothesis* (ERH) (Crawley, 1987 ; Elton, 1958). Cette hypothèse sous-tend que l'herbivorie est un facteur déterminant pour l'abondance des espèces dans une zone donnée, ce qui n'est pas évident pour tous les végétaux (Maron et al., 2001). Si cette hypothèse a largement été testée, les essais de synthèses sur ce sujet indiquent que l'ERH ne peut que partiellement expliquer le succès des plantes invasives (Colautti et al., 2004). Par ailleurs, Bossdorf et al. (2005) soulignent l'importance de distinguer entre herbivores spécialistes et généralistes. Si les comparaisons en populations naturelles révèlent des différences d'histoire de vie, elles ne permettent pas de dissocier les changements évolutifs de ceux liés à la plasticité phénotypique. Cette distinction implique des études en conditions homogènes.

4.2. Comparaisons de traits d'histoire de vie en conditions homogènes

S'il existe un compromis (*trade-off*) entre l'allocation de la plante à la croissance et à la défense, une autre hypothèse est que face à une diminution des ennemis naturels, ce compromis lui-même évolue. Les plantes de la zone introduite allouent plus de ressources à la croissance qu'à la défense, et ceci suite à une pression de sélection. Il y a alors de réels événements évolutifs, et on parle *d'Evolution of Increased Competitive Ability* (EICA *hypothesis*) (Blossey et al., 1995). Cette hypothèse a stimulé les recherches en biologie évolutive, faisant l'objet de nombreuses études en jardins communs (Bossdorf et al., 2004 ; Buschmann et al., 2005 ; Leger et al., 2003 ; Maron et al., 2004a). Si, comparées aux populations de l'aire native, les populations invasives ont une croissance plus soutenue, mais une résistance plus faible face aux herbivores et aux pathogènes, l'hypothèse EICA est validée (Blossey et al., 1995). Les résultats sont jusqu'à présent contrastés, et il paraît clair que d'autres hypothèses sont possibles pour expliquer les changements évolutifs observés chez les plantes exotiques (Hierro et al., 2005). En effet, des différenciations génétiques peuvent avoir lieu dans les populations introduites pour tout trait écologique propice dans le nouveau régime de sélection, pour autant qu'il existe une variation génétique pour ce trait. L'hypothèse de l'évolution de la capacité d'invasion (*Evolution of Invasiveness hypothesis*), plus générale qu'EICA, postule que des espèces exotiques ont pu atteindre leur niveau d'abondance et de dominance dans la zone d'introduction parce qu'elles ont connu des changements génétiques en réponse à des nouvelles pressions de sélection, liées à des facteurs biotiques ou abiotiques (Carroll et al., 1996 ; Hänfling et al., 2002 ; Lee, 2002 ; Maron et al., 2004b ; Sakai et al., 2001).

Parmi les traits d'histoire de vie pour lesquelles des différentiations évolutives ont été montrées, les plus communs sont la biomasse, le taux de croissance et la fécondité. Le taux de croissance et la biomasse sont généralement plus importants au sein de l'aire d'invasion que de l'aire d'introduction (Bastlová et al., 2002 ; Blossey et al., 1995 ; Bossdorf et al., 2008 ; Cano et al., 2008 ; Siemann et al., 2001). De même, les populations introduites montrent souvent une plus grande fécondité que les natives en conditions homogènes (Bastlová et al., 2002 ; Buschmann et al., 2005 ; Leger et al., 2007 ; Stastny et al., 2005 ; Wolfe et al., 2004), même si des exceptions existent (Bossdorf et al., 2008 ; Bossdorf et al., 2004 ; Buschmann et al., 2005). Des différenciations d'ordre génétique ont également été montrées pour d'autres traits de vie: taux de survie hivernale (Monty et al., soumis), résistance à l'herbivorie (différences qui, couplées à des différences de croissances, font référence à l'hypothèse EICA), régulation de l'eau (Cano et al., 2008) et physiologie foliaire (*Specific Leaf Area*)

(Cano et al., 2008). La plasticité phénotypique, *i.e.* la variabilité des traits de vie d'un génotype en réponse aux facteurs environnementaux, peut elle-même être différenciée entre les aires d'invasion et d'indigénat (Bossdorf et al., 2008 ; Cano et al., 2008). Enfin, les différenciations de systèmes de reproduction ont fait l'objet d'une récente revue bibliographique (Barrett et al., 2008), qui souligne que la sélection peut largement influer sur la période de floraison au cours de l'invasion.

5. Evolution de l'histoire de vie au sein de l'aire d'invasion

Encore relativement peu d'études ont été réalisées, à ce jour, sur l'évolution des plantes exotiques lors de leur invasion dans l'aire d'introduction. Les premières d'entre-elles se sont focalisées sur les phénomènes évolutifs le long de gradients latitudinaux. Ces gradients traduisent des variations progressives de climat (températures, précipitations) et de longueur de saison de végétation, à l'échelle continentale. Ils peuvent donc correspondre à des pressions de sélection graduelles sur l'histoire de vie. Des variations clinales de croissance (en hauteur, en volume estimé et/ou en biomasse) avec la latitude, en jardins communs, ont ainsi pu être montrées chez *Impatiens glandulifera* ROYLE (Balsaminaceae), *Hypericum perforatum* L. (Hypericaceae), *Eschscholzia californica* Cham. (Papaveraceae) et chez deux espèces de *Solidago* (Asteraceae) (Etterson et al., 2007 ; Kollmann et al., 2004 ; Leger et al., 2007 ; Maron et al., 2004b ; Weber et al., 1998). De façon comparable, des différences de délai de floraison (Kollmann et al., 2004 ; Montague et al., 2008 ; Weber et al., 1998), de taille d'inflorescence (Weber et al., 1998) et de fécondité (Maron et al., 2004b) en conditions homogènes ont été mises en relation avec la latitude des populations invasives. La plupart de ces études suggèrent qu'il y a eu évolution adaptative en relation avec les conditions environnementales rencontrées aux différentes latitudes. Une sélection d'ordre climatique agirait donc pour favoriser des génotypes pré-adaptés, ou de nouvelles recombinaisons génétiques apparues dans l'aire d'introduction. Si les gradients latitudinaux commencent donc à être relativement documentés, il n'en est pas de même pour les gradients altitudinaux, qui correspondent pourtant à des variations de climat comparables. A l'heure actuelle, seules deux études se sont penchées sur les différenciations d'espèces invasives le long de gradients d'altitude: l'une d'elle indique une différenciation de *Senecio inaequidens* DC. (Asteraceae) pour les traits de croissance (Monty et al., 2009, Monty et al., soumis), alors que l'autre suggère que *Verbascum thapsus* L. (Scrophulariaceae) colonise des zones altitudinales contrastées grâce à une grande plasticité (*general purpose genotype*) (Parker et al., 2003). Dans le cas de *S. inaequidens*, en conditions homogènes, la hauteur et la biomasse des plants diminue graduellement avec l'altitude des populations parentes. Cette différenciation, d'ordre génétique (Monty et al, soumis) est mise en relation avec les variations climatiques rencontrées par les populations parentes (Monty et al., 2009). Dans de nombreux cas (mais voir Monty et al., 2009), l'interprétation des différenciations observées le long de gradients est rendue difficile par le fait que les espèces étudiées ont été introduites maintes fois, en divers endroits, dans l'aire d'invasion. Ceci est notamment le cas pour les espèces d'intérêt ornemental. Dès lors, les patrons de variation observés peuvent en partie être attribués à l'historique d'introduction, en plus des phénomènes évolutifs tels qu'adaptation, sélection de génotypes pré-adaptés et dérive génétique.

Si les gradients environnementaux représentent les cas les plus évidents de sélection au sein d'une aire de répartition, certaines études se sont intéressées à d'autres situations où la sélection naturelle pouvait influencer l'histoire de vie des plantes introduites. Ainsi par exemple, Kevin Rice (2006) a mis en évidence, chez *Aegilops triuncialis* L. (Poaceae) des adaptations à des conditions édaphiques extrêmes (sols de serpentine).

A côté des phénomènes de sélection et de dérive génétique, des changements brutaux de traits de vie peuvent apparaître suite à des évènements d'hybridation dans l'aire d'introduction (Ellstrand et al., 2006). C'est notamment le cas dans les genres *Carpobrotus* (Aizoaceae) (Albert et al., 1997) et *Gossypium* (Wendel et al., 1990), où le phénomène s'est poursuivi jusqu'à l'introgression. Plus récemment, une étude sur le genre *Fallopia* (Polygonaceae) a montré que l'hybridation s'accompagnait de profonds changements d'histoire de vie, avec la restauration de la reproduction sexuée au sein de l'aire d'invasion (Tiébré et al., 2007b). Enfin, des phénomènes de polyploïdisation ont été montrés dans le genre *Spartina* au sein de l'aire d'invasion, qui semblent avoir induit le succès invasif du taxon alloploïde *Spartina anglica* C.E.Hubb.(Baumel et al., 2001).

6. Conclusion et perspectives

Les plantes exotiques envahissantes sont en général des modèles appropriés pour adresser des questions quant à l'évolution des traits d'histoire de vie. Elles présentent en effet des avantages indéniables, tels qu'un historique d'invasion documenté et inscrit dans un contexte temporel, une culture facile et une relative abondance. Parallèlement, la compréhension des mécanismes évolutifs impliqués dans le succès de ces espèces, et l'identification des traits de vie concernés, devrait permettre une meilleure compréhension du processus d'invasion. Deux principales approches ont pour l'instant été développées par les chercheurs : les comparaisons entre aires d'indigénat et d'invasion d'une part, et, d'autre part, les comparaisons de populations invasives en relation avec des facteurs environnementaux. La première approche, développée plus tôt, fait actuellement l'objet d'une littérature suffisante pour dégager des patrons généraux et synthétiques. La seconde, moins étaillée, devra encore faire l'objet de recherches avant toute généralisation. A l'avenir, les deux approches devront faire l'objet de synthèses dans une nouvelle vision, plus intégrée, du mécanisme complet d'invasion en relation avec l'évolution des espèces concernées. Il faudra, entre-autres, relier l'aspect fonctionnel des changements de traits d'histoire de vie aux études phylogénétiques retraçant les patrons d'invasion. Les changements évolutifs présentés dans cet article peuvent influencer la rapidité et les patrons de l'invasion. Ils présentent donc un intérêt tant pour la biologie évolutive que pour la compréhension et la gestion des invasions biologiques. Néanmoins, le rôle direct de ces changements d'histoire de vie dans les succès d'invasion est toujours imprécis (Barrett et al., 2008), et le lien de cause à effet entre l'évolution des traits et l'invasion n'est pas nécessairement évident. L'intérêt fonctionnel des différentiations évolutives observées au sein de l'aire d'invasion devra donc, lui aussi, faire l'objet de recherches approfondies.

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Article 2

Life history traits variation among native diploid, native tetraploid and invasive tetraploid forms of *Senecio inaequidens* (Asteraceae).

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Abstract. *Senecio inaequidens* is a rapidly spreading plant invader in Europe. In its native range, it occurs at two co-existing diploid and tetraploid cytotypes. To date, only tetraploids are reported in Europe, even though invasive diploids were recorded in other parts of the world. We compared native diploid and both native and invasive tetraploid populations in common gardens in Europe for a suite of life history traits. Diploids were able to develop, showed high biomass production and produced more flower heads than tetraploids. In contrast, winter survival was null for diploids. It was low for native tetraploids, but reached 40 % in invasive tetraploids. Results suggested that diploid cytotype tends to an annual life form when grown in Western Europe, with earlier and more abundant flowering. In contrast, the tetraploid cytotype was mainly perennial which may enhance its invasiveness.

Keywords. Senecio madagascariensis, Senecio inaequidens, *ploidy level, life history, common garden.*

Résumé. *Senecio inaequidens* est une plante exotique qui s'est rapidement répandue à travers l'Europe. Dans son aire d'indigénat, deux cytotypes coexistent: diploïde et tétraploïde. Jusqu'à présent, seuls des individus tétraploïdes ont été recensés en Europe, mais le cytotype diploïde est invasif dans d'autres parties du monde. Nous avons comparé des populations natives diploïdes, et des populations tétraploïdes, à la fois natives et invasives, dans un jardin commun installé en Europe. Nous y avons mesuré plusieurs traits d'histoire de vie. Les plants diploïdes se sont développés, ont montré des capacités de production de biomasse importantes et ont même produit plus de capitules que les plants tétraploïdes. Néanmoins, les diploïdes n'ont pas survécu aux conditions hivernales du site d'expérience. Le taux de survie hivernal fut faible pour les populations tétraploïdes natives, mais atteignit 40 % dans les populations invasives. Nos résultats suggèrent que le cytotype diploïde tend vers un cycle de vie annuel en Europe de l'Ouest, avec une floraison plus précoce et plus abondante. Les tétraploïdes, par contre, sont plutôt pérennes, ce qui semble favoriser leur potentiel invasif.

Mots-clés. Senecio madagascariensis, Senecio inaequidens, *niveau de ploïdie, histoire de vie, jardin commun.*

Introduction

Polyploidy, the possession of more than two sets of chromosomes, often leads to novel physiological and life history characteristics not present in diploids ancestors (Levin, 1983).
A growing body of studies indicates that polyploidy may contribute to invasive behaviour and spread of alien plant species (Ainouche et al., 2004 ; Pandit et al., 2006 ; Soltis et al., 2004 ; Vilà et al., 1998), and Benett et al. (1998) found that the proportion of polyploids were higher in weedy species than in other plant groups. However, to date, no general relationship between polyploidy and colonizing success has been demonstrated (Barrett et al., 1986).
Senecio inaequidens DC. is a dwarf shrub from the family Asteraceae, native to Southern Africa. It belongs to a sympatric species complex including *Senecio madagascariensis* Poir. and *Senecio harveianus* Mac Owan (e.g. Hilliard, 1977 ; Radford et al., 2000 ; Scott et al., 1998). But molecular studies suggested that the three taxa were the same species (Lafuma et al., 2003 ; Scott et al., 1998). Lafuma et al. (2003) showed that this complex occurred at two co-existing cytotypes in its South African native range and mapped populations of diploid and tetraploid cytotypes.
Species of the complex are presently invasive in Europe, Australia and South America. In Australia, Argentina and Mexico, only diploids are reported and generally referred to as *S. madagascariensis* (Lafuma et al., 2003 ; Michael, 1981 ; Radford et al., 2000 ; Scott et al., 1998 ; Verona et al., 1982). In Europe, the species (now only referred to as *S. inaequidens*) was accidentally introduced as a wool contaminant in several wool-processing centres during the late 19th – early 20th century (Ernst, 1998). Notably, the species invaded southern France from the city of Mazamet, where it was initially recorded in 1936 (Guillerm et al., 1990), and Belgium from Verviers where it was initially recorded in 1892 (Verloove, 2006). So far, Belgian and French invaded areas are still relatively unconnected (Monty A.,*unpublished*) and genetic pools introduced may differ between the two European regions (Bossdorf et al. 2005). It is not known if both cytotypes were introduced to Europe, but only tetraploids were recorded in Europe so far (Chichiricco et al., 1979 ; Lafuma et al., 2003). The absence of diploids due to non-introduction is rather unlikely, because of the relative sympatry with tetraploids (Lafuma et al., 2003). Another possibility is that both ploidy levels were introduced but diploids failed to establish whereas tetraploids became successful invaders.
In the present study we compared functional life history traits between (1) diploid populations (2) tetraploid populations from the native range and (3) tetraploid populations from two invaded ranges, *i.e.* Belgium and France, in North-western Europe.

Material and Methods

Seed collection

In February 2001 and 2002, achenes (therefore referred to as 'seeds') were collected in two diploid and two tetraploid *S. inaequidens* populations in the mountainous regions of South Africa (Table 1). The ploidy level of these populations is known from flow cytometry analysis (Lafuma et al., 2003). In November 2005 and November 2006 (for the 2006 and 2007 experiments, respectively; see below), seed collections were made in two Belgian and two French invasive tetraploid populations. In each population, a total of 20 maternal plant were sampled. Seeds were stored at 4°C in paper bags. Per parent individual (totalling 160), ten seeds without anomaly were sorted using a dissecting microscope.

Table 1. Characteristics of *Senecio inaequidens* DC (Asteraceae) populations within the four types (ND: native diploid; NT: native tetraploid; BIT: Belgian invasive tetraploid; FIT: French invasive tetraploid). N is the number of sampled individuals (over the two years of sampling). Native populations are the same as in Lafuma et al. (2003): corresponding population codes are given.

Country	Location	Coordinates	Altitude (m)	Geocyctoype	Population	Corresponding population code in Lafuma et al. 2003
South Africa	Rose haugh	25°22' S; 30°45' E	545	Native diploid	ND1	ARS
South Africa	Caver Sham	29°23' S; 30°01' E	1385		ND2	AXX
South Africa	Clarens	28°31' S. 28°25' E	2050	Native tetraploid	NT1	AGE
South Africa	Barkly Pass	31°09' S; 27°46' E	1917		NT2	AEL
Belgium	Paal	51°03' N; 05°09' E	35	Belgian invasive tetraploid	BIT1	-
Belgium	Malmédy	50°25' N; 06°00' E	320		BIT2	-
France	Caunes-Minervois	43°19' N; 02°32' E	175	French invasive tetraploid	FIT1	-
France	Nohèdes	42°37' N; 02°19' E	785		FIT2	-

Life history traits measurements

In spring 2006, a randomised 10-block common garden experiment was established on an open field in Gembloux (Belgium). We used seeds from ten parent individuals per population in this experiment. On March 17th and 18th, the ten sorted seeds per parent individual were collectively sown in pots containing 2.5 l of sand, 2.5 l of compost and 0.5 l of hydro-granulates. Each pot was placed in a 0.5 m x 0.8 m area for plant development without competition. Seedlings were counted every 2-3 days. The first emerged seedling in each pot was marked with a short rod for measurement. Other seedlings were removed after counting. Parent individuals were thus represented each by ten seeds for germination study, then by one descendant. Germination was considered complete when no additional seedling was counted for 10 days and germination rate was defined for each pot as the percentage of emerged seedlings. During summer drought, plants were watered periodically. Time to germination (days) since sowing and age flowering (days since germination) where recorded, based on daily observations. When flowering began, the number of flowering heads per plant was determined every 17-20 d, which is approximately the period between flower opening and fruit maturation in Belgium (Monty A., *unpublished*). Every flower head produced was thus on average counted one time and the sum of counted flower heads is an accurate quantification of total flower head production. Mid-December 2006, final height (cm) was recorded, plants were harvested at ground level and aboveground biomass (g) was measured after oven drying for 48 h at 60°C.

Winter survival analysis

In spring 2007, a similar experiment was set out at the same site, with seeds from the ten other parent individuals sampled per population. Sowing was carried out on April 17th

2007, and the plants were grown for more than one year. Winter survival was monitored in May–June 2008. Individuals were considered surviving if shoots sprouted.

Data analysis

Ploidy levels (diploid and tetraploid) and geographic origin (native range, Belgian and French invaded ranges) were combined in four *geocytotypes*: 'native diploid' (ND); 'native tetraploid' (NT); 'Belgian invasive tetraploid' (BIT) and 'French invasive tetraploid' (FIT)(Table 1). Each geocytotype was represented by 2 populations. A two-way ANOVA was performed on the measured life history traits. Populations (random) were nested within geocytotypes (fixed). When we detected a significant ($P < 0.05$) type effect, we further used ANOVA contrasts to test for three specific biological hypotheses. First (contrast C1), we tested for an effect of the ploidy level, by contrasting plant traits of ND vs the three tetraploid geocytotypes (NT, FIT and BIT). Second (C2), we tested if life history traits differed between African and European tetraploids, by contrasting traits of NT vs European geocytotypes (FIT and BIT). Third (C3), we tested for differences among European geocytotypes. Winter survival was not a continuous variable but a qualitative frequency within populations. We looked for a dependence of winter survival on geocytotype by performing a *Chi-square* test of independence with the four geocytotypes as rows and the two modalities of winter survival (dead or surviving) as columns. Analyses were performed using Minitab software version 14.20 (Minitab Inc. 2000). Aboveground biomass data were log-transformed to reach the assumptions of statistical analyses.

Results

Life history measurements

Geocytoypes significantly differed in age flowering, final height, above-ground biomass and flower head production, as revealed by the analysis of variance (Table 2). Contrasts among ploidy levels (C1) revealed that diploids flowered earlier, produced more biomass and more flower heads than tetraploids (Table 2, Figure 1). The contrast comparing African and European tetraploids (C2) was not significant. In Europe, French tetraploids were taller and produced more biomass and flower heads than Belgian ones (C3). Within types, population differed in time to germination and age flowering (Table 2).

Winter survival

Winter survival frequencies greatly varied among types. It was null for ND, equal to 15% for NT and reached 35% and 40 % for BIT and FIT, respectively (Figure 1). Chi-square test of independence with the four types revealed a global dependence of winter survival on plant types (Pearson Chi-Square test = 10.963; *DF* = 3; P = 0.012).

Table 2. ANOVA results depicting the effects of geocytotype and population. In the case of a significant effect of geocytotype, specific contrasts were tested. Significant P-values are in bold.

		Time to germination			Germination rate			Age flowering		
Source of variation	*df*	*MS*	*F*	*P*	*MS*	*F*	*P*	*MS*	*F*	*P*
Type	3	25.37	0.79	0.56	348.30	0.50	0.71	1 059.05	10.88	**0.02**
C1:Diploids vs tetraploids	1	-	-	-	-	-	-	1 363.50	33.52	**<0.001**
C2: African vs European tetraploids	1	-	-	-	-	-	-	68.27	1.68	0.20
C3: Belgian vs French tetraploids	1	-	-	-	-	-	-	156.80	3.85	0.05
Population (type)	4	32.23	3.08	**0.02**	702.50	1.01	0.41	97.34	2.39	**0.06**
Error	72	10.46			698.60			40.68		

		Final height			Above-ground biomass			Flower head production		
Source of variation	*df*	*MS*	*F*	*P*	*MS*	*F*	*P*	*MS*	*F*	*P*
Type	3	1 259.40	7.33	**0.04**	112 136.00	10.97	**0.02**	940 121.00	10.45	**0.02**
C1:Diploids vs tetraploids	1	332.08	3.14	**0.08**	46 122.06	9.20	**0.00**	944 765.98	10.63	**0.00**
C2: African vs European tetraploids	1	0.00	0.00	1.00	2 266.27	0.45	0.50	51 810.16	0.58	0.45
C3: Belgian vs French tetraploids	1	1 581.97	14.97	**0.00**	123 362.77	24.61	**<0.001**	413 604.75	4.65	**0.03**
Population (type)	4	171.90	1.63	0.18	10 224.00	2.04	0.10	89 995.00	1.01	0.41
Error	72	105.70			5 012.00			88 879.00		

Discussion

We compared native diploid populations and both native and invasive tetraploid populations under north-western European climate for a suite of functional life history traits. Germination traits did not differ according to ploidy level or geographic origin, whereas growth, phenology and winter survival appeared to strongly depend on ploidy level. The two European regions of introduction and invasion were also found to differ in growth and reproduction traits.

Ploidy level influence on life history

Polyploidy in *S.inaequidens* did not lead to better performance, during the experiment under north-western European climate. Diploids were able to germinate, develop and produced even more biomass and flower heads than tetraploids. Similarly, a recent study showed that polyploidy hardly enhanced growth of the invasive *Lythrum salicaria* (Kubátová et al., 2008), a species that also occurs at different ploidy levels in its native range, but only tetraploids are invasive. In contrast, monitoring winter survival in *S.inaequidens* showed that tetraploids were more resistant to harsh conditions in central Belgium, which is consistent with the higher resistance of polyploids to environmental extreme conditions (Bretagnolle et al., 1998 ; Levin, 1983). Diploids are described as short perennial in invaded areas (Radford and Cousens 2000, Rzedowski et al. 2003, Lopez et al. 2008). The fact that they do not survive winter in Europe can reduce to much their reproductive potential or can have prevented establishment in this self-incompatible species.

Differences in tetraploids across continents

The native and invasive tetraploid types did not differ in most measured traits. However, winter survival varied among continents, with better performances for the invasive than native tetraploids. These results are consistent with a recent study by Bossdorf et al. (2008), suggesting that among the panel of genotypes found in Africa, only a subset of pre-adapted ones managed to become successful invaders. During naturalisation in Western Europe (Belgium, France), a subset of winter resistant genotypes may have been selected.

Differences between French and Belgian tetraploids

In the common garden, French tetraploids were taller and produced more biomass and flower heads than Belgian ones. This suggested that present genetic pools differ between invaded areas. This may arise from the introduction of separated genetic pools, *i.e.* seeds brought to Belgium and to France came from different (groups of) native populations. Another (not exclusive) possibility is that evolutionary processes, namely founder effect, genetic drift and selection, lead to contrasting life history traits in the two invaded areas. As experiments were carried out in Belgium, it is surprising that Belgian populations showed lower flower head and biomass production, since these traits are proxies of fitness. A stronger founder effect and/or genetic drift, i.e. a lower genetic variation introduced to Belgium, was already suggested (Bossdorf et al., 2005 ; Lafuma et al., 2003). This could be responsible for the pattern observed. It has to be noted that as we grew the plants for only one season, our measurements may not reflect the actual fitness. Through high winter survival, potentially coupled with long life spans, Belgian populations may still exhibit a high flower head production. Much longer-term studies are needed to assess the actual fitness differences among invasive tetraploids.

Conclusions

Despite a limited experimental design, this study brought evidence of variation in life history traits among geocytotypes in the weed *S.inaequidens*. Diploids performed very well during the first growing season under north-western European climate. However, this cytotype showed no winter survival, which potentially points out a lower invasive potential than tetraploids. Among tetraploids, invasive populations showed higher winter survival. This indicated that winter resistance may be an important trait that promotes invasiveness in this species.

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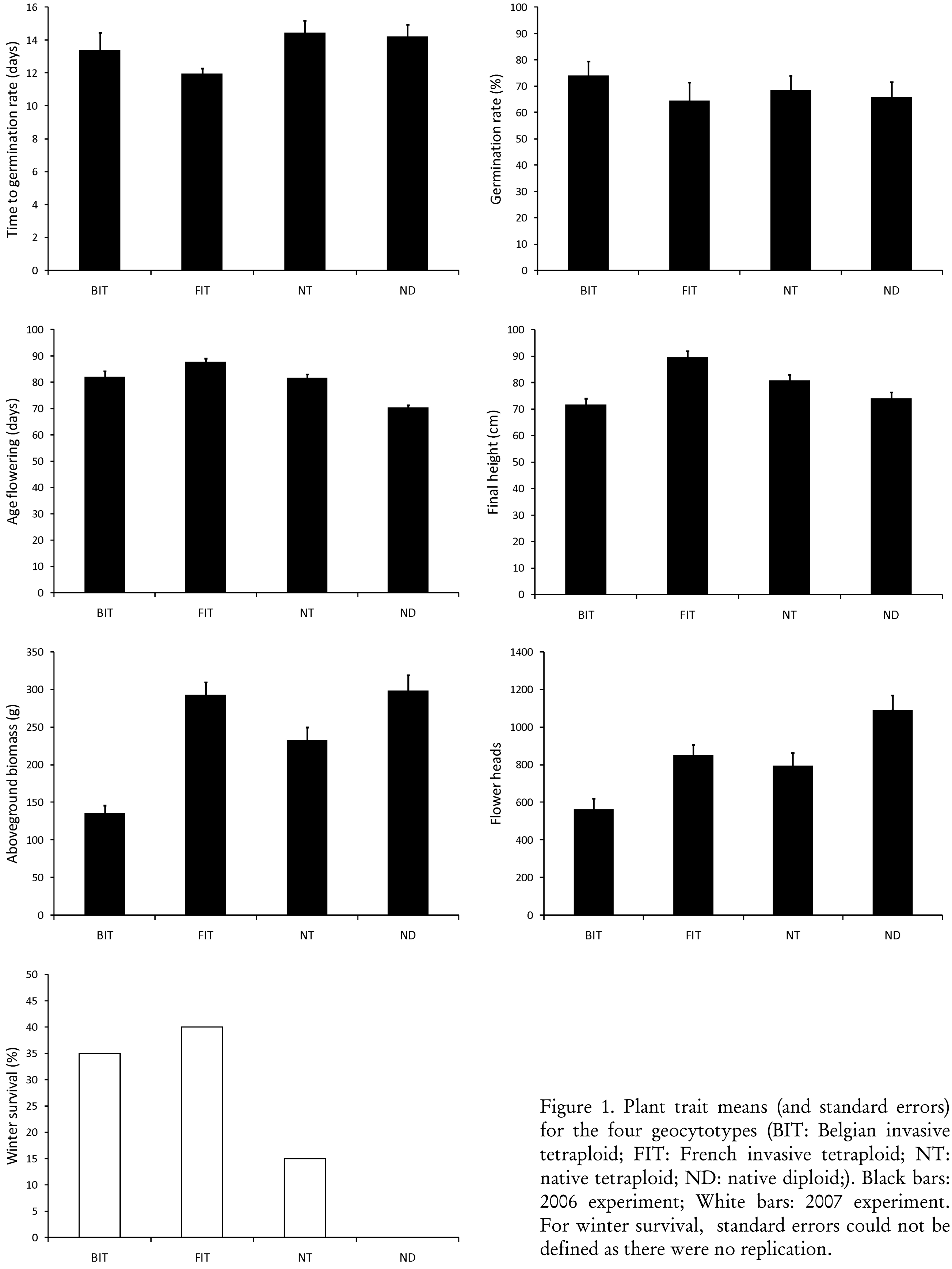

Figure 1. Plant trait means (and standard errors) for the four geocytotypes (BIT: Belgian invasive tetraploid; FIT: French invasive tetraploid; NT: native tetraploid; ND: native diploid;). Black bars: 2006 experiment; White bars: 2007 experiment. For winter survival, standard errors could not be defined as there were no replication.

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Article 3

Clinal differentiation during invasion: *Senecio inaequidens* (Asteraceae) along altitudinal gradients in Europe.

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Abstract. The dynamics of plant population differentiation may be integral in predicting aspects of introduced species invasion. In the present study, we tested the hypothesis that European populations of *Senecio inaequidens* (Asteraceae), an invasive species with South African origins, differentiated during migration from two independent introduction sites into divergent altitudinal and climatic zones. We carried out two years of common garden experiments with eight populations sampled from Belgian and ten populations from French altitudinal transects. The Belgian transect followed a temperature and precipitation gradient. A temperature and summer drought gradient characterized the French transect. We evaluated differentiation and clinal variation in plants germinated from field collected seed using the following traits: days to germination, days to flowering, height at maturity, final plant height and aboveground biomass. Results showed that *S. inaequidens* populations differentiated in growth traits during invasion. During the first year of sampling, the results indicated clinal variation for growth traits along both the Belgium and French altitudinal transects. Data from the second year of study demonstrated that with increasing altitude, a reduction in three growth traits, including plant height at maturity, final plant height and aboveground biomass was detected along the French transect, but no longer along the Belgian one. Phenological traits did not exhibit a clear clinal variation along altitudinal transects. The possible evolutionary causes for the observed differentiation are discussed.

Keywords. *Climatic gradient, altitude, genetic differentiation, clinal variation, invasive species.*

Résumé. La dynamique de différenciation des populations végétales est de première importance pour prédire les invasions biologiques. Dans la présente étude, nous avons testé l'hypothèse que les populations européennes de *Senecio inaequidens* (Asteraceae), une plante invasive originaire d'Afrique du Sud, s'étaient différenciées pendant la colonisation, à partir de deux sources d'introduction indépendantes, de zones altitudinales contrastées climatiquement. Deux années d'expériences en jardins communs ont été réalisées, avec huit populations sélectionnées le long d'un transect altitudinal belge et dix populations le long d'un transect altitudinal français. Ces gradients représentaient, respectivement, des variations graduelles de températures et précipitations annuelles, et des variations de températures annuelles et de sécheresse estivale. Nous avons évalué la différenciation et la présence de variations clinales, parmi les plants issus de graines collectées *in situ*, pour les traits suivants: le délai de germination, le délai de floraison, la hauteur à la maturité, la hauteur finale et la biomasse aérienne. Les résultats ont montré une différenciation dans les traits de croissance. La première année d'étude, des variations clinales dans ces traits ont été mises en évidence le long des deux transects. La seconde année, la diminution des traits de croissance avec l'altitude fut confirmée, pour le transect français seulement. Aucun patron de variation clair n'a été identifié pour les traits de phénologie de la reproduction. Les causes possibles des différenciations observées sont discutées.

Mots-clés. *Gradient climatique, altitude, différenciation génétique, variation clinale, espèce invasive.*

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POPULATION ECOLOGY - ORIGINAL PAPER

Clinal differentiation during invasion: *Senecio inaequidens* (Asteraceae) along altitudinal gradients in Europe

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Abstract The dynamics of plant population differentiation may be integral in predicting aspects of introduced species invasion. In the present study, we tested the hypothesis that European populations of *Senecio inaequidens* (Asteraceae), an invasive species with South African origins, differentiated during migration from two independent introduction sites into divergent altitudinal and climatic zones. We carried out 2 years of common garden experiments with eight populations sampled from Belgian and ten populations from French altitudinal transects. The Belgian transect followed a temperature and precipitation gradient. A temperature and summer drought gradient characterized the French transect. We evaluated differentiation and clinal variation in plants germinated from field-collected seed using the following traits: days to germination, days to flowering, height at maturity, final plant height and aboveground biomass. Results showed that *S. inaequidens* populations differentiated in growth traits during invasion. During the 1st year of sampling, the results indicated clinal variation for growth traits along both the Belgium and French altitudinal transects. Data from the 2nd year of study demonstrated that with increasing altitude, a reduction in three growth traits, including plant height at maturity, final plant height and aboveground biomass, was detected along the French transect, but no longer along the Belgian one. Phenological traits did not exhibit a clear clinal variation along altitudinal transects. The possible evolutionary causes for the observed differentiation are discussed.

Keywords Climatic gradient · Altitude · Genetic differentiation · Clinal variation · Invasive species

Introduction

Biological invasions, despite the harmful effects on biodiversity and economics (Pimentel et al. 2000), serve as an outstanding opportunity to address fundamental questions regarding evolution in response to environmental modification (Callaway and Maron 2006; Lee 2002; Sakai et al. 2001). When invasive species introduction and distribution history is well documented, invasions provide a rare chance to evaluate evolutionary change on a human time scale. Historical data provide information that was not intended for evolutionary studies, but is valuable metadata available to address more contemporary questions regarding environmental and evolutionary change (Sax et al. 2007).

The processes inherent in alien species establishment and migration, leading to an increase in abundance, can be divided into three distinct phases: establishment, naturalization and expansion (Kolar and Lodge 2001; Sakai et al. 2001; Sexton et al. 2002). At each phase, evolutionary processes may occur that are responsible for the success of the invasive taxon. A number of compelling studies reported phenotypic/genetic divergence between native and invasive populations (reviewed in Bossdorf et al. 2005). Dietz and Edwards (2006) argue that important selective regimes can also occur following the early stages of colonization, when an exotic plant encounters different habitat conditions. The non-native species may experience increased competition

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from the native flora (D'Antonio 1993), variable interactions with biotic factors (Siemann and Rogers 2001) or selection imposed by specific abiotic conditions (Mihulka and Pysek 2001). If ecological abiotic factors vary at the invasion fronts, changes in life history traits are expected, either due to plasticity or adaptation under selective pressures.

Climate is one of the most important abiotic factors in regard to species distribution. Expansion of the introduced species range will depend on how well exotic species cope with geographic heterogeneity in climate (Montague et al. 2008). Spread over broad climatic gradients is expected to involve clinal adaptive differentiation of populations (Cox 2004, Montague et al. 2008) because many climatic factors vary geographically in a continuous manner (Endler 1977). Large-scale latitudinal clines, interpreted as evolutionary adaptation to climate heterogeneity, has indeed been observed for different life history traits in an increasing number of introduced species (Etterson et al. 2007; Gilchrist et al. 2004; Huey et al. 2000; Kollmann and Banuelos 2004; Leger and Rice 2007; Montague et al. 2008; Weber and Schmid 1998). In contrast, analogous altitudinal life history variation has been studied much less frequently in invasive species (but see Parker et al. 2003 for an example with a plant species). Overall, altitudinal trends can be expected to mirror latitudinal trends in that with increasing altitude or latitude, the reproductive season gets shorter, and climate becomes harsher. Hence, examination of differentiation patterns along altitudinal gradients is very complementary to our recent knowledge on latitudinal variation in invasive species and will foster our understanding of adaptation in introduced ranges.

For the majority of exotic plant species, the evolutionary interpretation of life history trait variation across the invasion range may be difficult. The species introduction history and subsequent patterns of dispersal over time are largely unavailable. This is particularly true for plant species with horticultural interest, where long-distance seed exchange, anthropogenic gene flow and multiple introduction events take place (Ellstrand and Schierenbeck 2006). However, in Europe, the African ragwort *Senecio inaequidens* DC. (Asteraceae) is a highly invasive species with a well-documented invasion history. As a result of wool importation from South Africa during the late 19th century, recorded independent introduction sites chronicle the species migration routes. It has extended over a range of climatic zones in Europe, but its distribution remains disjunct, suggesting that species dispersal was primarily spontaneous, although helped by roads and railways (Ernst 1998). The species is not extensively propagated for ornamental purposes, reducing the risk of anthropogenic gene flow among regions. As the species is now present over broad altitudinal and climatic regimes, regional adaptations can be expected. The unique introduction history of *S. inaequidens* makes it an ideal model to study rapid differentiation in response to climatic variation in its introduced range.

In the present study, we tested the hypothesis that populations of *S. inaequidens* differentiated towards divergent altitudinal and climatic zones during invasion from two independent introduction sites. Field observations and common garden experiments using 18 populations of *S. inaequidens* from two altitudinal transects were carried out to address the following question: along the two transects, could an altitudinal cline in phenology and growth traits be identified?

Methods

Study species and invasion history

S. inaequidens is a herbaceous perennial shrub native to South Africa and Lesotho, distributed mainly on river edges and in stony meadows (Ernst 1998). It was accidentally introduced to Europe in the late nineteenth–early twentieth centuries, where only tetraploids are reported. In its native range, the species occurs as two co-existing cytotypes (Lafuma et al. 2003). The precise origin of the introduced tetraploid populations in the native range is unclear, but the high mountain regions of Lesotho and Free State areas are the most likely regions (Bossdorf et al. 2008; Lafuma et al. 2003). Several introductions occurred in Europe during the nineteenth–early twentieth century, and well-documented historical data are provided for two of them. In Belgium, herbarium records document the first occurrence near the wool factories of Verviers in 1892, near Liège (Verloove F., personal communication). The species was later recorded in the same wool-processing area in 1922 (Mosseray 1936). After 40 years restricted to this site, it started to spread rapidly. It reached Gent, Namur and Charleroi (Belgium) in the 1970s (Lebeau et al. 1978) and Amsterdam (The Netherlands) in 1985 (Ernst 1998). In southern France, the species was initially collected in 1936 in the wool-processing center of Mazamet (Guillerm et al. 1990; Senay 1944). It was only after 1950 that the species began its expansion. By the 1970s, the species was considered an agricultural weed in southwestern France, well within the first introduction region (Jovet and Vilmorin 1975). In the early 1980s, the species reached the Mediterranean coast from Mazamet (Guillerm et al. 1990). A decade later, even though the species was mainly distributed along roads and railways, it was considered a threat to natural habitats (Michez 1995). Other primary introduction sites were recorded in European wool centers: Bremen and Hanover in Germany (Kuhbier 1977), Edinburgh in Scotland (Lousley 1961) and Verona in Italy (Pignatti 1982).

The first records of the species, inferred as introduction events, were all tied to the wool industry, and for several decades, specimens were only documented in the vicinity of wool-processing centers (e.g., Ernst 1998; Lousley 1961). Therefore, these historical and herbarium data strongly suggested seeds were introduced with the sheep wool trade and that the colonization events, e.g., in Belgium and France, were independent. The present absence of the species in geographic areas between Southern Belgium and Southern France strengthen this interpretation (personal observation).

S. inaequidens extends to 1 m tall with numerous stems bearing yellow capitulae. Individuals can produce up to 1,500 capitulae over a flowering period, each capitulum bearing roughly 100 achenes (hereafter referred to as "seeds") (Lopez-Garcia and Maillet 2005). Sexual reproduction initiates in late spring (May–June), approximately 2 months after germination, and continues through late autumn (November–December). The species is considered self-incompatible, but some individuals were observed exhibiting partial self-fertility in France (Lopez-Garcia and Maillet 2005). Entomophilous pollination with generalist pollinators is most common.

Seed populations

Two transects were defined along altitudinal gradients in France and Belgium (Fig. 1). Each transect corresponded to a colonization event from an introduction site. Transects were 150 km (Belgium) and 200 km (France) long. They were divided into four (Belgium) and five (France) topographic and climatic zones and numbered consecutively from sea level to high elevation: Belgium: (1) Coastal Oceanic (altitudinal reference: 0 m), (2) Oceanic (50 m), (3) Sub-oceanic (200 m) and (4) Sub-oceanic (400 m); France: (1) Coastal Mediterranean (altitudinal reference: 0 m), (2) Sub-Mediterranean (200 m), (3) Sub-oceanic (400 m), (4) Low-elevation mountain (800 m) and (5) Mid-elevation mountain (1,600 m). Zone Number 3, in both countries, was the initial introduction zone for each colonization event. In each zone, two populations were selected at similar altitudes (Table 1) and were spaced at least 5 km apart. The 18 selected populations included at least 100 individuals and were all located along roadsides on rocky and/or gravel soils. In November 2005 and 2006, seeds were collected on two to three capitulae from ten randomly selected individuals per population. Based on large population size, sampled individuals were assumed not to be the same in 2005 and 2006. Seeds were stored at 4°C and sorted using a dissecting microscope: the ten largest seeds without anomaly per parent individual were preserved for the experiment as within-capitulum variability in seed mass has been shown for the species (Monty et al. 2008). The ten seeds per parent individually selected in 2006 were collectively weighed to the nearest 0.1 mg.

In November 2006, in situ measurements were performed in the 18 populations: the ten largest plants per population were recorded, and their height was measured. The largest individuals were considered instead of randomly selected ones in order to discard the effects of the demographic stage of the population, with the assumption that old plants (representative of the longevity of the species) were present in all populations.

Common garden experiments

In spring 2006 and 2007, a randomized block common garden experiment was established on an open field in Gembloux (Belgium, altitude: 160 m) with the seeds collected in 2005 and 2006, respectively. Each of the ten blocks consisted of two rows of nine pots. To prevent aboveground competition, rows were placed 80 cm apart and pots in the rows separated by 50 cm. The common garden was surrounded by two additional rows of pots, to prevent edge effects. On 17 (block 1–5) and 18 (block 6–10) March 2006 and on 17 April 2007, the ten sorted seeds per parent individual were collectively sown in pots containing 2.5 l of sand, 2.5 l of compost and 0.5 l of hydro-granulates. Pots were then covered with a protective light-permeable canvas until early May. Seedlings were counted every 2–3 days. The first emerged seedling in

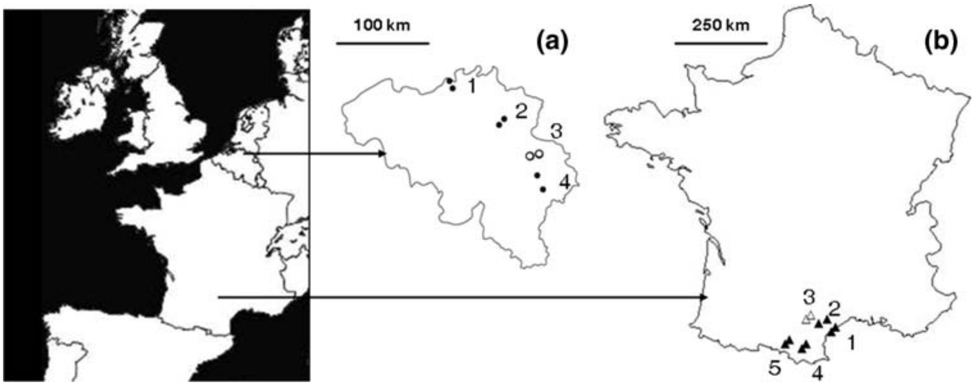

Fig. 1 Sample population locations of *Senecio inaequidens* DC (Asteraceae) from **a** Belgian and **b** French transects. *Numbers* correspond to transect altitudinal zones, and *symbols* correspond to populations. *Open symbols* represent populations from the first introduction sites

Table 1 Characteristics of *Senecio inaequidens* DC (Asteraceae) populations along the Belgian and the French transects, and corresponding climatic stations

Transect	Transect zone	Seed population					Climatic station				
		Population	Location	Altitude (m)	Latitude N	Longitude E	Altitude (m)	Latitude N	Longitude E	AnnT (°C)	AnnP (mm)
Belgium	B1	A	Ekeren	5	51°16′	4°23′	12	51°12′	4°28′	9.6	776
		B	Berendrecht	4	51°22′	4°17′					
	B2	A	Paal	35	51°03′	5°11′	55	51°10′	5°28′	9	799
		B	Hulst	22	51°04′	5°09′					
	B3	A	Verviers	195	50°36′	5°53′	186	50°39′	5°27′	7.4	1,127
		B	Goé	220	50°36′	5°57′					
	B4	A	Malmédy	320	50°25′	6°00′	564	50°28′	6°11′	5.7	1,326
		B	Kaiserbaracke	480	50°20′	6°05′					
France	F1	A	Narbonne	5	43°11′	3°02′	5	43°11′	3°01′	16.1	598
		B	Narbonne-Plage	2	43°10′	3°11′					
	F2	A	Rieux-Minervois	95	43°16′	2°37′	80	43°16′	2°31′	15	670
		B	Caunes-Minervois	175	43°19′	2°32′					
	F3[a]	A	Castaunouze	370	43°29′	2°23′	683	43°28′	2°21′	12.7	992
		B	Moulin de l'Oulne	330	43°29′	2°22′	232	43°33′	2°17′		
	F4	A	Nohèdes	785	42°37′	2°19′	1,000	42°38′	2°17′	10.3	888
		B	Taurynia	760	42°35′	2°25′					
	F5	A	La Llagone	1,695	42°32′	2°08′	1,600	42°31′	2°07′	6.3	708
		B	Egat	1,635	42°30′	2°01′					

AnnT and *AnnP* are annual mean temperature and annual cumulated rainfall, respectively

[a] Two climatic stations were considered for French transect third zone. Data used in the analysis are average values of both stations

each pot was marked with a short rod for measurement. Other seedlings were removed after counting. In 2006 and 2007, each of the 180 sampled parent plants (10/population) was thus represented by ten seeds for germination study, then by one descendant. Germination was considered complete on 15 May 2006 and on 1 June 2007 when no additional seedlings had emerged for 10 days. Measurements were conducted until mid-December 2006 and late November 2007. During summer drought in 2006, plants were watered periodically. All data were recorded block by block and included the following: time to germination (*d*) since sowing, time to flowering (*d*) since germination and height at maturity (cm), defined as the height of the plant at first flowering (based on daily observations). At the end of each experiment, plants were harvested at ground level, final height (cm) was recorded and aboveground biomass (*g*) was measured after oven drying for 48 h at 60°C. Final plant height was measured for half the blocks (90 individuals) in 2006. Aboveground biomass was measured for half the blocks (90 individuals) both years.

Climatic data

Along each transect, a climatic station per zone was selected nearest the two populations per zone, at comparable elevation (Table 1). Available data included monthly mean temperature and rainfall over the period 2000–2005 for the French transect and over the last 25 years for the Belgian study site. The average values of two stations were used for the French third zone (F3) to be representative of the populations. In order to reduce the number of variables and control for autocorrelation, principal component analyses (PCA) were performed on the climatic data. First PCA axes will therefore be referred to as "PCA 1 CLIMATE." Climatic interpretation of the altitudinal gradients was made using Pearson's correlation coefficients between climatic data and principal components scores (Supplementary Table S1).

Data analysis

Evidence of clinal altitudinal differentiation was tested by calculating Pearson's correlation coefficients between the mean population values for each of the measured traits and the altitude of seed populations. To help visualization, corresponding linear regressions were performed in Fig. 2. Individual plant values were indirectly analyzed through the population mean because individuals within populations were not independent samples. In order to take into account maternal carry-over effects due to differences in

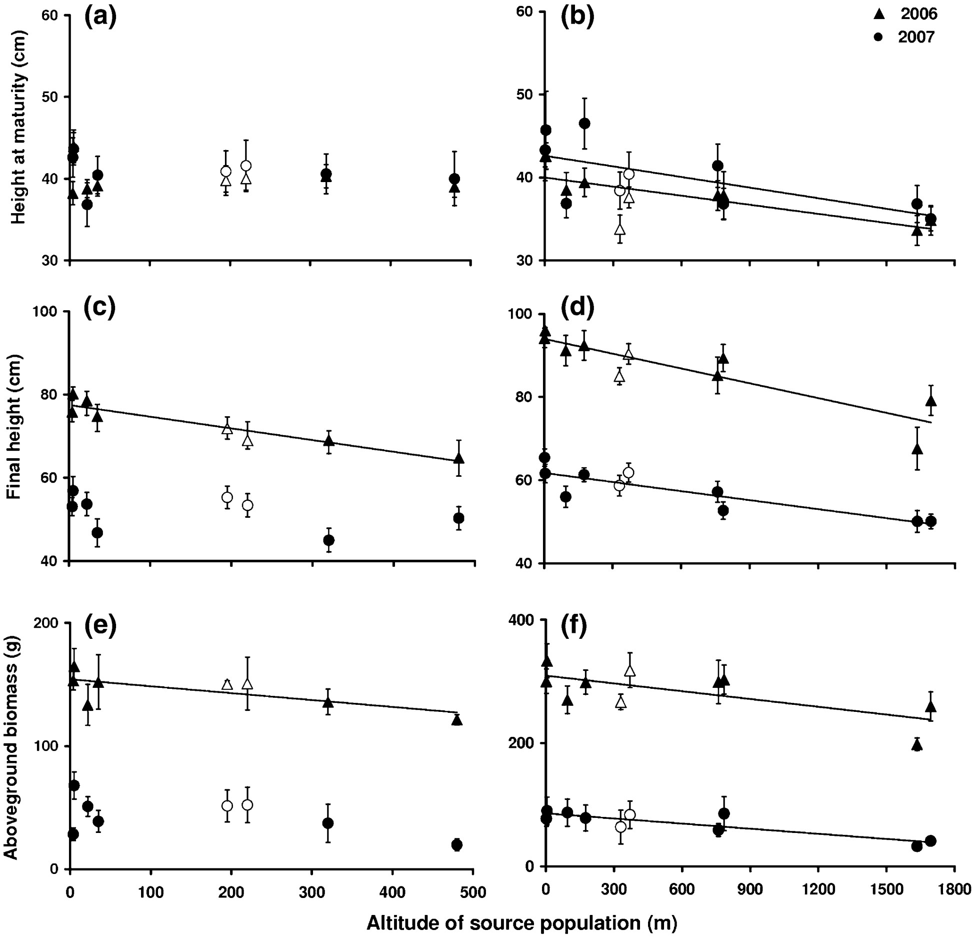

Fig. 2 *Senecio inaequidens* populations in the common garden experiments in 2006 and 2007: **a**, **b** height at maturity, **c**, **d** final height and **e**, **f** aboveground biomass against altitude of source populations Belgian (**a**, **c**, **e**) and French (**b**, **d**, **f**) transects. *Symbols* represent population trait means, with standard errors. *Solid lines* represent significant regressions ($P < 0.05$) along the transects. *Open symbols* represent populations from the first introduction site (Verviers in Belgium and Mazamet in France)

seed provisioning among populations, in 2007, we used seed mass as a covariate in correlation analysis. In addition, we assessed whether populations were significantly different among altitudinal zones for the same traits employing mixed-model ANOVA (2006) and ANCOVA (2007). Populations (random) were nested within altitudinal zones (fixed), which were nested in the factor region (fixed), i.e., Belgium and France. The factor block was random. Altitudinal pattern of seed mass variation was tested by calculating Pearson's correlation coefficient between seed mass and population altitude.

As plant traits tended to be correlated (Supplementary Table S2), in order to assess the global differentiation of plants in relation to climate, a PCA was performed on the plant traits data for each transect and each year separately. The scores of the four resulting first PCA axes (referred to as "PCA 1 PLANT TRAIT") were regressed against the corresponding PCA 1 CLIMATE scores (Fig. 3).

In situ measurements were analyzed by calculating Pearson's correlation coefficients (and corresponding linear regressions) between mean population height and (1) population altitude and (2) mean population height in the common garden.

Analyses of variance and covariance were performed using the general linear model (GLM) in Minitab software version 14.20 (Minitab Inc. 2000). Correlations analysis and PCA were made using Statistica software version 6.1 (Statsoft Inc., 2004). Aboveground biomass data were log-transformed to reach the assumptions of statistical analyses.

Results

Climatic description of the transects

Climatic data for increases in elevation along the French transect showed annual mean temperature decreased gradually from 16.1 to 6.3°C. Annual precipitation rose from sea level (598 mm) to the initial introduction zone (992 mm) and then fell at higher elevations. In Belgium, annual mean temperature decreased gradually from 9.6°C at sea level to 5.7°C at the highest altitude, and annual precipitation increased from 726 to 1,326 mm (Table 1). The first two PCA axes generated from the monthly climatic variables were informative. In France, the first axis explained 62.5% of the variance and was described as a temperature and

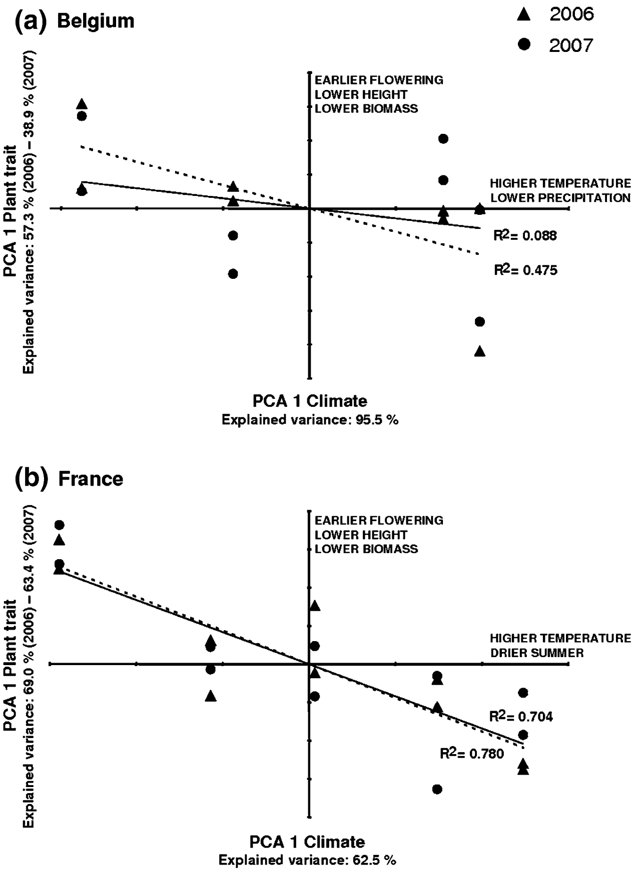

Fig. 3 Population scores for PCA 1 of climate and plant traits for (**a**) Belgian and (**b**) French transects in 2 years. *Lines* represent regressions for each year. *Triangles* and *dotted lines*: 2006. *Circles* and *plain lines*: 2007. Regressions were significant for the French transect in 2006 ($r^2 = 0.780$, $P = 0.001$) and 2007 ($r^2 = 0.704$, $P = 0.002$)

summer-drought axis (Supplementary Table S1). In Belgium, the first axis explained 95.5% of the variance and was positively correlated to all the monthly temperatures and negatively correlated to all monthly precipitation levels. No significant correlation was found for the second axis. Along both the French and Belgium transects, the first axis was strongly and negatively correlated to altitude (France: $r = -0.959$, $P < 0.001$, Belgium: $r = -0.963$, $P < 0.001$), while the second axis revealed a lack of correlation (France: $r = 0.266$, $P = 0.457$, Belgium: $r = 0.091$, $P = 0.831$). This indicated that the French altitudinal transects followed a temperature and summer-drought gradient, with the warmest and most summer-dry zones at lower altitudes. The Belgian transect followed both a decrease in temperature and an increase in rainfall levels with increasing elevation. Most traits correlated with altitude were also correlated with each transect and the corresponding first PCA axis (Table 2).

Population differentiation in the common gardens

Germination occurred in every pot in the experimental gardens, and all selected plants survived and bloomed. ANOVA (2006) and ANCOVA (2007) (Table 3) revealed significant differences between the two regions (Belgium and France) for the aboveground plant biomass and final height in 2006. Within each transect, there were significant differences between altitudinal zones in height at maturity and aboveground biomass in 2006 and final plant height in 2006 and 2007. Populations within altitudinal zones differed only for time to germination in 2007. Seed mass in the 2007 ANCOVA explained a significant part of the variability in time to germination and final plant height.

In 2006 and 2007, populations from the French transect showed significant correlations with altitude for three traits in the common garden: height at maturity, final height and aboveground biomass. All trait values decreased with increasing elevation of the source populations (Fig. 2). A similar trend was found for time to flowering in 2006 that was not statistically confirmed in 2007. For the Belgian populations, final plant height and aboveground biomass also showed a significant decrease with altitude in 2006.

Table 2 Pearson's coefficient of correlation between population trait means in the common gardens 2006 and 2007, and altitude of source population along both transect and corresponding PCA 1 CLIMATE axes

Trait	Belgium								France							
	2006				2007				2006				2007			
	Altitude		PCA 1		Altitude		PCA 1		Altitude		PCA 1		Altitude		PCA 1	
Time to germination	−0.590	Ns	**0.709**	*	−0.123	Ns	0.255	Ns	0.476	Ns	−0.592	Ns	0.570	Ns	0.470	Ns
Time to flowering	−0.537	Ns	0.441	Ns	−0.289	Ns	0.284	Ns	**−0.654**	*	**0.718**	*	−0.401	Ns	0.340	Ns
Height at maturity	−0.155	Ns	0.125	Ns	−0.147	Ns	0.151	Ns	**−0.726**	*	**0.827**	**	**−0.670**	*	**0.731**	*
Final height	**−0.940**	**	**0.919**	**	−0.390	Ns	0.435	Ns	**−0.890**	**	**0.872**	**	**−0.870**	**	**0.868**	**
Aboveground biomass	**−0.712**	*	0.643	Ns	−0.514	Ns	0.406	Ns	**−0.695**	*	**0.647**	*	**−0.868**	**	**0.809**	**

Bold values are significant correlations

Ns not significant

* $0.01 \leq P < 0.05$, ** $0.001 \leq P < 0.01$

Table 3 Results of nested ANOVA (2006) and ANCOVA (2007, covariate: seed mass) for traits measured in the common gardens

Trait	Source of variation	2006 (ANOVA)			2007 (ANCOVA)		
		df	*F*	*P*	*df*	*F*	*P*
Time to germination	Seed mass	–	–	–	1	5.505	**0.020**
	Region	1	2.131	0.188	1	4.390	0.074
	Altitudinal zone (region)	7	2.434	0.107	7	0.705	0.670
	Population (altitudinal zone)	9	0.836	0.584	9	2.304	**0.019**
	Block	9	1.567	0.130	9	2.758	**0.005**
	Error	153			153		
Time to flowering	Seed mass	–			1	0.072	0.789
	Region	1	4.248	0.078	1	3.865	0.090
	Altitudinal zone (region)	7	0.910	0.539	7	0.826	0.590
	Population (altitudinal zone)	9	1.161	0.324	9	1.847	0.064
	Block	9	0.722	0.689	9	1.831	0.067
	Error	153			153		
Height at maturity	Seed mass	–	–	–	1	1.223	0.270
	Region	1	1.524	0.257	1	0.548	0.483
	Altitudinal zone (region)	7	4.345	**0.023**	7	2.325	0.119
	Population (altitudinal zone)	9	0.908	0.520	9	0.999	0.444
	Block	9	1.588	0.123	9	0.724	0.687
	Error	153			153		
Final height	Seed mass	–	–	–	1	6.988	**0.009**
	Region	1	6.827	**0.035**	1	0.699	0.431
	Altitudinal zone (region)	7	7.795	**0.003**	7	5.111	**0.014**
	Population (altitudinal zone)	9	0.672	0.731	9	1.626	0.112
	Block	4	1.820	0.135	9	3.266	**0.001**
	Error	68			153		
Aboveground biomass	Seed mass	–	–	–	1	0.000	0.985
	Region	1	56.030	**<0.001**	1	3.973	0.086
	Altitudinal zone (region)	7	4.624	**0.019**	7	1.984	0.167
	Population (altitudinal zone)	9	0.355	0.952	9	1.331	0.237
	Block	4	1.319	0.272	4	1.078	0.374
	Error	68			68		

Populations (random) were nested within altitudinal zones (fixed), which were nested in the factor region (fixed), i.e., Belgium and France. Block was a random factor. *P*-values in bold are significant

Those trends were not confirmed in 2007 as no significant correlations were found between traits and population altitude for Belgian populations. Both years, correlation signs were however similar: growth traits decreased with increasing altitude of the source populations (Table 2).

Despite a marginally significant correlation of seed mass and altitude along the French transect ($r = 0.590$, $P = 0.072$) and a significant influence on plant height in 2007 ANCOVA, including seed mass as a covariate in the correlation analysis in 2007 did not change the outcome of the analysis. Height at maturity, final height and aboveground biomass were still significantly correlated with altitude when seed mass was used as a covariate ($P = 0.024$, $P = 0.004$, $P = 0.011$, respectively).

From the PCA performed on plant traits, only the first axis was kept for further consideration. In Belgium, PCA 1 PLANT TRAIT explained 57.3 and 38.9% of the variance, respectively, in 2006 and 2007. In France, the explained variance was 69.0% in 2006 and 63.4% in 2007. Correlations of plant traits with PCA 1 PLANT TRAIT axes are presented in Supplementary Table S3. Figure 3 allows visualization of the global plant differentiation in relation to climate.

Phenotypic variation in the field

Mean population heights recorded in situ were significantly correlated to altitude (Fig. 4) along the French transect ($r = -0.651$, $P = 0.042$), but not along the Belgian transect

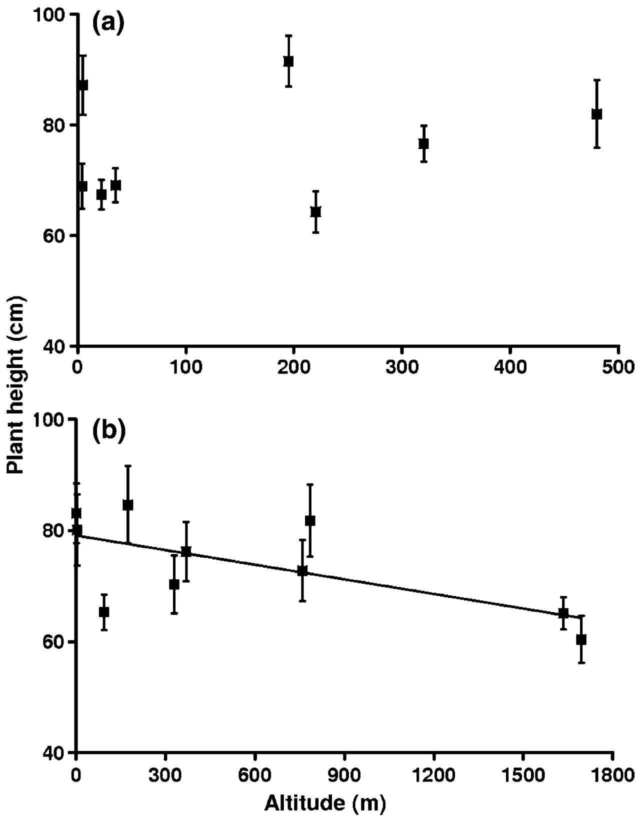

Fig. 4 In situ variation of plant height with altitude for **a** Belgian and **b** French transects. *Symbols* represent population means, with standard errors. The *line* represents the significant regression along the French transect

($r = -0.265$, $P = 0.527$). Similarly, there was a significant correlation between population mean height in the field and in the common garden for French populations ($r = 0.704$, $P = 0.023$), but not for the Belgian ones ($r = -0.0591$, $P = 0.889$).

Discussion

In this study, we addressed whether populations of the invasive species *S. inaequidens* diverged during range expansion along altitudinal gradients, ca. 1 century after introduction, using field measurements and common garden experiments. We evaluated life-history traits by measuring characteristics of germination, flowering phenology and growth. We used two independent transects differing in their altitudinal range and repeated the experiments over 2 years with independent seed sources.

Clinal variation in the common garden experiments

Our most noteworthy result was a significant clinal reduction in plant height (height at maturity and final height) and aboveground biomass associated with increasing altitude in plants from the French transect. This study area exhibited the greatest altitudinal range. These results were consistently observed over 2 years of common garden experiments and parallel the variation observed in wild populations. A similar significant reduction of final height and biomass with increasing altitude was also observed for Belgian populations, the study area with a lower range of altitudinal variation. However, significant results were only observed for the 1st year of study. Altitudinal trends were globally less clear along the Belgian than along the French transect. The shorter experimental period in the 2nd year of the experiment may therefore be a reason for the lack of significant differentiation in growth traits along this transect.

Phenological traits, particularly time to flowering, are also considered important life-history traits, likely to influence invasive plant success (Griffith and Watson 2006). Among invasive plants, genetically based clinal variation for flowering time has been reported along large latitudinal gradients (e.g., Kollmann and Banuelos 2004; Montague et al. 2008; Weber and Schmid 1998). However, we did not detect such differentiation for *S. inaequidens* along altitudinal transects. Height at maturity gradually decreased with increasing elevation in France, but time to flowering did not exhibit consistent patterns of differentiation over the 2 years of study and among the transects. In *S. inaequidens*, this may be due to increased phenotypic plasticity for this trait than for growth traits. Selection pressure on flowering time may also be lower than on growth traits and size at maturity. This could be counteracted by gene flow, which is expected to be higher at the geographical scale of our study (100 km) rather than over latitudinal ranges (1,000 km).

Regressing principal component scores of plant traits against climate (Fig. 3) showed that globally, plant altitudinal differentiation was related to climatic variation along the French transect. Climatic interpretations revealed that the French transect followed a temperature and summer-drought gradient.

Non-adaptive mechanisms leading to clinal variation

Clines are typically interpreted as the product of local adaptation across an ecological gradient. However, alternative, non-exclusive hypotheses cannot be excluded on the basis of common garden experiments. Clines can originate from demographic processes, notably as a by-product of colonization routes and repeated founder events (Endler 1977) that are likely to occur in invasive species. Genetic drift associated with repeated bottlenecks may result in significant differences among populations and reduced genetic variation within populations (Barrett and Husband 1990; Parker et al. 2003). In our case, however, because source populations were in the middle of colonization routes, genetic drift should have led to opposite effects in the two colonization directions. We detected an increased frequency of larger individuals towards low altitudes versus an increased frequency of smaller individuals towards higher altitudes, an improbable scenario under complete genetic drift. Another possibility is the introduction of preadapted genotypes from climatically similar regions of the native range (climate matching, e.g., propagules from higher altitude in the native range are introduced directly into higher altitude localities in the introduced range) (Maron et al. 2004; Montague et al. 2008). Consequently, divergence in introduced regions would reflect prior divergence of source populations in the native range. Historical records of *S. inaequidens* range expansion make this explanation unlikely, particularly in regards to the transects explored in this study, where the species migration progression has been well documented by botanists (e.g., Ernst 1998; Guillerm et al. 1990; Jovet and Vilmorin 1975; Lebeau et al. 1978). In both regions, the species was first restricted to local sites of introduction (vicinity of wool factories) and started to spread to new localities in the 1970s. Hence, the expansion of the species was initiated following the end of the main known source of introduction, notably wool importation. In addition, differences in altitudinal range of the tetraploid *S. inaequidens* between native and introduced ranges make it unlikely that coincidental introduction is the cause for the observed clinal trait variation. Lafuma and Maurice (2007) reported that invasive tetraploid populations of *S. inaequidens* in Europe probably originated from South African high mountain regions, while invasive populations in Europe cover a range from sea level to mountain altitudes. Nevertheless, phylogeographic analysis including native and introduced populations coupled with estimates of genetic diversity along altitudinal transects would be required to elucidate the role of immigration history and/or genetic drift in geographical differentiation of life-history traits (e.g., Kliber and Eckert 2005; Maron et al. 2004).

Because our seed source was from field collections, environmental maternal effects could have contributed to among-progeny variation in the common garden (Roach and Wulff 1987). Such effects could result from different seed provisioning among populations because of variation in the maternal growth environment. To overcome this limitation, levels of differentiation in traits should be further assessed using seeds generated under controlled conditions to ascertain genetic origins. However, different lines of evidence make it unlikely that maternal effects play an important role in the observed patterns of divergence. Height differentiation along both transects was low in juvenile stages and more discernable in mature individuals (data not shown). This indicated low maternal effect contributions as these are generally marked during primal growth (Roach and Wulff 1987). Moreover, controlling for seed mass in ANCOVA and correlation analyses between seed traits and altitude showed negligible changes in the results. Differences between altitudinal zones, as well as clinal variations, existed exclusive of the influence of seed mass. However, we cannot exclude maternal effect because of adaptive transgenerational plasticity that improves progeny fitness in maternal environments and may not involve differential provisioning (Galloway 2005).

Adaptive mechanisms leading to clinal variation

With our current knowledge of the species history, a significant role of evolutionary process due to selection along colonization routes may be the most parsimonious hypothesis to explain the observed altitudinal cline in growth traits along the French transect. Correspondence between the performance of populations in the field and common gardens would be expected if geographic variation in life-history traits had a genetic component. We detected a similar trend of decreasing height with higher altitude both in the field and in the common gardens. Variation in life-history traits among introduced populations of *S. inaequidens* closely matched specific theoretical expectations for selection on growth traits across an altitudinal gradient and also corresponded to a climatic gradient. Small size is expected to reflect adaptation to harsher conditions and to shorter growing seasons, and is a common feature of alpine ecotypes in native species (e.g., Blanckenhorn 1997; Clausen et al. 1941; Galen et al. 1991; Körner 1999). Along elevationnal gradients, native herbaceous species were found to show a decrease in plant size and growth rate (Hemborg and Karlsson 1998; von Arx et al. 2006). This parallels clinal variation in size that has been observed in different invasive species, including both plants and animals, along latitudinal gradients that may represent similar climatic variation (e.g., Huey et al. 2000; Kollmann and Banuelos 2004; Weber and Schmid 1998). In *S. inaequidens*, the pattern of covariation between growth traits and elevation was stronger in France than in Belgium. Climatic analysis indicated the French transect was mainly along a temperature and summer-drought gradient and had the broadest temperature ranges. The Belgian transect covered a smaller range of altitude and temperature. Selection pressures might therefore be lower under these conditions. If the correlation analysis along the French transect is performed after removing the two highest populations (results not shown), although the overall pattern remains similar (smaller plants at higher altitudes), altitudinal trends in most traits (but final height) are no longer statistically significant. Altitudinal limitation in Belgium might therefore be a reason for the lack of significance in the results.

If adaptation occurred, different processes of selection may have shaped the observed differentiation among populations. Individuals dispersed from their native range may have already contained all the genetic variation that was later expressed among populations in the introduced localities. In that case, natural selection would have filtered among the available genotypes and favored those best suited to the new environments. This ‘sorting’ process would not have required the formation of new genotypes and evolutionary processes other than differential mortality after introduction (Sakai et al. 2001). Bossdorf et al. (2008) advocated such a ‘sorting’ process to explain trait differences between native and introduced populations of *S. inaequidens* in Central Europe, as they observed that invasive populations were less genetically variable than natives in a common garden experiment. However, the authors only included low altitude European populations, which did not encompass the full range of genetic variation in growth traits reported in the current study. In addition, for the traits showing an altitudinal cline, we did not detect evidence for a reduction of within-population trait variation along the study transects: the coefficients of variation were not correlated to altitude (data not shown). This would have been an indicator of selection of a subset of introduced genotypes. Alternatively, as the species is known to be largely self-incompatible (Lopez-Garcia and Maillet 2005), sexual reproduction may have resulted in the origin of novel genotypes in the introduced range for natural selection to favor or cull from the non-native populations. Lafuma and Maurice (2007) provided evidence that the *S. inaequidens* population of Mazamet (site of introduction, French transect) may have been founded by a mixture of several South African populations as they exhibited a higher number of compatible crosses compared to South African populations. Some authors have suggested that gene recombination in the introduced range between populations previously isolated in the native range may be an important force for rapid evolution in invasive species (Lee 2002; Sakai et al. 2001). The differential mortality and reproduction of genotypes resulting from sexual reproduction, dictated by environmental conditions, could lead to the observed clinal variation. A similar evolutionary scenario was proposed by Weber and Schmid (1998) to explain latitudinal differentiation in size of European *Solidago altissima* and *Solidago gigantea*.

Conclusions

Selection of adapted genotypes during migration from a colonist source seems the most likely explanation for the observed clinal patterns in growth traits for *S. inaequidens*. However, different evolutionary processes invoked are not mutually exclusive, and the immigration history of invaders is often complex. In addition, it is not yet clear how much the observed differences among populations for growth traits may affect fitness. A comprehensive evaluation of divergence patterns is made difficult by the use of only one common garden site. If genetically based differentiation can be linked to altitude and temperature/summer-drought conditions, reciprocal transplants would be a more viable test of the adaptive significance of the observed clinal variation (Lacey 1988; Rice and Mack 1991). For this reason, additional studies are needed to fully understand the evolutionary basis of the clinal trend observed in our study. Nevertheless, our study contributes to the growing body of evidence regarding the potential for rapid differentiation of invasive species populations as they disperse through their non-native range. The documentation of phenotypic and genotypic variation of exotic plants within their invasive range is vital to understand the evolutionary potential of these species. In the case of successful invaders such as *S. inaequidens*, adaptive differentiation, if confirmed by further studies, may be an important factor that promotes establishment and subsequent invasions.

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Electronic supplementary material - Appendix legends

S1: Principal component analysis performed on climatic data from the climatic stations along each transect. Correlations of each climatic variable with the two first PCA axes (referred to as PCA 1 CLIMATE and PCA 2 CLIMATE) are presented. Significant correlations are in bold. For each axis, the eigenvalue and the explained variance are given.

Climatic trait	France				Belgium			
	PCA 1 CLIMATE		PCA 2 CLIMATE		PCA 1 CLIMATE		PCA 2 CLIMATE	
January mean temperature	**0.985**	***	-0.107	n.s.	**0.994**	***	-0.084	n.s.
February mean temperature	**0.994**	***	-0.068	n.s.	**0.989**	***	-0.134	n.s.
March mean temperature	**0.983**	***	-0.169	n.s.	**0.994**	***	-0.097	n.s.
April mean temperature	**0.994**	***	-0.099	n.s.	**0.997**	***	-0.073	n.s.
May mean temperature	**0.994**	***	-0.091	n.s.	**0.999**	***	-0.033	n.s.
June mean temperature	**0.992**	***	-0.096	n.s.	**0.999**	***	-0.017	n.s.
July mean temperature	**0.995**	***	-0.026	n.s.	**0.999**	***	-0.030	n.s.
August mean temperature	**0.992**	***	-0.104	n.s.	**0.997**	***	-0.073	n.s.
September mean temperature	**0.993**	***	-0.101	n.s.	**0.991**	***	-0.136	n.s.
October mean temperature	**0.986**	***	-0.158	n.s.	**0.990**	***	-0.137	n.s.
November mean temperature	**0.986**	***	-0.142	n.s.	**0.996**	***	-0.087	n.s.
December mean temperature	**0.980**	***	-0.167	n.s.	**0.985**	***	-0.133	n.s.
January cumulated rainfall	-0.101	n.s.	**-0.897**	***	**-0.984**	***	0.169	n.s.
February cumulated rainfall	0.143	n.s.	-0.576	n.s.	**-0.986**	***	0.158	n.s.
March cumulated rainfall	-0.562	n.s.	**-0.814**	**	**-0.993**	***	0.113	n.s.
April cumulated rainfall	-0.420	n.s.	**-0.704**	*	**-1.000**	***	0.002	n.s.
May cumulated rainfall	**-0.697**	*	-0.391	n.s.	**-0.986**	***	-0.163	n.s.
June cumulated rainfall	**-0.781**	**	-0.574	n.s.	**-0.942**	***	-0.335	n.s.
July cumulated rainfall	**-0.968**	***	-0.191	n.s.	**-0.872**	**	-0.489	n.s.
August cumulated rainfall	-0.427	n.s.	**-0.822**	**	**-0.842**	**	-0.540	n.s.
September cumulated rainfall	0.333	n.s.	**-0.797**	**	**-0.930**	***	-0.361	n.s.
October cumulated rainfall	0.351	n.s.	**-0.817**	**	**-0.995**	***	-0.044	n.s.
November cumulated rainfall	0.513	n.s.	-0.477	n.s.	**-0.989**	***	0.121	n.s.
December cumulated rainfall	-0.096	n.s.	**-0.917**	***	**-0.985**	***	0.158	n.s.
Eigenvalue	*15.0*		*6.0*		*22.9*		*1.0*	
Explained variance (%)	*62.5*		*25.1*		*95.5*		*4.2*	

Note. *** P<0.001, ** 0.001≤ P<0.01, * 0.01≤ P<0.05, n.s. not significant

S2: Pearson's coefficient of correlation among the measured plant traits, calculated on mean population values, for both transects and both years altogether. Significant correlations are in bold.

	Time to germination		Time to flowering		Height at maturity		Final height	
Time to flowering	0.120	n.s.						
Height at maturity	0.301	n.s.	**0.449**	**				
Final height	- **0.791**	***	0.180	n.s.	- 0.081	n.s.		
Aboveground biomass	- **0.790**	***	0.162	n.s.	- 0.232	n.s.	**0.952**	***

Note. *** P<0.001, ** 0.001≤ P<0.01, * 0.01≤ P<0.05, n.s. not significant

S3: Principal component analysis performed on plant traits, for the two years of experiment and along the two transects. Correlations of each trait with the first PCA axis (referred to as PCA 1 PLANT TRAIT) are presented. Significant correlations are in bold. For each PCA 1 PLANT TRAIT axis, the eigenvalue and the explained variance are given.

Plant trait	PCA 1 PLANT TRAIT							
	Belgium				France			
	2 006		2 007		2 006		2 007	
Time to germination	- 0.253	n.s.	0.521	n.s.	0.492	n.s.	**- 0.729**	*
Time to flowering	**- 0.920**	***	- 0.442	n.s.	**- 0.875**	***	**- 0.760**	*
Height at maturity	**- 0.771**	*	**- 0.748**	*	**- 0.956**	***	**- 0.864**	***
Final height	**- 0.837**	**	- 0.619	n.s.	**- 0.924**	***	**- 0.836**	**
Aboveground biomass	**- 0.812**	**	- 0.732	n.s.	**- 0.822**	**	**- 0.785**	**
Eigenvalue	*2.87*		*1.95*		*3.45*		*3.17*	
Explained variance (%)	*57.31*		*38.93*		*69.03*		*63.41*	

Note. *** P<0.001, ** 0.001≤ P<0.01, * 0.01≤ P<0.05, n.s. not significant

Article 4

An explicit test for the contribution of environmental maternal effects to rapid clinal differentiation in an invasive plant.

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Abstract. Population differentiation of alien invasive plants within their non-native range has received increasingly more attention. Common gardens are typically used to assess the levels of genotypic differentiation among populations. However, in such experiments, environmental maternal effects can influence phenotypic variation among individuals if seed sources are collected from field populations under variable environmental regimes. In the present study, seeds were collected from *Senecio inaequidens* (Asteraceae) populations along an altitudinal gradient in southern France. In addition, seeds from the same populations were generated by intra-population crossings in a climatic chamber. The two seed lots were grown in a common garden in Central Belgium to identify any evidence of environmentally induced maternal effects and/or an altitudinal cline in a suite of life-history traits. Results failed to detect any environmental maternal effects. However, an altitudinal cline in plant height and above ground biomass was found to be independent of the maternal environment.

Keywords. *Maternal environment, climatic gradient, clinal variation, seed mass, altitude, invasive plant.*

Résumé. De plus en plus d'études récentes s'intéressent à la différenciation des populations de plantes invasives au sein de leurs aires d'introduction. Typiquement, ces études utilisent des expériences en jardins communs pour évaluer l'importance de la différenciation génétique parmi les populations. Néanmoins, dans de telles expériences, les effets maternels environnementaux peuvent influencer la variation phénotypique si les graines utilisées proviennent de populations naturelles se développant dans des conditions environnementales variées. Dans cette étude, nous avons collecté des graines de *Senecio inaequidens* (Asteraceae) dans des populations naturelles, le long d'un gradient d'altitude dans le sud de la France. De plus, nous avons produit des graines par croisements contrôlés en chambre de culture, à partir de plants provenant des mêmes populations. Les deux types de graines ont été semés dans un jardin commun en Belgique, afin d'identifier la présence d'effets maternels environnementaux et/ou la présence d'une variation clinale pour différents traits d'histoire de vie. Aucun effet maternel n'a été détecté. Par contre, un cline altitudinal dans les traits de croissance (hauteur et biomasse aérienne) a été montré, indépendemment de l'environnement maternel de production des graines.

Mots-clés. *Environnement maternel, gradient climatique, masse des graines, altitude, plante invasive.*

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An explicit test for the contribution of environmental maternal effects to rapid clinal differentiation in an invasive plant

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Keywords:

altitude;
climatic gradient;
clinal variation;
invasive plant;
maternal environment;
seed mass.

Abstract

Population differentiation of alien invasive plants within their non-native range has received increasingly more attention. Common gardens are typically used to assess the levels of genotypic differentiation among populations. However, in such experiments, environmental maternal effects can influence phenotypic variation among individuals if seed sources are collected from field populations under variable environmental regimes. In the present study, we investigated the causes of an altitudinal cline in an invasive plant. Seeds were collected from *Senecio inaequidens* (Asteraceae) populations along an altitudinal gradient in southern France. In addition, seeds from the same populations were generated by intra-population crossings in a climatic chamber. The two seed lots were grown in a common garden in Central Belgium to identify any evidence of environmentally induced maternal effects and/or an altitudinal cline in a suite of life-history traits. Results failed to detect any environmental maternal effects. However, an altitudinal cline in plant height and above-ground biomass was found to be independent of the maternal environment.

Introduction

The way and the speed organisms will cope with, react or adapt to rapid environmental changes is a prime question for evolutionary ecology in the context of global change. Biological invasions are an outstanding opportunity to address fundamental questions concerning rapid species evolution in relation to environmental change (Sakai *et al.*, 2001; Lee, 2002; Callaway & Maron, 2006). As a result, the evolution of invasive species during range expansion is receiving increasingly more attention, and questions of population differentiation within introduction ranges of invasive species have been addressed in several common garden studies in the last decade (Weber & Schmid, 1998; Parker *et al.*, 2003; Kollmann & Banuelos, 2004; Maron *et al.*, 2004; Leger & Rice, 2007; Montague *et al.*, 2008). Such studies often indicated latitudinal (Weber & Schmid, 1998; Kollmann & Banuelos, 2004; Maron *et al.*, 2004; Montague *et al.*, 2008) or altitudinal (Monty & Mahy, 2009) clines, most often interpreted as the result of rapid adaptive processes. However, seeds used in those common garden studies were all obtained from natural populations (but see Weber & Schmid, 1998, where clones were used), and future studies are also likely to use field-collected propagules for practical facility. Therefore, environmental maternal effects (EMEs) cannot be ruled out, even if most results suggest *a posteriori*, a low maternal influence on measured traits. Yet, the role of EME on such clinal variation has never been explicitly tested. This is, however, important because if most studies use seeds collected in the field, an insufficient understanding of the influence of EME can lead to misinterpretation of the results and overestimation of the capacity of rapid adaptation to changing environment.

Alien species expansion into introduced ranges depends on the species capacity to cope with environmental heterogeneity. Population expansion over climatic gradients is possible due to the presence of phenotypic variability within a species. Phenotypic variability among populations can be the result of one or more of the following factors: phenotypic plasticity (Annapurna & Singh, 2003; Parker *et al.*, 2003), evolutionary differentiation (Cox, 2004) and/or maternal

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effects (Roach & Wulff, 1987). The latter are influences of the maternal parent on the phenotype of its offspring via mechanisms exclusive of the genetic information carried on chromosomes (Roach & Wulff, 1987). Maternal effects are determined by either genetic or environmental factors (Roach & Wulff, 1987; Platenkamp & Shaw, 1993).

Nongenetic or environmental maternal effects are elicited when the maternal plant environment influences the expression of traits in its offspring (Roach & Wulff, 1987). For example, the maternal tissue environment in which the zygote develops into a sporophyte can influence the phenotype of the offspring following germination (Roach & Wulff, 1987; Kirkpatrick & Lande, 1989; Schmid & Dolt, 1994). Previous studies demonstrated that EME imposed a greater influence on offspring than genetic maternal effects (Platenkamp & Shaw, 1993; Schmid & Dolt, 1994; Byers *et al.*, 1997). EME on individual plant growth often appears to be transitory (Miao *et al.*, 1991; Wulff & Bazzaz, 1992; Schmid & Dolt, 1994), but may still play a role if the period of influence is important to plant fitness (Alexander & Wulff, 1985). EME are often mediated by seed size or seed mass, and several studies found a positive relationship between seed size, the probability and speed of germination, and subsequent seedling size (e.g. Weis, 1982; Hendrix, 1984; Schmid & Dolt, 1994). However, other studies did not detect these same properties (Dolan, 1984; Schmitt & Antonovics, 1986) and emphasized the possible role of other seed qualities (Hereford & Moriuchi, 2005). EME studies have generally considered one or two specific sources of environmental variation on maternal plants, which can influence the seeds and/or the offspring. Sources of variation in soil nutrient levels and water availability have been thoroughly addressed (Stratton, 1989; Aarssen & Burton, 1990; Philippi, 1993; Schmid & Dolt, 1994; Luzuriaga *et al.*, 2005), and other studies focused on the effects of photoperiod (Munir *et al.*, 2001), intra- or inter-specific competition (Platenkamp & Shaw, 1993; Donohue, 1999) or herbivory (Weiner *et al.*, 1997). However, few studies have questioned the effects of climatic conditions on seed and seedling characteristics (Alexander & Wulff, 1985; Blödner *et al.*, 2007). Most studies experimentally induced variations in the maternal plant environment and ascertained the consequences of phenotypic maternal variation on seeds and seedlings. Studies on EME in natural populations are scarce (but see Philippi, 1993; Galloway, 1995, 2001) and little is known about their contribution to phenotypic variation under field conditions.

Environmental maternal effect can complicate the interpretation of common garden experiments. Phenotypic variation among plants in a common garden is generally attributed to genetic differentiation (Clausen *et al.*, 1940). However, environmental conditions in seed production can result in maternal carry-over effects. To minimize this source of error, plants should be grown for one or several generations in a common garden prior to the experiment (Roach & Wulff, 1987). However, many studies on plant differentiation fail to do so for practical reasons.

The invasion history of the African ragwort *Senecio inaequidens* DC. (Asteraceae) in southern France suggests that all the populations originated from a single introduction site and dispersal was largely spontaneous and/or aided by roads and railway traffic (Guillerm *et al.*, 1990; Ernst, 1998). Differences between French populations are therefore not attributable to multiple introduction events. Seeds from French *S. inaequidens* populations collected *in situ* and grown in a common garden environment showed elevational clinal variation in growth traits, i.e. plants from higher elevations showed lower height and above-ground biomass (Monty & Mahy, 2009). The nature of this cline could, however, not be ascertained, as both genetic differentiation and EME could have lead to the pattern observed. Therefore, the species represents a suitable model to assess the importance of EME in natural populations, and compare it to genetic differentiation along an altitudinal gradient during range expansion.

In this study, we conducted a common garden experiment with populations of *S. inaequidens* known to present a clinal phenotypic differentiation along an altitudinal gradient (Monty & Mahy, 2009) with the aim to examine the role of EME in phenotypic variation in plant traits. We specifically addressed the following questions: (i) to what extent does seed size explain phenotypic variation? (ii) exclusive of seed size, can phenotypic differences between plants from different maternal environments, i.e. natural and controlled, be detected? and (iii) is the clinal pattern dependent on the maternal environment?

Materials and methods

Senecio inaequidens natural history

Senecio inaequidens is an herbaceous perennial native to South Africa and Lesotho (Ernst, 1998). The species was accidentally introduced to Europe in the late 19th to early 20th century, where only tetraploids are reported. In its native range, the species occurs as two co-existing cytotypes (Lafuma *et al.*, 2003). The first records of the species are considered introduction events tied to the wool industry. For several decades, specimens were only documented in the vicinity of wool-processing centres in Europe (Ernst, 1998): Hannover (D) in 1889 and Bremen (D) in 1886; Edinburgh (UK) in 1928; Liège (B) in 1892 (Verloove, 2006); and Mazamet (F) in 1936 (Guillerm *et al.*, 1990). In southern France, Guillerm *et al.* (1990) documented the progressive colonization of the species, and found that for decades, it was only reported in the vicinity of the wool-processing centre of Mazamet. However, it was not until after 1950 that it steadily expanded throughout areas geographically removed from existing wool industrial centres. By the 1970s, the species had colonized areas of south-western France well within the first introduction region and was considered an agricultural weed. In the early 1980s, the species reached the Mediterranean coast from Mazamet. A decade later, although the species was largely distributed along roads and railways, it was considered a threat to natural habitats (Michez, 1995). Today, *S. inaequidens* is still absent from several areas in central France, indicating that invasion fronts from Mazamet and other introduction sites have not yet converged (A. Monty, unpublished data). Historical data and the present distribution of the species strongly suggest seeds were introduced with the sheep wool trade and that several different independent colonization events occurred.

Senecio inaequidens reaches 1 m tall at its maximum height and possesses numerous stems bearing yellow capitula. Each plant can produce up to 1500 flowering heads during the reproductive season, each one bearing roughly 100 achenes (Lopez-Garcia & Maillet, 2005). Flowering initiates in late spring and continues through late autumn. The species is considered self-incompatible, but in France, some individuals were observed exhibiting partial self-fertility (Lopez-Garcia & Maillet, 2005). Entomophilous pollination with generalist pollinators is most common. The fruit is an achene bearing a pappus to aid in wind-dispersal (Monty *et al.*, 2008).

Seed collection and production

We used the same populations as Monty & Mahy (2009), located along an altitudinal gradient in Southern France. The transect was located within the geographic area representing populations known to originate in Mazamet (Guillerm *et al.*, 1990). It encompassed the maximal altitudinal range of the species in its introduced range. Along the transect, we selected five reference altitudes: 0, 200, 400, 800 and 1600 m. Two seed populations were selected at each altitudinal reference (Table 1; Fig. 1). All populations chosen for the study were located on similar soil environment along roadsides on rocky, sandy and/or gravely soils and were at least 2 km apart. The two populations occupying the central part of the transect (400 m) were located in the initial introduction area and, most probably, derived from parental plants involved in the initial introduction to Mazamet.

In November 2005, seeds were collected on 2–3 capitula from 10 randomly selected individuals per population and stored in paper bags at 4 °C. In November 2006, the seeds were sown in pots. Each of the 10 field sampled individuals in each of the 10 populations was represented by one offspring. The 100 plants were grown under controlled conditions in an insect-proof climatic chamber (18 °C constant temperature; 16 h photoperiod; 70–80% humidity). In March 2007, hand pollinations were performed when all capitulum florets were receptive (flowering is centripetal within a capitulum but stigmas remain receptive for several days if not pollinated). Manual crossings were made by gently rubbing two capitula together. Six individuals per population were randomly assigned as the maternal parent and received pollen from one (or two) other individual(s) in the population. The resulting seeds were sorted using a dissecting microscope to discard aborted seeds or those with obvious abnormalities. Ten seeds per maternal individual were randomly selected for a common garden experiment and collectively weighted to the nearest 0.1 mg. These seeds corresponded to the 'controlled' maternal environment.

Table 1 Seed population coordinates and corresponding climatic stations, climatic data and seed mass data.

	Seed population				Climatic station								Mass of 10 seeds (10^{-4} g)	
Code	Location	Altitude (m)	Latitude N	Longitude E	Location	Altitude (m)	Latitude N	Longitude E	RR	TN	TM	TX	Natural	Controlled
A1	Narbonne-Plage	2	43°10′	3°11′	Narbonne	5	43°11′	3°01′	41.4	15.3	19.2	23.1	30.67 ± 1.20	31.00 ± 1.83
A2	Narbonne	5	43°11′	3°02′									33.67 ± 2.94	33.83 ± 2.85
B1	Rieux-Minervois	95	43°16′	2°37′	Laure-Minervois	80	43°16′	2°31′	38.1	14.3	18.4	22.4	29.00 ± 1.57	32.50 ± 2.50
B2	Caunes-Minervois	175	43°19′	2°32′									37.83 ± 1.11	35.00 ± 2.35
C1	Mazamet (Moulin de l'Oulne)	330	43°29′	2°22′	Rouairoux	345	43°28′	2°35′	57.7	12.2	16.6	20.9	30.17 ± 1.66	31.17 ± 1.28
C2	Mazamet (Castaunouze)	370	43°29′	2°23′									32.50 ± 2.17	40.17 ± 2.12
D1	Taurynia	760	42°35′	2°25′	Nohèdes	1000	42°38′	2°17′	37.4	9	13.1	17.2	27.17 ± 1.85	33.83 ± 1.60
D2	Nohèdes	785	42°37′	2°19′									28.83 ± 1.62	31.00 ± 1.39
E1	Egat	1635	42°30′	2°01′	Valcebollere	1420	42°23′	2°02′	46.7	5.5	11.9	18.4	31.83 ± 0.70	33.50 ± 2.58
E2	La Llagone	1695	42°32′	2°08′									38.50 ± 0.81	31.83 ± 0.91

Seed data are the population average mass (10^{-4} g) and standard error of the six lots of 10 seeds collectively weighted per parent population prior to the common garden experiment, for both maternal environment (natural vs. controlled), separately. Climatic data are the cumulative rainfall (RR, mm), mean minimal temperature (TN, °C), mean maximal temperature (TX, °C) and mean temperature (TM, °C) during October 2006.

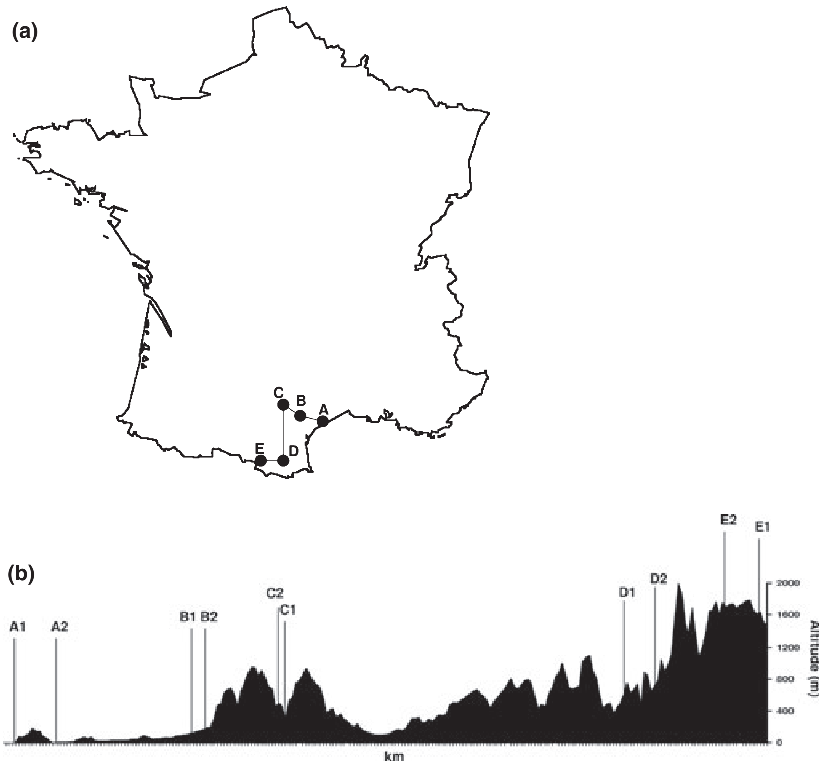

Fig. 1 (a) Transect location in southern France, with the five altitudinal references (A: 0; B: 200; C: 400; D: 800 and E: 1600 m); (b) Seed population locations along the altitudinal transect. The transect in (b) correspond to linear sections between consecutive populations. Altitude was measured every km on the *x*-axis.

In early November 2006, seeds on 2–3 capitula were field collected from 10 randomly selected individuals and stored in paper bags at 4 °C. The seeds were subsequently sorted and 10 seeds (without obvious anomalies, i.e. abortion or malformation) per parent individual were randomly selected and collectively weighted to the nearest 0.1 mg. These seeds represented the 'natural' maternal environment. The characteristics of the two sets of seeds are presented in Table 1. Monthly mean minimal (TN) and maximal (TX) temperatures during October 2006, as well as cumulative rainfall were obtained from climatic stations located within 20 km or less from seed populations at each altitude (Table 1). Monthly mean temperature was defined as (TN + TX)/2. These data represented the climatic conditions in the field for the maternal plants during flowering and fruit maturation ('natural' maternal environment).

Common garden experiment

In spring 2007, a common garden was established using a randomized block experimental design on an open area in Gembloux (Belgium; 50°33′N; 4°42′E; alt. 160 m). Six north–south-oriented blocks consisted of two rows of 10 pots each. Pots contained 2.5 L sand, 2.5 L compost and 0.5 L hydro-granulates (Argex®, Argex NV, Zwijndrecht, Belgium). The 20 pots per block were randomly assigned to the 20 combinations of the maternal environments and the populations (two maternal environments, i.e. natural and controlled × 10 seed populations). To prevent above-ground competition, rows were placed 80 cm apart and within each row, pots were separated by 50 cm. The common garden was surrounded by two additional rows of potted individuals to prevent edge effects.

On 4 June 2007, all 10 seeds per parent were sown together in each pot. Pots were covered with a protective light-permeable canvas for 10 days. Seedlings were counted every 2–3 days. The first seedling that emerged in each pot was marked with a short rod and retained in the pot for measurement. Remaining seedlings were removed after counting. Germination was considered complete on 23 June, 10 days after no additional seedlings had emerged. The following data were recorded: time to germination (d) since sowing; time to flowering (d) since germination (based on daily observations); and height at maturity (cm), defined as the height of the plant at first flowering. Plant height was measured on 6 August (63 days after sowing; henceforth referred to as 'intermediate height') and on 19 November (168 days after sowing and close to winter senescence; 'final height'). On 23 November, plants were harvested at ground level and above-ground biomass (g) was measured after oven drying for 48 h at 60 °C.

Data analysis

A two-way analysis of variance (ANOVA) was performed on the seed mass data. Maternal environment was treated as a fixed factor with two modalities represented by the controlled and natural seed maternal environments. The second fixed factor was the source population (10 modalities), and the two fixed factors were crossed. In the case of an interaction between the two factors, a one-way ANOVA was performed with the factor population, for each maternal environment separately. The presence or absence of clinal variation was assessed by testing for a linear contrast on the population mean seed masses against population altitude (Dagnelie, 2003).

Phenotypic variation among individuals has two possible sources under homogeneous conditions: genetic variation and maternal effects. Those two sources of variation can interact, as the influence of maternal conditions can differ according to genotype. A mixed model ANCOVA was used to distinguish the different sources of phenotypic variation observed among individuals in the common garden. Maternal environment (two modalities) and source population (10 modalities) were fixed factors. Block (six modalities) was treated as a random factor and all factors were crossed. The mass (mg) of the 10 seeds sown per pot served as the covariate. A significant covariate effect indicates that seed mass influences phenotypic variation, and consequently reveals EME mediated by seed mass, whereas a significant effect of the maternal environment indicates maternal effects not mediated by seed mass. For traits demonstrating a significant population effect, testing for a linear contrast between the population trait means and population altitude would provide evidence of an altitudinal cline. In addition, a significant maternal environment × population interaction would suggest that the cline was dependent on the maternal environment, and therefore indicate EME.

Analyses of variance and covariance were performed using the general linear model in MINITAB software version 14.20 (Minitab Inc., State College, PA, USA). Biomass data were log-transformed for distribution normality.

Results

Seed mass analysis

The two-way ANOVA performed on seed mass revealed no significant effect of maternal environment ($F_{1,100}$ = 2.68, P = 0.105), but a significant maternal environment by population interaction ($F_{9,100}$ = 2.51, P = 0.012). We therefore analysed separately the seed masses of the 'controlled' and the 'natural' maternal environment. Seeds from controlled conditions did not significantly differ in mass, according to population ($F_{9,50}$ = 1.84, P = 0.083). In contrast, the population effect was highly significant for the 'natural' maternal environment ($F_{9,50}$ = 4.95, P < 0.001). The altitudinal linear contrast tested for this maternal environment was not significant ($F_{1,50}$ = 1.78, P = 0.189; Table 2).

Table 2 Results of analyses of variance on seed mass: (a) two-way ANOVA depicting the effect of maternal environment and population on the whole data set. As the maternal environment × population interaction was significant, one-way ANOVA were separately performed for (b) the seeds produced under controlled conditions and (c) the seeds sampled in the field. As the latter showed a population effect, a linear contrast was tested against the altitude of the source population.

Source of variation	d.f.	MS	P
(a)			
Maternal environment	1	56.03	0.105
Population	9	77.61	**< 0.001**
Maternal environment × population interaction	9	52.63	**0.012**
Error	100	20.93	
(b)			
Population	9	45.67	0.083
Error	50	24.78	
(c)			
Population	9	84.57	**< 0.001**
Altitudinal linear contrast	1	30.32	0.189
Error	50	17.08	

Significant results are in bold.

Common garden experiment

Germination was successful in every pot in the experimental garden and all 120 selected plants survived until harvest. However, 18 plants, evenly distributed among the two maternal environments and the 10 populations (*data not shown*) failed to flower. Therefore, time to flowering and height at maturity were only recorded for 102 of 120 plants. ANCOVA (Table 3) revealed no significant covariate influence, indicating that seed mass failed to explain phenotypic variation in the measured traits. Furthermore, the maternal environment factor was never significant, i.e. no differences were detected between plants grown from *in situ* collected seeds and seeds produced under controlled conditions. The remaining two factors (source population and block) were not significant for time to germination, time to flowering and height at maturity. Significant variation among populations for intermediate height ($F_{9,94}$ = 2.28, P = 0.023), final height ($F_{9,94}$ = 2.36, P = 0.019) and above-ground biomass ($F_{9,94}$ = 2.26, P = 0.025) was observed. A linear contrast for these traits was applied and revealed an altitudinal cline in final plant height ($F_{1,94}$ = 7.38, P = 0.008) and above-ground biomass ($F_{1,94}$ = 8.81, P = 0.004). The altitudinal cline in phenotypic traits was not dependent on maternal environment, as no significant maternal environment × population interaction was indicated (Table 3). The block factor was significant for intermediate height ($F_{9,94}$ = 7.81, P < 0.001), final height ($F_{9,94}$ = 6.01, P < 0.001) and above-ground biomass ($F_{9,94}$ = 3.36, P = 0.008). Table 4 presents means and standard deviations of the different plant traits, distributed by populations and maternal

Table 3 Results of ANCOVA analyses depicting the effect of seed mass, maternal environment, source population and block on the measured traits.

		Time to germination		Time to flowering		Height at maturity		Intermediate height		Final height		Above-ground biomass	
Source of variation	d.f.	MS	*P*	MS	*P*	MS	*P*	MS	*P*	MS	*P*	MS	*P*
Seed mass	1	2.44	0.627	20.40	0.785	7.53	0.753	60.77	0.400	289.1	0.152	0.0064	0.807
Maternal environment	1	3.90	0.540	203.3	0.389	13.51	0.673	53.72	0.416	50.6	0.547	0.0204	0.662
Population	9	8.25	0.615	237.8	0.550	111.6	0.170	193.7	**0.023**	327.2	**0.019**	0.2394	**0.025**
Altitudinal linear contrast	1	–	–	–	–	–	–	287.6	0.0688	1021.5	**0.010**	0.9338	**0.004**
Block	5	23.79	0.050	377.2	0.237	83.87	0.360	662.3	**< 0.001**	844.2	**< 0.001**	0.3564	**0.008**
Maternal environment × population interaction	9	12.30	0.306	342.2	0.272	68.92	0.517	49.9	0.804	70.7	0.864	0.0268	0.985
Error	94*	20.28		271.3		75.3		84.4		138.4		0.106	

In the case of a population effect, the statistics of the corresponding linear contrast against altitude are given. Significant results are in bold.
*For time to flowering and height at maturity, the degree of freedom of the error term was 76.

Table 4 Means ± standard deviations of the plant traits measured in the common garden, distributed by populations and maternal environments.

Population code	Altitude (m)	Time to germination (days)	Time to flowering (days)	Height at maturity (cm)	Intermediate height (cm)	Final height (cm)	Above-ground biomass (g)
Natural							
A1	2	7.2 ± 0.41	95.2 ± 23.1	41.0 ± 12.0	30.8 ± 15.2	49.7 ± 14.9	41.2 ± 32.5
A2	5	7.0 ± 0.00	103.6 ± 22.5	45.0 ± 10.8	27.8 ± 11.3	47.0 ± 14.3	51.9 ± 36.9
B1	95	7.0 ± 0.00	110.2 ± 15.3	51.2 ± 7.5	25.2 ± 12.7	48.7 ± 16.1	45.4 ± 50.4
B2	175	7.0 ± 0.00	114.0 ± 15.1	39.5 ± 6.9	23.7 ± 7.3	41.3 ± 8.2	21.8 ± 5.6
C1	330	7.0 ± 0.00	115.0 ± 1.4	44.8 ± 9.4	17.2 ± 8.1	39.0 ± 17.2	26.3 ± 20.0
C2	370	7.0 ± 0.00	95.0 ± 9.3	43.6 ± 9.2	29.7 ± 9.7	43.0 ± 14.9	30.1 ± 22.0
D1	760	7.0 ± 0.00	102.4 ± 19.1	43.2 ± 15.2	24.0 ± 15.4	44.3 ± 18.4	28.4 ± 19.6
D2	785	7.0 ± 0.00	111.7 ± 13.5	42.7 ± 7.3	21.7 ± 6.9	44.7 ± 7.2	26.3 ± 17.9
E1	1635	10.8 ± 8.91	89.3 ± 13.0	41.5 ± 5.7	23.7 ± 12.6	32.5 ± 17.3	23.9 ± 15.6
E2	1695	7.0 ± 0.00	102.0 ± 7.6	41.8 ± 5.0	28.3 ± 7.7	43.2 ± 5.1	20.3 ± 7.4
Controlled							
A1	2	7.0 ± 0.00	103.6 ± 10.7	46.8 ± 3.3	30.3 ± 6.6	48.8 ± 9.9	33.9 ± 22.1
A2	5	7.3 ± 0.82	111.3 ± 10.8	49.3 ± 5.3	25.3 ± 10.7	41.7 ± 18.6	49.4 ± 21.6
B1	95	7.2 ± 0.41	97.3 ± 27.9	45.0 ± 9.0	32.5 ± 10.7	54.2 ± 8.0	52.3 ± 33.5
B2	175	7.2 ± 0.41	120.3 ± 16.5	43.3 ± 11.2	23.8 ± 9.0	45.5 ± 10.9	17.2 ± 8.1
C1	330	11.7 ± 11.43	95.0 ± 26.5	36.3 ± 9.8	15.7 ± 11.2	32.3 ± 17.9	25.5 ± 24.1
C2	370	7.8 ± 2.04	104.0 ± 9.1	46.8 ± 8.6	26.5 ± 12.6	41.3 ± 16.2	54.4 ± 53.7
D1	760	7.0 ± 0.00	109.3 ± 12.9	46.5 ± 11.0	20.8 ± 11.7	40.5 ± 14.3	34.7 ± 30.0
D2	785	7.5 ± 1.22	109.2 ± 12.5	40.0 ± 6.0	24.8 ± 13.6	44.5 ± 8.1	20.4 ± 14.0
E1	1635	7.0 ± 0.00	114.8 ± 16.0	39.2 ± 5.5	21.3 ± 8.5	39.0 ± 5.0	16.6 ± 4.5
E2	1695	7.5 ± 1.22	105.2 ± 20.6	34.2 ± 6.8	19.3 ± 5.4	37.7 ± 8.7	17.4 ± 8.8

'Natural' refers to plants grown from seeds collected in the field, 'controlled' refers to plants grown from seeds produced under controlled conditions.

environments. Figure 2 illustrates the altitudinal pattern of variation in final plant height and above-ground biomass, for both maternal environments.

Discussion

Origin of the clinal phenotypic variation

Geographic variation in life-history traits associated with climate has been documented in plants within their native ranges, and clinal variation with latitude is common in wide-ranging species (e.g. Winn & Gross, 1993; Van Dijk *et al.*, 1997; Li *et al.*, 1998; Jonas & Geber, 1999; Clevering *et al.*, 2001; Olsson & Agren, 2002). Other studies have reported variation in life-history traits among native plants along altitudinal gradients, e.g. development time and leaf morphology (Galen *et al.*, 1991; Jonas & Geber, 1999). Furthermore, evidence of genetic differentiation in the form of geographic clines in growth traits has been shown in

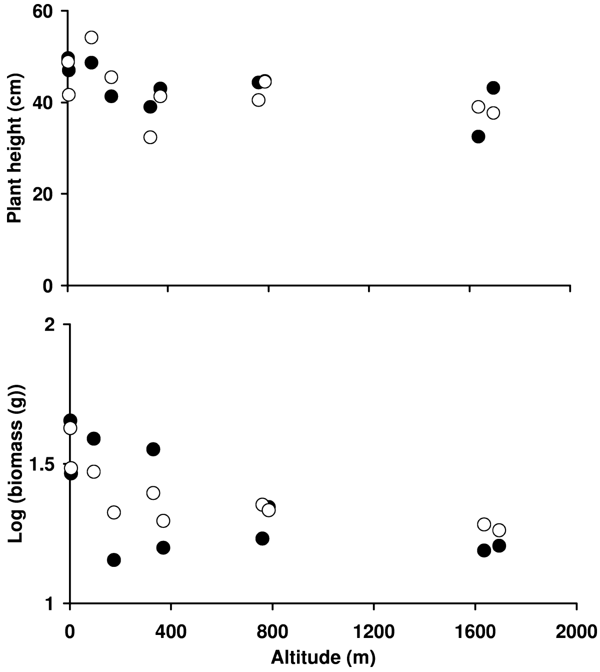

Fig. 2 *Senecio inaequidens* populations and maternal environment in the common garden: population mean final height and above-ground biomass against altitude of source populations for the two maternal environments. Open symbols: plants grown from seeds collected from natural populations; closed symbols: plants from seeds produced under controlled conditions.

invasive species (Weber & Schmid, 1998; Del Pozo *et al.*, 2002; Sexton *et al.*, 2002; Kollmann & Banuelos, 2004; Maron *et al.*, 2004). Though, the importance of EME in such clines is unclear.

This study is one of the first to evaluate EME by growing an invasive species in a common garden from (a) seeds collected *in situ* and (b) seeds produced under controlled conditions. The cline observed by Monty & Mahy (2009) in plant size was used as a basis to investigate the importance of EME, when compared with genetic differentiation, on clinal variations observed in invasive plants. The experiment we report here clearly suggested that *S. inaequidens* experienced population genotypic differentiation during range expansion from the site of introduction towards higher and lower altitudinal areas, whereas no evidence for EME was detected. When grown under controlled conditions in the common gardens, plants from lower elevations were taller and larger than those from higher elevation, and varied gradually, independently on the maternal seed production environment. Surprisingly, however, plants grown from seeds produced under controlled conditions were more variable in some traits, e.g. above-ground biomass (Fig. 2). This pointed out the different behaviour of populations B2 and C1 (located at altitudes of 175 and 330 m, Table 4).

Seed mass analysis

Seed mass did not statistically differ between the two maternal environments. However, the pattern of variation between populations was different in the 'controlled' and the 'natural' maternal environment, as revealed by the maternal environment × population interaction. When environmental variability was controlled, seed mass did not significantly differ between populations. But in the field, the population influence was highly significant. This suggests a plastic response in seed mass to environmental conditions, at least in some populations. As the pattern of variation was found not to be linear with altitude, the plastic response in seed mass may arise from other environmental conditions than climate, e.g. soil nutrient levels. Our results are in line with those of Hereford & Moriuchi (2005), who found that maternal environment significantly affected seed weight in field populations of *Dioda teres* (Rubiaceae). It must be noted, however, that we collectively weighed each set of 10 seeds instead of recording individual seed mass. This lowers the precision of the analysis.

Lack of evidence for EME

In this study, we found no evidence for EME on phenotypic variation. Seed mass influence on the measured traits was not statistically significant. The effect of the maternal environment of seed production was neither on plant traits nor on the clinal variation observed in plant height and biomass. The lack of detectable maternal effects is not consistent with most published research on EME: when they are experimentally induced, differences in environmental conditions of seed production generally influence germination, due to seed size (Alexander & Wulff, 1985; Stratton, 1989; Aarssen & Burton, 1990; Platenkamp & Shaw, 1993; Schmid & Dolt, 1994) or irrespective of seed size (Munir *et al.*, 2001). Even under natural (e.g. field) conditions, Galloway (2001) found the maternal environment influenced germination percentage in *Campanula americana* (Campanulaceae). In our study, we did not experimentally induce phenotypic variation among maternal plants to examine how the maternal environment influences progeny phenotype. Instead, we tested for maternal effects as expressed under natural conditions, due to different environmental (e.g. climatic) parameters encountered during seed formation.

Seed collection during November ensured that temperature conditions strongly varied along the transect. During October 2006, minimal and maximal mean temperatures were 15.3 and 23.1 °C at the lowest elevations, and decreased to 5.5 and 18.4 °C at the highest altitudes respectively (Table 1). Collecting seeds on a temporal basis would be an interesting approach to assess seasonal variation in seed characteristics. However, as no significant differences were found between seeds produced under varied field conditions, and seeds produced at a constant temperature (18 °C), our results merely suggested a very minor influence of maternal climatic conditions on the offspring phenotype. Despite our samplings cover the whole altitudinal range of the species in France, the absence of maternal effect along the transect might be ascribed to an environmental gradient not sufficiently strong to induce EME.

Our analysis of intermediate and final plant height was congruent with low EME. The longer the plants remained in the common garden, the more evident a linear trend in plant height. The linear contrast in plant height was only marginally significant (Table 3) in early August 2007 and corresponded to a reduction in mean population height by 15.4% (from 26.6 to 22.5 cm) from the lowest to the highest populations. In contrast, it was highly significant in November 2007 and corresponded to a reduction by 19.4% (from 44.3 to 35.8 cm). As maternal effects are known to be more pronounced in early life-history stages (Roach & Wulff, 1987), this supports the altitudinal cline as one of genetic origin, and not the result of maternal effects.

The absence of maternal environment effects on offspring phenotype is congruent with most interpretations from studies on invasive species differentiation within their non-native ranges. Environmental maternal influence appears to be negligible compared with the evolutionary differentiation along an environmental gradient. Previous studies have addressed the importance of EME on phenotypic differentiation in native plant species across environmental gradients. Olsson & Agren (2002) reported that EME were barely detectable compared with genetic differentiation over a latitudinal cline in *Lythrum salicaria* across Sweden. Other studies on EME in native plants generally concluded that EME were discernable, especially on seed and seedling characters, but had a relatively weak influence on offspring phenotypes compared with other factors influencing offspring fitness (Weiner *et al.*, 1997). The magnitude of EME appears to be so low that they play an insignificant role on species differentiation in response to climate.

Evolutionary interpretation of the clinal variation

Variation in life-history traits among introduced populations of *S. inaequidens* closely matched the theoretical expectations for selection on growth traits across an altitudinal and climatic gradient. Small plant size is expected to reflect adaptation to harsher conditions and shorter growing seasons, and is a common and expected feature of native species in mountain ecosystems (e.g. Galen *et al.*, 1991; Blanckenhorn, 1997; Körner, 2003). However, the clinal variation observed in the present study can also originate from demographic processes induced by colonization constraints. If repeated founder effects occurred during range expansion, genetic drift could be responsible for the genotypic differentiation of *S. inaequidens*. Reciprocal transplants is the next step to test the adaptive significance of the observed cline (Lacey, 1988; Rice & Mack, 1991). Furthermore, genetic structure analyses within the invaded range of *S. inaequidens* could be used to further elucidate the relative importance of genetic drift and adaptation.

Conclusions

A common garden experiment was used to study the influence of EME on a clinal variation in plant traits, among invasive populations of *S. inaequidens* selected along an altitudinal gradient in southern France. Plants were grown from two maternal seed environments, represented by (i) *in situ* collected seeds and (ii) seeds produced under controlled conditions. Seed mass did not differ according to the maternal environment, but the pattern of variation among population in the field was different from that under controlled conditions, indicating a plastic response of seed mass to environmental conditions. However, seed mass did not influence phenotypic variation of the offspring. As no difference were found between plants grown from the two maternal environments, this study brought to light that EMEs played a negligible role on the observed cline.

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Article 5

Environmental maternal effects influence *Senecio inaequidens* (Asteraceae) phenotypes under harsh climatic conditions.

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Abstract. In a previous study, we tested for the contribution of environmental maternal effects (EME) on an altitudinal cline in the invasive plant *Senecio inaequidens*, by performing a common garden experiment in central Belgium. Here, we used reciprocal transplants to show that the expression of EME depends on the climatic conditions under which the experiment is set out. Under mild spring conditions in the Mediterranean, narrow germination window prevented EME to influence life history. Whereas in Pyrenean mountains, seed mass affected germination timing, which in turn influenced fitness-related traits. Other sources of phenotypic variation, considered in the experiment, are discussed.

Keywords. *Harsh climate, reciprocal transplants, time to germination, seed mass.*

Résumé. Dans une étude récente, nous testions si les effets maternels environnementaux (EME) contribuaient à une variation clinale observée le long d'un gradient d'altitude chez l'espèce invasive *Senecio inaequidens*. Nous utilisions pour cela un jardin commun, installé en Belgique. Dans la présente étude, nous avons utilisé des transplantations réciproques pour montrer que l'influence des EME dépend des conditions climatiques dans lesquelles l'expérience se déroule. Dans les conditions climatiques douces de la Méditerranée, au printemps, la germination fut groupée. Ceci empêcha l'expression des EME. Par contre, en conditions plus rudes, dans les Pyrénées, la masse des graines affecta le délai de germination, qui influença les traits d'histoire de vie liés à la valeur adaptative (*fitness*) des individus. D'autres sources de variations ont été considérées, qui sont également discutées.

Mots-clés. *Climat rigoureux, transplantations réciproques, délai de germination, mass des graines.*

Introduction

The sources of phenotypic variation in alien plant within their non-native range has received increasing attention (Callaway et al., 2006). Notably, an increasing body of research has focussed on evolutionary processes related to plant invasions with respect to latitude (Kollmann et al., 2004 ; Maron et al., 2004 ; Montague et al., 2007 ; Weber et al., 1998) or altitude (Monty et al., 2009). Those studies used common garden experiments to assess the level of genetic differentiation among populations. However in such experiments, the way environmental maternal effects (EME) can influence phenotypic variation because seed sources are collected from field populations, is still unclear. To partly reduce EME and for practical facility, most studies used seedlings instead of seeds in the common gardens: seedling were previously obtained under controlled conditions, then transplanted altogether at the beginning of the experiment. As a result, the role of EME in phenotypic variation among invasive plants is even more poorly understood, contrary to the role of genetic differentiation. In a previous common garden study (Monty et al., submitted), we explicitly tested for the contribution of EME on an altitudinal cline in the invasive *Senecio inaequidens* DC. (Asteraceae). We found no evidence for EME, but this may be attributed to a very narrow germination window under favourable spring climatic conditions, during the year of study in central Belgium (Galloway, 2001). In the present study, we used a reciprocal transplants experiment with two climatically contrasted gardens and nine populations to test the hypothesis that environmental maternal effects were only expressed under harsh conditions, i.e. mountainous climate, within the invasion range of *S. inaequidens* in southern France. Specifically, we asked the following questions: 1) under mild climatic conditions, does a narrow germination window prevent EME to influence fitness-related traits? 2) Under harsher climatic conditions, does seed mass variation lead to variable time to germination? And at the end of the growing season, do differences in time to germination lead to differences in fitness-related traits? Besides EME, we discussed the other sources of phenotypic variation found among populations.

Method

Study species and invasion history

S. inaequidens is an herbaceous perennial native to South Africa and Lesotho (Ernst 1998). The species was accidentally introduced to Europe in the late 19th - early 20th century, where only tetraploids are reported. In its native range, the species occurs as two co-existing cytotypes (Lafuma et al., 2003). The first records of the species are considered introduction events tied to the wool industry (Ernst, 1998 ; Lousley, 1961). In southern France, Guillerm et al. (1990) documented the progressive colonization of the species. For decades after the first specimens were recorded in 1936, *S. inaequidens* was only reported in the vicinity of the wool-processing centre of Mazamet. After 1950 it steadily expanded throughout areas geographically removed from existing wool industrial centres. In the early 1980s, the species reached the Mediterranean coast from Mazamet. Today, *S. inaequidens* is found at altitudes up to 1700 m in the Pyrenean mountains (Cottrel et al., 1998). It is still absent from several areas in central France, indicating that invasion fronts from Mazamet and other introduction sites have not yet converged (Monty, unpublished).

All populations in this study are therefore assumed to originate in Mazamet. *S. inaequidens* extends to 1 m tall, with numerous stems bearing yellow capitula. Individuals can produce up to 1500 flower heads over a flowering period, each capitulum bearing roughly one hundred achenes (Lopez-Garcia et al., 2005). In France, flowering occurs from April to December. Achenes are mainly dispersed by wind (Monty et al., 2008). Massive germination occurs all year long, with the highest rates being spring and autumn. *Ex situ* germination tests indicated that germination was null under 2°C (Lopez-Garcia et al., 2005).

Seed populations

We collected seeds in three distinct altitudinal zones (henceforth referred to as *zones*): the primary introduction area in the ancient wool-processing zone of Mazamet, the Mediterranean plain, and the Pyrenean mountains. Three populations were selected at similar altitude in each zone (Table 1). 15 plants were haphazardly selected per population, on which one ripe capitulum was collected and stored in a paper bag at room temperature.

Table 1. Characteristics of the three seed populations of *Senecio inaequidens* sampled per zone.

Altitudinal zone	Population	Location	Altitude (m)	Latitude N	Longitude E
Mediterranean plain	A	Narbonne	5	43°11'	3°02'
	B	Narbonne-Plage	0	43°10'	3°10'
	C	Narbonne	9	43°09'	2°59'
Mazamet (primary introduction wool centre)	A	Castaunouze	370	43°29'	2°23'
	B	Mazamet	330	43°29'	2°22'
	C	Hautpoul	425	43°28'	2°24'
Pyrenean mountains	A	La Llagone	1695	42°32'	2°08'
	B	Egat	1635	42°30'	2°01'
	C	Enveitg	1416	42°28'	1°54'

Experimental design and study sites

Two identical random block common gardens were established in contrasting climatic areas for this study. One, in Montpellier, was representative of the Mediterranean zone (*Mediterranean garden*; alt.: 55 m; 43°38’ N; 3°52’ E). The other was located in La Cabanasse, in the Pyrenean zone (*Pyrenean garden*; alt.: 1509 m; 42°30’ N; 2°07’ E). Figure 1 is representative of the climatic conditions in the two gardens. On each collected capitulum, we haphazardly selected two lots of 10 achenes without obvious anomaly (henceforth referred to as *seeds*) that we weighted to the nearest 0.1 mg. Each of the two lots was randomly assigned to a garden, so that to each plant at a particular place in one garden corresponds a maternal sib (or full sib) at the same place in the other garden. Each garden was composed of 15 blocks and surrounded by an additional row of pots to prevent edge effect. Each block included nine pots, representative of the nine populations sampled. The pots were placed 80 cm apart in all directions and contained 2.5 l of sand, 2.5 l of compost and 0.5 l of hydro-granulates. On March 20th 2008 (Mediterranean garden) and

March 21st 2008 (Pyrenean garden), the 10 seeds per lots were collectively sown in the pots. Pots were then covered with a protective light-permeable canvas until germination was complete, to prevent seed predation and transportation by wind. Pots were lightly watered weekly during the first month of experiment. Seedlings were counted every 2-3 d. The first emerged seedling in each pot was marked with a short rod for measurement. Other seedlings were removed.

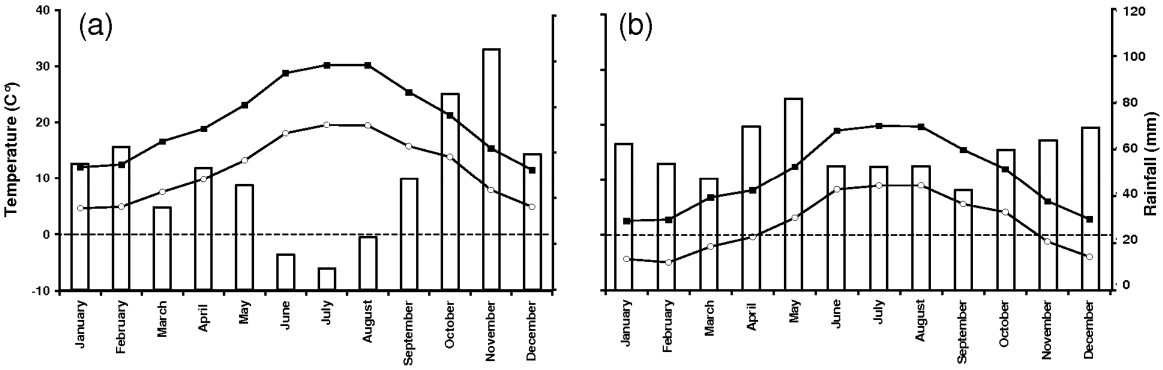

Figure 1. Monthly mean maximum temperatures (°C, black squares), monthly mean minimum temperatures (C°, white circles) and monthly rainfalls (mm, white bars) in the two common gardens. (a) Mediterranean garden; (b) Pyrenean garden. Dotted lines correspond to 0°C. Data used for this graphics are average values for the years 2001 to 2005, obtained from climatic stations in the vicinity of the gardens. See Monty and Mahy (2009) for details.

Measurements

We will refer to the cumulated mass of the 10 seeds sown in each pot as *seed mass*. The *time to germination* is the number of days between sowing and the emergence of the first seedling in the pot. Every month, from mid-June to late November, every ripe capitulum was counted and clipped on each plant. At the last counting, buds and flowering heads were counted in additions to ripe capitulae. The sum of counted heads, the *flower head production*, was used as a proxy of fitness. When a plant died during the experiment, flower head production was still considered over the lifespan of the plant. On mid-October 2008, maximum height (h) and two perpendicular diameters (d1 and d2) were measured on each (surviving) plant. *Plant volume* (V) was calculated as

$$V=\pi*\frac{h}{3}*\left(\frac{d1+d2}{4}\right)^2$$

Data analysis

Seed mass data were analysed using ANOVA with zone (fixed) and population (random, nested within zone) as factors. In the case of a significant zone effect, pairwise comparisons of means were made using Tukey Simultaneous Tests.

We then analysed phenotypic traits from the reciprocal transplants experiment using ANCOVA with seed mass as the covariate. For plant volume and flower head production, time to germination was also included as a covariate. Zone was a fixed factor, and population within zone was random. Garden was fixed, with two modalities (Pyrenean and Mediterranean), and crossed to both zone and population. The factor block, nested within garden, was random. Interactions with the factor block were not calculated. However, we calculated the interactions between the covariates and the factor garden. A significant effect of these latter interactions would indicate a different pattern of influence of the seed mass and the germination timing on phenotypic traits in the two gardens. We then performed a similar ANCOVA, separately for each garden. The correlation matrix between all traits was calculated, separately for each garden.

Results and discussion

Plants from the Pyrenean produced heavier seeds

The seed mass analysis revealed a significant effect of both the factor zone (df = 2; F = 9.26; P = 0.015) and the factor population (df = 6; F = 4.15; P =0.001). Tukey Simultaneous pairwise comparisons between zones showed that seeds collected in the Pyrenean were significantly heavier than those collected in Mazamet (T = 8.03; $P < 0.001$) or the Mediterranean zone (T = 7.08; $P < 0.001$), but the two latter were not significantly different (T = -0.952; P = 0.608). These results are consistent with previous studies (Monty et al., 2009, Monty et al., submitted, Monty and Mahy, submitted). In particular, Monty and Mahy (submitted) suggested that a plastic response, not a genetic differentiation, lead to heavier seeds in mountainous populations.

Seed mass influence differed between gardens

Spring climatic conditions influenced germination, as revealed by the garden effect on time to germination (df = 1; F = 97.83; $P < 0.001$). However, the influence of seed mass on time to germination depended on the garden, as revealed by the significant garden x seed mass interactions (df = 1; F = 10.73; $P < 0.001$). In fact, there was no variation in time to germination in the Mediterranean garden. The interaction garden x time to germination could therefore not be calculated. In contrast, germination window reached 60 days in the mountain garden (Figure 2), where spring climate is much harsher (Figure 1). There were no correlation between seed mass and time to germination in Montpellier (as there was no variation), but a strong negative correlation in the Pyrenean (r = -0.269; P = 0.002) was revealed. Figure 2 shows the plot of seed mass against time to germination for both gardens. The garden x seed mass interaction was also significant for plant volume (df = 1; F = 3.94; P = 0.049). Plant volume and flower head production significantly differed between gardens (respectively df = 1; F = 7.57; P = 0.006 and df = 1; F = 9.72; P = 0.002). Further analysis was made separately for the two gardens.

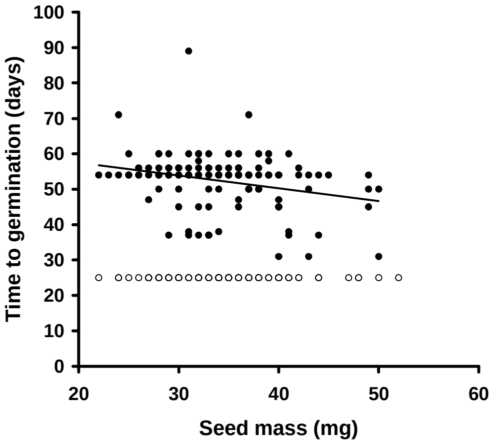

Figure 2. Time to germination against seed mass in the two common gardens. Black circles: Pyrenean garden; white circles: Mediterranean garden. The plain line is the linear regression representative of the significant correlation between the two traits in the Pyrenean garden.

EME influenced fitness-related traits in the Pyrenean, not in the Mediterranean

In the Pyrenean garden, time to germination depended on seed mass and significantly influenced fitness-related traits (Table 2, Figure 3). Heavier, earlier-germinating seeds, produce larger individuals that eventually produced more flower heads over the growing season. Time to germination was significantly correlated to plant volume (r = -0.539; P < 0.001) and flower head production (r = -0.541; P < 0.001), as shown in Figure 3. EME therefore appeared to play a significant role on the fitness of *S. inaequidens* in Pyrenean mountains, mainly through the timing of germination. Beyond the influence of time to germination, heavier seeds still produced larger plants (as both covariates were significant, Table 2), but seed mass itself had no significant influence on flower head production. EME influenced fitness related traits in the same way for the different populations and zones (no significant effect of zone/population on time to germination, Table 2). In the mountain garden, however, EME were not the only source of phenotypic variation in plant volume. A significant effect of the factor zone and a Tukey Simultaneous pairwise comparison indicated a genetic difference between mountain plants on one hand and Mazamet and Mediterranean plants on the other hand (Pyrenean – Mediterranean zone: T = -3.678; P = 0.0011; Pyrenean – Mazamet zone: T = -2.571; P = 0.031; Mazamet – Mediterranean zone: T = -1.111; P = 0.510)

In Montpellier, much milder germination conditions led to a extremely narrow germination window. Therefore, no analysis of time to germination could be conducted. There were no relation between seed mass and time to germination, and EME played a negligible role on the observed phenotypic variation. In contrast, significant zone and population effects confirmed a genetic differentiation between altitudinal zones and between populations within zones, as previously shown (Monty et al., 2009). Our results are in line with previous studies on insects that already pointed that EME were more evident in more adverse environmental conditions (Fox et al., 1996).

Table 2. Results of ANCOVA depicting the effects of seed mass (covariate), time to germination (covariate), zone (fixed), population (random, nested within zone) and block (random) on measured traits, in both common gardens. Significant P-values are in bold. As there was no variation in time to germination, this trait could not be analysed or used as a covariate in the Mediterranean garden.

	Time to germination			Plant volume			Flower head production		
Source of variation	*df*	*F*	*P-value*	*df*	*F*	*P-value*	*df*	*F*	*P-value*
Mediterranean garden									
Seed mass	-	-	-	1	0.49	0.543	1	0.45	0.575
Time to germination	-	-	-	-	-	-	-	-	-
Zone	-	-	-	2	9.51	**0.011**	2	0.21	0.812
Population (zone)	-	-	-	6	2.18	**0.050**	6	2.53	**0.025**
Block	-	-	-	14	0.83	0.637	14	0.92	0.536
Error	-			105			110		
Pyrenean garden									
Seed mass	1	12.41	**0.001**	1	4.9	**0.029**	1	2.02	0.158
Time to germination	-	-	-	1	54.6	**<0.001**	1	56.3	**<0.001**
Zone	2	0.21	0.817	2	11.78	**0.004**	2	1.49	0.286
Population (zone)	6	1.35	0.242	6	0.51	0.802	6	1.16	0.332
Block	14	1.56	0.104	14	2.54	**0.004**	14	2.49	**0.004**
Error	129			121			105		

Different sources of phenotypic variation interacted

Phenotypic variation in *S. inaequidens* over its invasion range in southern France appeared to originate in many factors. Phenotypic plasticity in response to climate was important, as revealed by the garden effect on all traits. Besides, genetic differentiation between zones (and populations) was observed in both gardens. It was more evident in the Montpellier garden as in this experiment, EME were negligible. Indeed, when there is (almost) no variation in germination time, genetic trends are much more expressed in a common garden. This is the case in most common garden studies that first produced synchronized seedlings in controlled conditions (glasshouse, incubator) prior to open air common gardens (e.g. Kollmann et al., 2004 ; Maron et al., 2004). Under harsher conditions in La Cabanasse, both genetics and EME influenced phenotypic variation, and the influence of time to germination was so strong on phenotypic traits that the genetic differentiation was rather blurred. Laura F. Galloway (Galloway, 1995), in a study on *Mimulus guttatus*, also found that environmental maternal effects masked genetic differentiation in life-history characters. This emphasizes the importance to perform common garden experiments from seeds, not seedlings, if the interest is to study the actual causes of phenotypic variation in various environments.

The influence of seed mass in mountainous conditions, mainly through germination timing, has to be related to the plastic response in that trait observed in previous studies (Monty and Mahy, submitted). If plants growing in harsher environments produce heavier diaspores, due to a plastic response, increasing offspring's fitness, then it is a case of transgenerational adaptive plasticity (Galloway, 1995 ; Galloway, 2005 ; Mousseau et al., 1998 ; Roach et al., 1987).

Understanding the causes of genetic differentiation was not the very focus of this paper. However, it was interesting to compare the performance of plants from recently invaded zones to that of Mazamet, when grown in the garden representative of their “local” climate. This improved the understanding of the importance of genetic drift and adaptation. In the Mediterranean garden, plants from Mediterranean populations performed better (plant volume, flower head production) than those from the initial introduction zone of Mazamet (even if the higher flower head production of the Mediterranean zone was not significant, Tukey Simultaneous pairwise comparisons revealed that one Mediterranean population produced significantly more flowers than all three populations from Mazamet). Local adaptation thus seemed to play a role in the genetic differentiation observed. In the Pyrenean, however, the pattern was different. As compared to plants from Mazamet, Pyrenean plants showed comparable flower head production and lower plant volume. These results were not in line with a local adaptation pattern (Kawecki et al., 2004), and merely suggested that genetic drift occurred during progressive invasion in mountains. Nevertheless, our understanding of the importance of local adaptation is limited by our assumption that the flower head production over the first growing season is an accurate proxy of fitness. A different pattern of variation in fitness may indeed be found if flower head production was monitored over several years, e.g. because of differential winter mortality. Also, measuring the total seed mass production could bring further understanding of the genetic and plastic sources of variation.

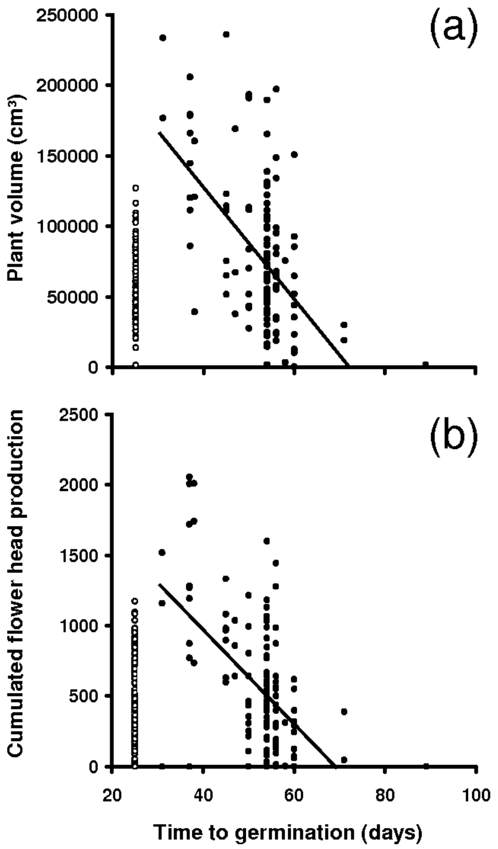

Figure 3. Time to germination against plant volume (a) and flower head production (b) in the two common gardens. Black circles: Pyrenean garden; white circles: Mediterranean garden. The plain lines are the linear regressions representative of the significant correlations between the traits in the Pyrenean garden

Conclusion

This study brought to light that different sources of phenotypic variation were related to mountain and plain invasion for *S. inaequidens* in southern France. In mountains, adverse spring climatic conditions led to a great variation in time to germination, which led to differences in fitness-related traits. EME were therefore important in such conditions, and even masked the genetic trends that exist among altitudinal zones and among populations. In contrast, the very narrow germination window in milder Mediterranean conditions allowed the expression of genetic differences in the absence of environmental maternal effects. Local adaptation thus seems to help invasion in plain, whereas seed mass appears to be an important feature for the differential success of individuals in mountain invasion. Our results emphasized the importance of using seeds directly sown in common gardens to identify the causes of phenotypic variation under natural conditions. Further research is ongoing to better understand the causes of genetic differentiation in the species.

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Article 6

Seed rain pattern of the invasive weed *Senecio inaequidens* (Asteraceae)

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Abstract. Dispersion capacity of alien invasive plants is a key feature for understanding invasion processes and risks. Here, we present an experimental study focussing on the seed rain pattern of *Senecio inaequidens*, an African plant widespread throughout Europe, under common favourable dispersal conditions. One hundred achenes from two Belgian populations underwent a drop time in still air experiment in order to assess linear correlation between several morphological traits and terminal velocity. Variation in morphological traits was measured for 250 additional achenes per population. The trait best correlated to terminal velocity was the square root of the plume loading. We then used this trait to model terminal velocity using a linear regression (r^3 of 79.7%). With this regression and imposing ecological parameters such as wind speed, turbulences and height of release to be representative of favourable dispersal conditions, we established a Gaussian tilted plume model to predict the seed rain pattern of the species. A wind tunnel experiment consequently permitted to validate the model. Under a wind speed of 5 m s^{-1} with 2% turbulences along the vertical axis, and height of release of 0.40 m, 99.8 % of achenes were dispersed within 100 meters, with a maximum deposition rate at 5.2 m from the parent plant. Uplifted achenes, not forecasted by the model, represent 6.25% of all achenes.

Keywords. Senecio inaequidens, *Gaussian model, invasive plant, plume loading, seed rain pattern.*

Résumé. Les capacités de dispersion des espèces exotiques invasives sont des caractères-clés dans la compréhension et l'anticipation des processus d'invasion. Nous présentons une étude expérimentale de la pluie de graines de *Senecio inaequidens*, une espèce d'origine africaine répandue en Europe, en conditions normales de dispersion. Cent akènes, provenant de deux populations belges, ont été utilisés dans des mesures de vélocités terminales en tube. Des mesures morphologiques supplémentaires ont été réalisées sur 250 akènes par population. Les traits morphologiques des akènes ont été corrélés avec la vélocité terminale afin d'identifier le trait le mieux corrélé : la racine carrée du *plume loading*. Nous avons utilisé ce trait pour modéliser, par régression linéaire (r^3 de 79.7%), la vélocité terminale. Avec cette régression, en imposant des paramètres environnementaux comme la vitesse du vent, les turbulences et la hauteur de lâcher, nous avons établi un modèle Gaussien pour prédire la pluie de graines (akènes). Celui-ci fut ensuite validé en tunnel à vent. Selon le modèle, avec un vent de 5 m s^{-1} et 2% de turbulences selon l'axe vertical, si la hauteur de lâcher est de 40 cm, 99.8% des akènes tombent dans les 100 premiers mètres, avec un maximum à 5.2 m de la source. Les akènes subissant des courants ascendants (*uplift*), non-modélisés, représentent 6.25% des akènes.

Mots-clés. Senecio inaequidens, *modèle Gaussien, plante invasive, plume loading, pluie de graine.*

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SEED RAIN PATTERN OF THE INVASIVE WEED *SENECIO INAEQUIDENS* (ASTERACEAE)

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ABSTRACT. — Dispersion capacity of alien invasive plants is a key feature for understanding invasion processes and risks. Here, we present an experimental study focussing on the seed rain pattern of *Senecio inaequidens*, an African plant widespread throughout Europe, under common favourable dispersal conditions. One hundred achenes from two Belgian populations underwent a drop time in still air experiment in order to assess linear correlation between several morphological traits and terminal velocity. Variation in morphological traits was measured for 250 additional achenes per population. The trait best correlated to terminal velocity was the square root of the plume loading. We then used this trait to model terminal velocity using a linear regression (r^2 of 79.7%). With this regression and imposing ecological parameters such as wind speed, turbulences and height of release to be representative of favourable dispersal conditions, we established a Gaussian tilted plume model to predict the seed rain pattern of the species. A wind tunnel experiment consequently permitted to validate the model. Under a wind speed of 5 m s^{-1} with 2% turbulences along the vertical axis, and height of release of 0.40 m, 99.8 % of achenes were dispersed within 100 meters, with a maximum deposition rate at 5.2 m from the parent plant. Uplifted achenes, not forecasted by the model, represent 6.25% of all achenes.

KEY WORDS. — *Senecio inaequidens*, Gaussian model, invasive plant, plume loading, seed rain pattern.

INTRODUCTION

Biological invasions and seed dispersal are two major topics in plant ecology. As they are considered as the second cause of biodiversity loss worldwide (CRONK & FULLER 1995, PARKER *et al.* 1999), biological invasions have attracted much attention in the last decade (PYSEK *et al.* 2004). Seed dispersal can be regarded as a key process in the survival and distribution of plant species. Hence, dispersal analysis is important to understand the success of invasive species. Studies on alien species dispersal abilities are of crucial interest to understand plant invasion patterns and dynamics in the field (HARPER 1977, DAUER *et al.* 2006). Although much has been written on the dispersal capacities of invaders, the number of detailed studies of dispersal ability and realized dispersal patterns is surprisingly low (Higgins *et al.* 2001).

The spatial distribution of seeds around their source is called a 'seed shadow' or 'seed rain pattern', a density curve of landing probabilities (Willson & Traveset 1993, Jongejans & Schippers 1999). Models used to predict seed shadows range from simple phenomenological to complex mechanistic models. Phenomenological models are based on fit to observed data on seed dispersal. Those models generally describe the seed number-distance relationships as leptokurtic (with a higher peak and longer tail than a normal distribution). From the peak outwards, seed densities generally fit a negative exponential curve or sometimes a negative power function (Okubo & Levin 1989, Willson & Traveset 1993). Although these empirical models are simple and fit some datasets reasonably well, they are only based upon parameters that are not measured or controlled independently, such as wind speed, release height or terminal velocity. Therefore, these models do not allow extrapolation from one situation to another nor do they provide any understanding of the underlying mechanisms. Detailed mechanistic models were developed to predict dispersal of anemochorous plants (Okubo & Levin 1989, Greene & Calogeropoulos 2002, Nathan *et al.* 2002, Tackenberg 2003, Soons *et al.* 2004). Unlike phenomenological models, these mechanistic models describe the probability distributions of seeds and should be applicable over infinite distances and under any circumstances (Bullock & Clarke 2000). Some of these models have the advantage to take into account the seed uplift, which is supposed to be responsible for very long distance dispersal. Unfortunately, they have to be very accurate to give satisfactory results. Another shortcoming is that validating patterns with real data and obtaining basic input data is complicated (Nathan *et al.* 2002, Tackenberg 2003, Soons *et al.* 2004).

Between phenomenological and mechanistic approaches, Gaussian models represent simple and yet poorly explored seed shadow models. These models generally compute the horizontal and vertical distribution of wind-borne particles under the influence of diffusion and advection. For particles with non-negligible weight, such as most plant diaspores, the Gaussian tilted plume model is used, which takes gravitational settling into account (e.g., Csanady 1963). The most important species-specific characteristic used in wind dispersal models is the terminal velocity (Vt), i.e., seed fall velocity in still air. It is relatively easy to measure and has been used as an index of relative dispersal ability among species (Sheldon & Burrows 1973, Matlack 1987, Schulz *et al.* 1991, Andersen 1992, 1993, Kahrs 1994, Askew *et al.* 1997, Tackenberg *et al.* 2003). Dispersal capability of wind-dispersed propagules, including terminal velocity, can often be successfully predicted from their morphological characteristics (Sheldon & Burrows 1973, Morse & Schmitt 1985, Matlack 1987, Cody & Overton 1996, Soons & Heil 2002). Besides the selection of a pertinent, and yet pragmatic model of seed dispersal, the problem of model validation is of crucial importance. Wind tunnels provide repeatable controlled experimental conditions to generate seed shadows and validate seed dispersal models.

Senecio inaequidens DC. is a perennial herbaceous Asteraceae. It was originally distributed in Lesotho and on the South African west coast, on river edges and in stony meadows (Ernst 1998). It was accidentally introduced in Europe in the early 20th century via the wool trade. During several decades it was only found in the vicinity of a few wool-processing areas (Guillerm *et al.* 1990, Ernst 1998). From 1950 onwards, *S. inaequidens* expanded throughout western, central and southern Europe, and to environments outside the wool industry. By the 1970s, the species was considered as a weed in agricultural areas in France, especially in vineyards (Guillerm *et al.* 1990), as well as in road ditches and in grazing meadows where it is unpalatable to livestock. By the 1990s, it was also recorded in natural habitats (Michez 1995). *S. inaequidens* has a highly effective production of capitula over a long flowering period (i.e., six months). Tubula and ligula become wind-dispersed achenes, provided with an unbeaked pappus (Fig. 1). Some individuals can produce up to 1500 capitula during a flowering period (Monty, unpublished data). Each capitulum bears roughly one hundred achenes (LOPEZ-GARCIA & MAILLET 2005).

With this study we aimed at evaluating the dispersal capacity of the achenes of the invasive *S. inaequidens*, on a hundred-metre scale*,* using a Gaussian modelling approach. We then tested the model with wind tunnel data. Also, our aim was to test the adequacy of this approach under common favourable dispersal conditions in order to provide the basis for future studies on the factors affecting dispersal of this species.

MATERIAL AND METHODS

ANALYSIS OF MORPHOLOGICAL TRAITS AND DETERMINATION OF A PROXY FOR TERMINAL VELOCITY

To run a Gaussian model, terminal velocity of the particle is the only characteristic needed. However, our approach included a validation test of the selected model in a wind tunnel. In this validation experiment, achenes were captured on a sticking paper and were no longer available for direct terminal velocity measurements. We thus needed to find a proxy for terminal velocity based on morphological traits.

S. inaequidens propagules were collected in December 2004 in two populations located in waste lands in Belgium: Seraing (50°36'9.1"N; 05°29'46.0"E; alt.120 m; population area 350 m^2) and Antwerpen (51°14'37.4"N; 04°23'12.3"E; alt. 2 m; population area 700 m^2). In both populations, five capitula were randomly collected on five randomly selected individuals. Capitula were stored at ambient temperature in pierced boxes insuring uniform spread of plume fibres. Terminal settling velocity was measured on two achenes per capitulum: one external (at the periphery of the receptacle) and one internal (near the centre of the receptacle) (50 achenes per population). The achene-pappus unit was dropped down a transparent vertical Plexiglas tube (diameter 139 mm; height 2 m). The Plexiglas tube was connected to the ground with a copper wire to discard static electricity interferences. The descent was timed by an observer with an electronic stopwatch (precision: 0.01 s) along one meter, where terminal velocity was assumed to be reached (SHELDON & BURROWS 1973).Three replicate measurements were made per achene. From the same capitula, five external and five internal additional achenes were randomly picked and measured (250 achenes per population). The following morphological traits were measured on all achenes: (1) total propagule mass (achene-pappus unit, in mg; precision: 0.1mg); (2) achene mass (without pappus, in mg); (3) pappus mass (mg) calculated as the difference (1)-(2); (4) pappus length (mm; precision: 0.1 mm); (5) pappus radius (radius of the wider circle made by the pappus, in mm); (6) achene length (without pappus, in mm); (7) maximal and (8) minimal achene diameters (without pappus, in mm) Fig. 1). The following trait combinations were calculated: (9) achene flattening [(8)/(7)]; (10) pappus mass-radius ratio [(3)/(5)]; (11) achene-pappus mass ratio [(1)/(3)]; (12) plume loading. The latter was defined as the ratio of the total propagule mass (1) to the area of the horizontal projection of the wider circle made by the pappus (Fig. 1) (AUGSPURGER & FRANSON 1987, MATLACK 1987). It was preferred to the square root of the seed mass divided by the horizontal projection of the wider circle made by the pappus, which is sometimes used instead (SOONS & HEIL 2002), to allow comparison with a more abundant literature. Both trait combinations were highly correlated anyway ($r = 0.949$).

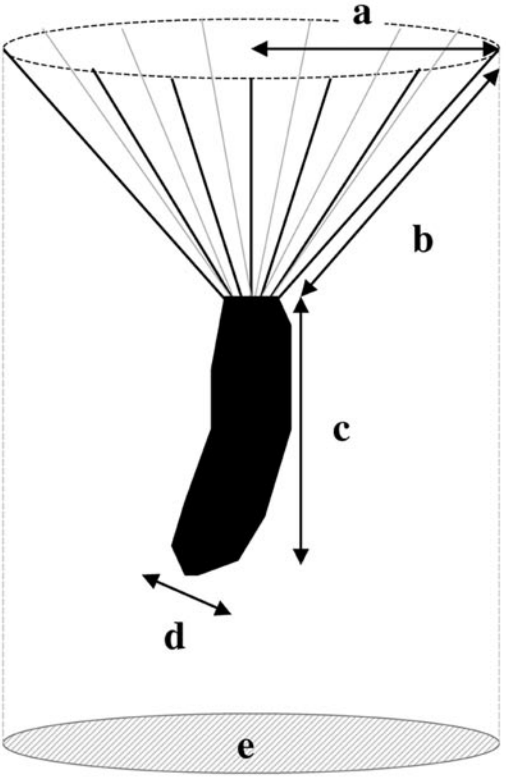

Fig. 1. *S. inaequidens* propagule with (a) pappus radius; (b) pappus length; (c) achene length; (d) one achene diameter and (e) area of the horizontal projection of the wider circle made by the pappus, used to calculate the plume loading.

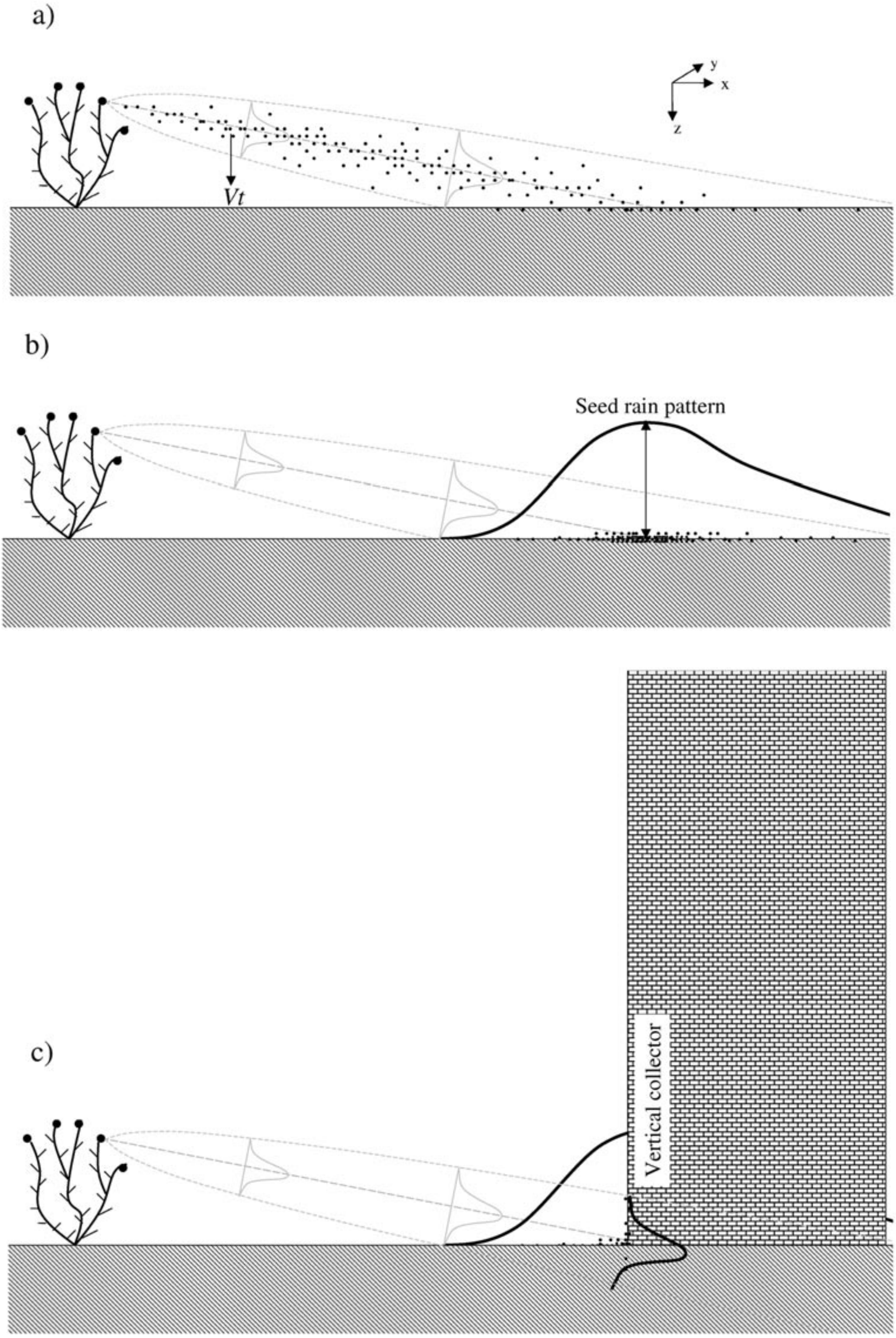

Fig. 2. Schematic representation of (a) tilting plume model dispersion; (b) seed rain pattern and (c) seed rain pattern intercepted horizontally and vertically in the wind tunnel.

Population, individual, capitulum and position (internal/external) differences for those morphological traits were analysed using nested ANOVA on the 500 achenes dataset with position nested within capitulum, capitulum nested within individual and individual nested within population, all of them being random factors. We calculated the linear correlation (Pearson's *r*) coefficient between morphological traits on the one hand, either directly measured or mathematically transformed (square, square root, logarithm, exponential) and terminal velocity on the other hand, to find the best morphological proxy trait of terminal velocity. In addition, linear regression (with stepwise selection) using least-square estimation was used to model terminal velocity by morphological proxy. All statistical analyses were executed using MINITAB ver. 13.20 (Minitab Inc., State College, Pennsylvania).

Modelling seed rain pattern

Model construction

A Gaussian tilted plume model was selected with the aim to predict the seed rain pattern of *S. inaequidens* based on terminal velocity estimations. Gaussian models (Pasquill 1962, Hanna *et al.* 1982) are commonly used to study very light particle dispersal, such as atmospheric pollutants or aircraft spray drift (e.g., Stainier *et al.* 2006). However, they have also been applied to spore dispersal (Gregory *et al.* 1961, Fitt & McCartney 1986). With some adaptations they can be used for heavier particle dispersal (Okubo & Levin 1989) where the wind direction is considered as the centre line of the propagule flow. On the perpendicular direction to the wind, the distribution has a Gaussian shape with an increase of amplitude while moving downwind from the source. This is the Gaussian tilted plume model (Fig. 2a), which extends the Gaussian models to cases where particles have a non-negligible settling velocity. A major shortcoming of these models is that they cannot explicitly simulate particle uplift.

In computing the deposition rate for those particles, this model ignores bouncing or resuspension of particles hitting the ground and assumes that deposition due to diffusion is negligible compared to sedimentation. So, the deposition rate on the ground is given by:

$$D = S(x, y, 0)Vt \qquad \text{(eq. 1)},$$

where *Vt* is the terminal deposition velocity. The expression for the concentration of particles at the ground level is (Okubo & Levin 1989):

$$D = Q(x,y) = \frac{nVt}{2\pi u \sigma_y \sigma_z} \exp\left\{ \frac{y^2}{2\sigma_y^2} - \frac{\left(H - Vt\frac{x}{u}\right)^2}{2\sigma_z^2} \right\} \qquad \text{(eq. 2)},$$

where *n* is the number of particles at source, *u* is the mean wind speed along X direction at height of release *H*. Generally, $n = n_x$ (the effective number of particle at distance *x*) and standard deviations σ_y and σ_z depend on *x* (Pasquill 1962), but one can simplify by ignoring the decay in n and assuming that n is constant (Okubo & Levin 1989).

Since our main interest is to study how far *S. inaequidens* achenes move downwind, we suppressed the crosswind distribution (along y axis, which is Gaussian) by integrating in that direction (Okubo & Levin 1989). The result, called the crosswind-integrated deposition (CWID), is given by (eq. 3) and represents the seed rain pattern along the X axis.

$$CWID = Q(x) = \frac{nVt}{u\sigma_z\sqrt{2\pi}} \exp\left\{ \frac{-\left(H - Vt\frac{x}{u}\right)^2}{2\sigma_z^2} \right\} \qquad \text{(eq. 3)}$$

To model the seed rain pattern of *S. inaequidens*, environmental parameters were imposed to mimic favourable and generally encountered dispersal circumstances, with steady winds on a flat open field. Height of release was 0.4 m, similar to the mean capitula height observed in Belgian populations (Pieret, unpublished data). A constant wind speed of 5 m s^{-1} was selected, corresponding to the minimal wind speed needed to detach all the achenes from the receptacle (Pieret & Stainier, unpublished data). It represents a frequently encountered wind in open areas over the flowering season in northwestern Europe. For instance, in the Lonzée (central Belgium) meteorological station, such a wind was reached (at 0.4 m high) 68 days (31.7%) over the 2005 flowering season (June – December). Turbulences along the Z axis were fixed at 2% of wind speed along the X axis. As it appeared that terminal velocity exhibited a high intraspecific variation among achenes, the model was adapted to take into account this variability within achene populations rather than simply considering the mean observed terminal velocity. In order to be consistent with the experimental conditions imposed in the wind-tunnel validation, the terminal velocity of achenes was derived from the morphological proxy trait using the linear regression fitted above. The values used by the model are no direct estimations but the centres of proxy trait classes given in Table 1.

Table 1. Plume loading classes (kg m^{-2}) considered for terminal velocity estimations from morphological measurements.

Class	Minimum	Maximum
1	0	0.005
2	0.005	0.01
3	0.01	0.015
4	0.015	0.02
5	0.02	0.025
6	0.025	0.03
7	0.03	-

The model was set up using MATLAB ver. 7.1 (MATHWORKS Inc., Natick, Massachusetts). It provided a curve of crosswind-integrated deposition along X axis, i.e., the seed rain pattern of the species in the considered environmental conditions.

Model validation

The model was validated in a controlled dispersal experiment in the low-speed wind tunnel of the Mechanics and Construction Department of the Gembloux Agricultural University. The environmental conditions in the tunnel for validation were imposed to be the nearest to those used for model fitting. The tunnel is a closed loop re-circulating wind tunnel designed around a 1200 mm diameter axial blower driven by a 22 kW electric motor with a variable speed controller. To stabilize turbulence intensity, the wind tunnel has a 1 m long honeycomb of 200 mm edge square tubes with a monoplane windbreak grid upstream and a porous textile sheet downstream. The test section of the wind tunnel is 2 m wide, 2 m high, 6 m long and located 1 meter downstream of the fabric. The blower located at the loop opposite to the test section draws air through the tunnel at the desired velocity within the range 0-6 m s^{-1}. The wind speed and the turbulences are controlled by a triaxial supersonic anemometer (precision: 0.05 m s^{-1}).

Three capitula were collected in Gembloux on December 2004 (50°34'27.1"N; 04°41'15.8"E; alt. 140 m, population area 400 m^2) and stored at ambient temperature in pierced boxes. A capitulum was maintained vertically ahead of the blowing engine on a pedestal at 0.40 m above the ground and hidden by a cylindrical plastic bowl. The ground was covered with a 6-metre-long adhesive paper reaching the end of the test section of the tunnel. The bottom of the test section (at the opposite of the blowing engine) was covered with a vertical collector (textile), which allowed achene fixation. During the experiment, turbulences, defined as the ratio of the standard deviation of the wind speed along the axis considered to the average wind speed along the wind direction (X axis), reached 7% (Y axis), 2% (Z axis) and 3% (X axis). The experiments began when the wind reached 5 m s^{-1} and remained constant. The bowl was then lifted up so that the achenes could disperse. After measuring their distance from the capitulum on the adhesive paper, or their distance from the floor on the vertical collector, achenes were collected for the same morphological measurements as described above. Besides morphological measurements, aborted or non-aborted aspect was noted for each achene (binary character). Aborted achenes are typically flat and whitish. Data of the three capitula were pooled for analysis. As there were no replications, no direct adjustment test could be statistically conducted.

With the morphological proxy trait of the achenes, along with the regression equation of *Vt* previously described, we defined a prediction interval around this regression with α = 0.2. By definition this interval, limited by hyperboles presented in equations (4) and (5), contains 80% of the data of the drop time in still air experiment

$$Vt_{\max} = \hat{V}T\,(x_0) + t_{1-\alpha/2} * \sqrt{\sigma^2_{VT.x}} * \left[(n+1)/n + (x_0 - x)^2 / SCE_x\right. \quad \text{(eq. 4)}$$

$$Vt_{\min} = \hat{V}T\,(x_0) + t_{1-\alpha/2} * \sqrt{\sigma^2_{VT.x}} * \left[(n+1)/n + (x_0 - x)^2 / SCE_x\right. \quad \text{(eq. 5)}$$

Using these two equations instead of the terminal velocity regression in the constructed model, we produced two crosswind deposition curves, "model min" and "model max". These curves encompass the seed rain pattern provided by the model in the imposed environmental conditions. If 80% of the real dispersion data in the wind tunnel are situated between equations (4) and (5), henceforth referred to as the 80% interval, the model is considered as valid (Fig. 3).

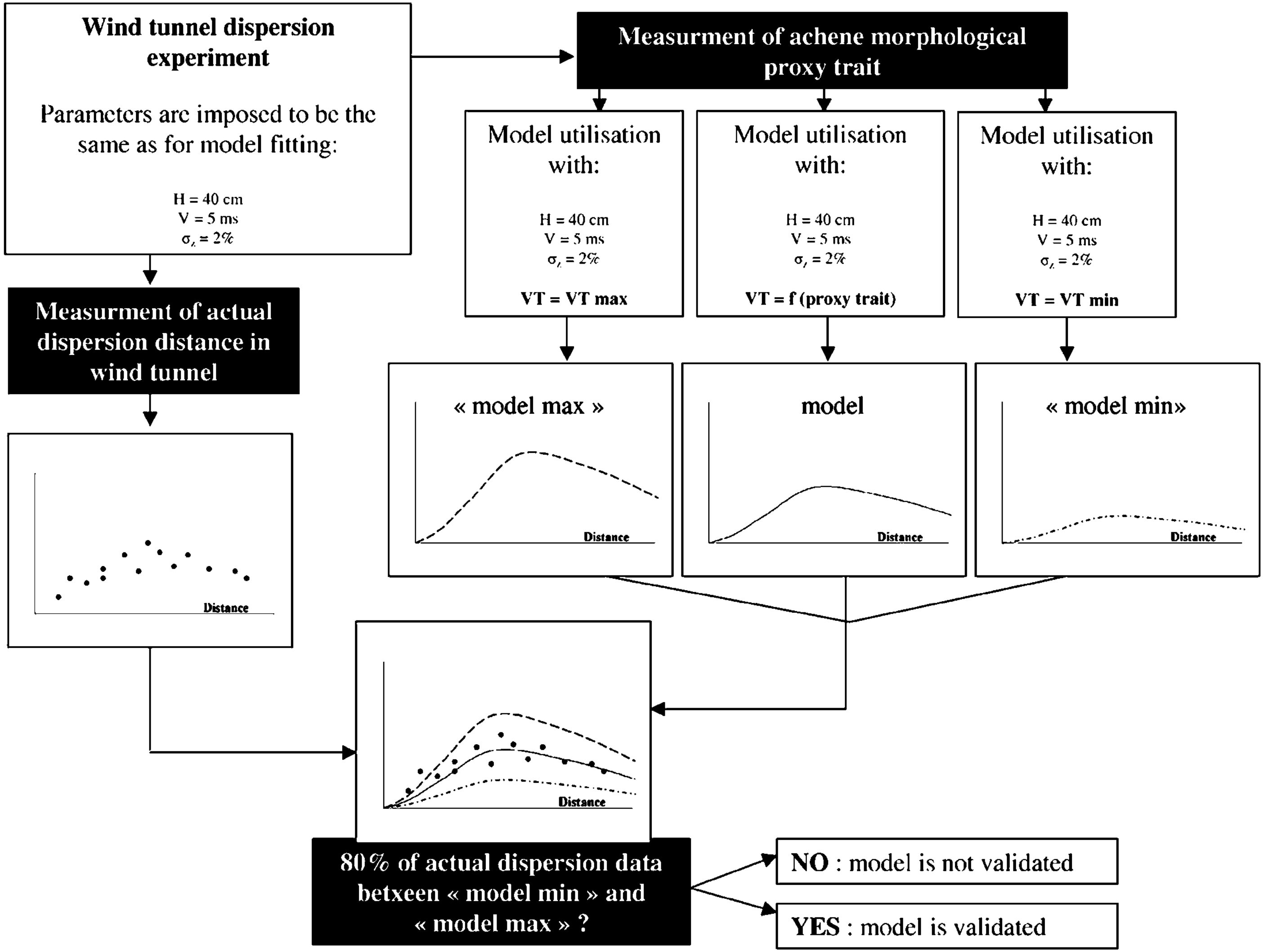

Fig 3. Schematic representation of model validation.

Table 2. Nested ANOVA to investigate the effects of individual, capitulum and propagule position (centre vs. edge) on morphological traits and terminal velocity.

Trait	Individuals (population)				Capitulum (individuals)				Position (capitulum)			
	df	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*	*df*	*MS*	*F*	*P*
Total propagule mass (mg)	8, 400	0.0397	3.358	**	40, 400	0.0118	3.886	***	50, 400	0.003	1.921	***
Achene mass (mg)	7, 360	0.03[a]	3.98	**	36, 360	< 0.01[a]	3.12	***	45, 360	< 0.01[a]	1.95	***
Pappus mass (mg)	7, 359	0.002[a]	6.15	***	36, 359	< 0.01[a]	1.77	*	45, 359	< 0.01[a]	1.64	**
Pappus length (mm)	8, 399	9.66[a]	9.2	***	40, 399	1.05	1.13	n.s.	50, 399	0.9324	6.02	***
Pappus radius (mm)	8, 400	4.926	2.948	*	40, 400	1.6709	1.124	n.s.	50, 400	1.487	6.83	***
Achene length (mm)	8, 400	2.3412	11.69	***	40, 400	0.2003	3.966	***	50, 400	0.0505	5.00	***
Maximal achene diameter (mm)	8, 400	0.0083	3.639	**	40, 400	0.0023	1.199	n.s.	50, 400	0.0019	1.348	n.s.
Minimal achene diameter (mm)	8, 399	0.02[a]	5.19	***	40, 399	< 0.01[a]	1.25	n.s.	50, 399	< 0.01[a]	1.19	n.s.
Achene flattening	8, 399	0.150[a]	2.74	*	40, 399	0.0548	1.12	n.s.	50, 399	0.0489	0.97	n.s.
Pappus mass-radius ratio (g m^{-1})	7, 359	< 0.01[a]	8.82	***	36, 359	< 0.01[a]	1.97	*	45, 359	< 0.01[a]	0.76	n.s.
Achene-pappus mass ratio	7, 359	19102[a]	0.99	n.s.	36, 359	19362[a]	0.99	n.s.	45, 359	19468	1.11	n.s.
Plume loading (mg mm^{-2})	8, 400	< 0.01[a]	2.319	*	40, 400	< 0.01[a]	1.099	n.s.	50, 400	< 0.01[a]	4.554	***
Terminal velocity (m s^{-1})	8, 200	2.586	2.336	*	40, 200	1.11	0.74	n.s.	50, 200	1.5	61.98	***

Note. ***: $P < 0.001$; **: $0.001 \leq P < 0.01$; *: $0.01 \leq P < 0.05$; n.s.: not significant; [a] not an exact F-test.

RESULTS

Population, individual, capitulum and position effect on propagule traits and terminal velocity

In the drop time in still air experiment, no difference was found between populations for any morphological trait or for terminal velocity (Table 2). A significant part of the variability in morphological traits was explained either by individuals, by capitula and/or by the position of the achene on the receptacle. Individual differences were significant (at 0.05 level) for most traits, showing important intra-population variability. External and internal achenes significantly differed for most traits. These differences were particularly marked for pappus morphology (31.5% and 45.7% of the variance explained by achene position for pappus length and pappus radius, respectively). External achenes had a smaller pappus but a similar mass, and, therefore, a greater plume loading. Terminal velocity differed among individuals and positions. The major part (85.0%) of the variability was explained by the achene position, with lower *Vt* for central achenes.

Morphological proxy traits and terminal velocity regression

Terminal velocity values in the drop time in still air experiment ranged from 0.171 to 0.513 m s^{-1} (mean = 0.309 m s^{-1}; SD = 0.066 m s^{-1}).

The quantitative morphological trait best correlated with terminal velocity was the square root of plume loading (SRPL) ($r = 0.891$; Table 3). This trait is thus a suitable proxy trait for terminal velocity estimations. Using this proxy trait, terminal velocity regression was fitted as follows:

$$Vt = -0.0192 + 2.53 * SRPL \qquad \text{(eq. 6)}$$

The coefficient of determination was 0.797. Any additional variable in the model did not increase the coefficient by more than 0.025 so this equation was selected and used in the model to provide terminal velocity values estimated from achene morphology (Fig. 4).

Table 3. Pearson's coefficient of correlation (*r*), calculated between all morphological traits on the one hand, as measured or mathematically transformed, and terminal velocity on the other hand.

Morphological trait	Mathematical transformation				
	None	Square	Square root	Logarithm	Exponential
Total propagule mass (mg)	0.3012	0.2571	0.3233	0.3433	0.2904
Achene mass (mg)	0.3652	0.3251	0.3837	0.398	0.3571
Pappus mass (mg)	-0.3060	-0.2742	-0.3072	-0.2882	-0.3051
Pappus length (mm)	0.0531	-0.2735	0.0786	0.0829	-0.3648
Pappus radius (mm)	-0.6681	-0.6277	-0.686	-0.7017	-0.567
Achene length (mm)	-0.1961	-0.2013	-0.1929	-0.1893	-0.2051
Maximal achene diameter (mm)	0.0184	0.0149	0.0199	0.0213	0.0166
Minimal achene diameter (mm)	0.0441	0.0387	0.0486	0.0543	0.0424
Achene flattening	0.0276	0.0254	0.0306	0.0351	0.0268
Pappus mass-radius ratio (kg 10^{-3} m^{-1})	0.0855	0.0855	0.0854	0.0842	0.0855
Achene-pappus mass ratio	0.2459	0.0486	0.3695	0.4601	-0.0602
Plume loading (kg m^{-2})	0.8849	0.8325	0.8905	0.8794	0.8842

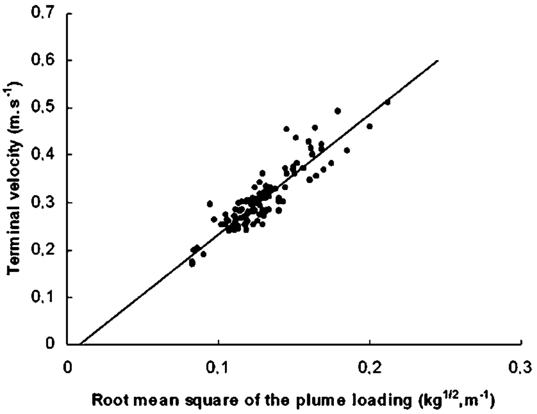

Fig. 4. Model equation for predicting terminal velocity using the square root of the plume loading.

MODEL VALIDATION

Among the 192 dispersed propagules in the three pooled wind-tunnel experiments, 101 (52.6%) fell on the horizontal 6-metre-long collector and 91 (47.4%) on the vertical one. Among the latter, 21 were collected above 0.4 m and cannot be considered for the Gaussian tilted plume model, as the model cannot simulate uplift. The case of those uplifted achenes is treated separately. Fig. 5 shows the horizontal and vertical repartition of the remaining 171 achenes considered in the wind tunnel, along with the modelled curve and the earlier described 80% interval. Horizontally 77.2% and vertically 75.7%, were inside this 80% interval. The model, therefore, does not reach proper validation, but can be considered as reasonably fitting the achene dispersal in the wind tunnel.

SEED RAIN PATTERN

Once the model constructed, the seed rain pattern under the selected environmental conditions was assessed. Fig. 6 shows the curve of the cumulated crosswind-integrated deposition for each plume loading class (Table 1) against the distance from the source (step 0.05 m). On a flat open area, under a 5 m s^{-1} stable wind and for an average height of release, 32.10% of the dispersed achenes are deposited in the nearest 6 m from the mother plant, with a peak at 5.2 m; 63.19% are dispersed within 10 m, and 99.83% within 100 m.

The 21 uplifted achenes (10.94% of all dispersed achenes) were collected from 0.43 to 0.86 m above ground. Nine of them were aborted. Viable uplifted achenes represented 6.25% of all dispersed achenes. They were not significantly different from non-uplifted achenes for achene mass (Mann-Whitney test; $W = 12036$; $P = 0.431$), pappus surface (Mann-Whitney test; $W = 12019$; $P = 0.752$) and plume loading (Mann-Whitney test; $W = 12132$; $P = 0.688$).

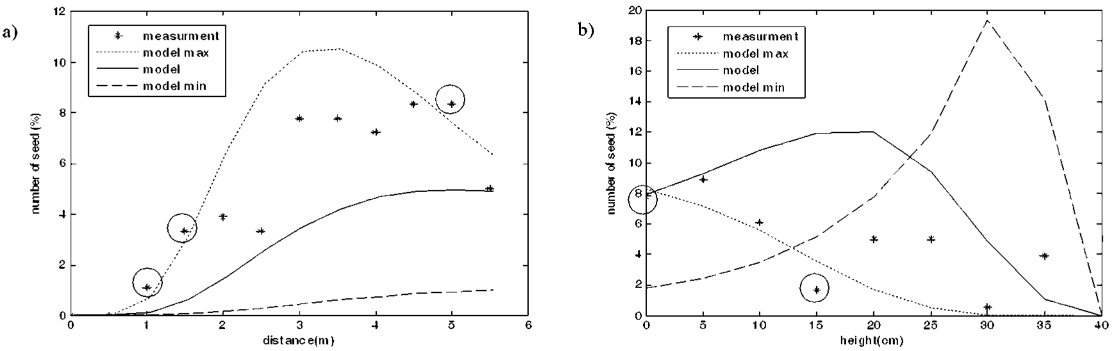

Fig. 5. Horizontal (a) and vertical (b) repartition of the 171 non-uplifted achenes dispersed in the wind tunnel, along with the modelled curve and the 80% interval ("model min" and "model max"). Dots represent the proportion of achenes per 5 cm class along the horizontal and vertical collectors. Circled measurement dots are outside the 80% interval. In the second figure, height is along the horizontal axis whereas it is vertical in the wind tunnel.

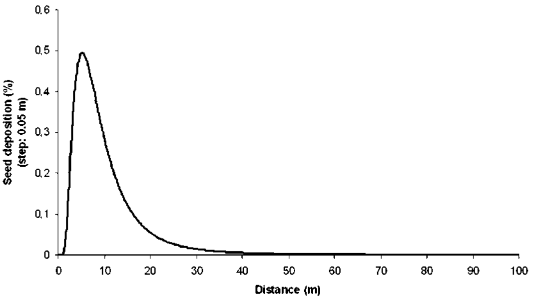

Fig. 6. Seed rain pattern of *S. inaequidens*. The curve is shown for the nearest 100 m from the parent plant. Percentage of deposition was calculated every 0.05 m.

DISCUSSION

The Gaussian tilted plume model provided a seed rain pattern for the environmental conditions that we imposed. It did not reach proper validation at the 80% level, but fitted the data obtained in the wind tunnel reasonably well. The produced pattern corresponds to a simplified situation as compared to field conditions, as several hypotheses were assumed for model fitting (no resuspension of seeds, *n* constant, negligible deposition due to diffusion). The validation experiment was limited to three dispersal events and showed a good agreement between predicted and mesured data only for short distances. The seed rain pattern is only efficient under the environmental conditions considered for model construction: flat area, wind speed around 5 m s^{-1}, low turbulences and release height of 0.40 m. Under these conditions, most achenes deposit in the vicinity of the parent plant: 63.2% within 10 m; 99.8% within 100 m. The maximum deposition rate is reached at 5.2 m. In the field, maximum winds and turbulences can be higher than those considered. Under those conditions the global seed rain pattern may be more spread out and the proportion of uplifted seeds can be higher, especially in the case of gusts. Nevertheless, the wind speed considered is such that most (if not all) achenes are detached from the receptacle. If wind speed increases gradually, most achenes are already dispersed when faster winds occur, even if achene abscission was shown not to be independent of wind speed (GREENE 2005). If wind speed increase is not gradual, gusts may however make the seed rain pattern different. Other aspects, such as topography or surrounding vegetation and shape of the parent plant (NIKLAS 1984) may also impact seed rain pattern in the field, so that the real pattern may dramatically differ from that modelled here. Besides, the invasion pattern of *S. inaequidens* was shown not to be isotropic: the species seems to disperse preferentially along roadsides and railways, which act like corridors along which stronger winds are more often reached (ERNST 1998).

The settling terminal velocities measured for *S. inaequidens* in this study are comparable to those given by ERNST (1998) for populations in the Netherlands. However, they are relatively low compared to other Asteraceae: among 39 species for which terminal velocity values were found in the literature and ranked increasingly, *S. inaequidens* ranked eleventh (SHELDON & BURROWS 1973, MATLACK 1987, SCHULZ *et al.* 1991, ANDERSEN 1992, 1993, KAHRS 1994, ASKEW *et al.* 1997, TACKENBERG *et al.* 2003). This indicates that *S. inaequidens* has a relatively high potential for exploiting favourable wind conditions. As generally reported for Asteraceae, the square root of the plume loading was the best proxy trait for terminal velocity (AUGSPURGER 1986, MATLACK 1987, ANDERSEN 1993, but see SOONS & HEIL 2002). Our assessment of terminal velocity by regression on the square root of the plume loading showed an acceptable precision compared to other studies (MATLACK 1987, ANDERSEN 1993, CODY & OVERTON 1996). As terminal velocity of a wind-dispersed diaspores is a major component of its dispersal ability (GREENE & JOHNSON 1986, MATLACK 1987), our results indicate that *S. inaequidens* achenes with low plume loading have a longer dispersal distance, i.e., light propagule and/or large pappus will be the best characteristics for dispersion.

In the context of plant invasion, estimating the tail of the seed shadow is of particular interest as it can have very important consequences on the colonisation rate of new sites (PORTNOY & WILLSON 1993). Although our model and experimental approach are not explicitly designed to evaluate rare long distance events, some interesting insights may be gained from both the modelled seed shadow and the observed dispersal pattern in the wind tunnel. Our model suggests a capacity for dispersal up to 200 m under favourable and relatively common wind conditions over the flowering season. Measuring seed dispersal capacity beyond tens of metres directly in the field is usually constrained by experimental design. Because of the necessity to keep sampling effort constant over increasing distances, the number of seed traps should be doubled when doubling distances (BULLOCK & CLARKE 2000). This results in unmanageable experimental designs. We demonstrated that simple mechanistic models, such as the Gaussian plume model, with morphological seed characteristics that are relatively easy to measure such as plume loading, might help to understand the vulnerability of neighbouring unoccupied sites to colonisation from established populations.

In addition to seed included in the tail of the seed shadow, it has long been known that seed uplift is a key mechanism for long distance dispersal (NATHAN *et al.* 2002). In our experiment 6.25% of viable achenes were uplifted. These were not forecasted by the model. Long-distance dispersal may therefore represent up to 6.25% even under wind conditions with low turbulences. In comparison, in a study of another Asteraceae with a high dispersal capacity, *Cirsium vulgare*, KLINKHAMER *et al.* (1988) found that just over 10% of the dispersed seeds were caught by updrafts and were carried out of the study area (more than 32 m). Studying the dispersal of *Crepis praemorsa*, SKARPAAS *et al.* (2004) found that 4% of the seeds were uplifted and carried away from the population. In a recent study on the wind-dispersed invasive *Conyza canadensis* (Asteraceae), DAUER *et al.* (2006) found that seeds regularly dispersed at least 500 m from the source population despite that 99% of seeds were found in the nearest 100 m. As one *S. inaequidens* plant can produce up to 1500 capitula annually (MONTY, unpublished data) bearing roughly 100 achenes each (LOPEZ-GARCIA & MAILLET 2005), the seed rain (total amount of achenes produced per year) of *S. inaequidens* can reach 150 000 achenes plant^{-1} y^{-1} under favourable conditions. A single large *S. inaequidens* individual can thus potentially generate more than 9 000 uplifted achenes out of the population (i.e., more than 200 m away from the parent). This value is found for low turbulent conditions in the wind tunnel; hence it may be an underestimation compared to natural conditions. This represents an important colonisation potential, but information on post-dispersal survival is needed to predict long-distance colonisation. Diaspore weight and dispersal structure are linked to separate ecological functions, which may lead to contrasting forms of selection during invasion.

Diaspores with lower plume loadings will be the best disperser for colonisation of new areas, but heavier seeds are more likely to produce large and vigorous seedlings (Black 1956, Dolan 1984), and, therefore, presumably confer an advantage in competition and fitness. According to this, one may expect that seeds with low plume loading and heavier seeds, i.e., larger pappus surface, would be favoured during invasion, thanks to a higher probability to found new populations. Yet, our results do not support this hypothesis because uplifted achenes were not morphologically different from others.

The present study shows how a seed dispersal modelling approach resulted in valuable information about further *S. inaequidens* invasion. The Gaussian tilted plume model might be generalised to other anemochorous alien species if long-distance dispersal is not the major focus, at least if one acknowledges the limitations of the model about seed uplift.

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Article 7

Evolution of dispersal traits along an invasion route in the wind-dispersed *Senecio inaequidens* (Asteraceae)

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Abstract. In introduced organisms, dispersal propensity is expected to increase during range expansion. This prediction is based on the assumption that phenotypic plasticity is low compared to genetic diversity, however an increase in dispersal can be counteracted by the Allee effect. These hypotheses, although viable, lack the empirical evidence necessary to elucidate the dynamics of species invasion. Therefore, the present study tested for evidence of differentiation in dispersal-related traits and the Allee effect in the wind-dispersed invasive *Senecio inaequidens* (Asteraceae). We collected capitula from individuals in ten field populations, along an invasion route including the original introduction site in southern France. In addition, we conducted a common garden experiment from field-collected seed and obtained capitula from individuals representing the same ten field populations. We analysed phenotypic variation in dispersal-related diaspore and capitulum traits between field and common garden environments and the distance between dispersed populations to the introduction site. Our results revealed low levels of phenotypic differentiation among populations for all traits. However, significant clinal variation in dispersal traits was demonstrated in common garden plants representing the invasion route. In field populations similar trends in dispersal-related traits, and evidence of an Allee effect were not detected. In part, our results supported expectations of increased dispersal capacity with range expansion, and emphasized the contribution of phenotypic plasticity under natural conditions.

Keywords. *Dispersal, achene, plume loading, invasive plant, colonization gradient, Allee effect.*

Résumé. En théorie, la propension à la dispersion est censée augmenter durant l'expansion d'aire d'un organisme introduit. Cependant une telle prédiction peut être contrecarrée par un effet Allee, le cas échéant, et suppose que la plasticité phénotypique est suffisamment faible en comparaison de la diversité génétique dans les traits de dispersion. Enfin, ce genre de prédiction souffre d'un manque de validations empiriques. Nous testons l'hypothèse d'une différenciation dans les traits de dispersion et la présence d'un effet Allee chez *Senecio inaequidens* (Asteraceae), le long d'un gradient de colonisation où les populations sont connues pour être originaires d'un seul site d'introduction. Nous avons collecté des capitules à la fois dans dix populations naturelles, et sur des plants provenant de ces mêmes populations mais cultivés en jardin commun. Nous avons analysé la variation phénotypique dans les traits des diaspores et des capitules, en relation avec l'environnement de croissance et la distance des populations avec le site d'introduction. Le niveau de différenciation, entre populations, s'est révélé faible pour l'ensemble des traits mesurés. Néanmoins, une variation clinale a été observée dans les traits de dispersion le long du gradient, pour les diaspores collectées en jardin commun. En conditions naturelles, une telle variation clinale n'a pas été trouvée. Nous n'avons par ailleurs pas observé d'effet Allee dans les populations naturelles du front d'invasion. Globalement, les prévisions théoriques ont donc été partiellement validées, mais la plasticité semble jouer un rôle contraire dans les populations naturelles.

Mots-clés. *Dispersion, akène, plume loading, plante invasive, gradient de colonisation, effet Allee.*

Introduction

The range of an increasing number of plant species is expanding due to introductions, migration, and/or climate change (e.g. Pitelka, 1997 ; Vitousek et al., 1997). The success of range expansion is strongly influenced by dispersal strategies. Therefore, an enhanced understanding of dispersal dynamics can improve invasion predictions (Travis et al., 2002). Seed dispersal is an integral component of plant life history, and fundamental in determining spatial patterns, population growth rates, rates of species advance following climatic or other environmental change, and is the basis of species survival (Clobert et al., 2001 ; Levin et al., 2003). Several scenarios explain the evolution of dispersal strategies. In a competitive framework, a specific mode of dispersal may be favoured even if it is costly, provided that it results in establishment of the disperser over the non-disperser (Hamilton et al., 1977). This intrinsic advantage to dispersers is enhanced due to reduced competition among close relatives, especially sibs (Dieckmann et al., 1999). Furthermore, dispersal can reduce inbreeding and the potential for deleterious genetic effects (e.g. Dieckmann et al., 1999 ; Moore et al., 1984).

In wind-dispersed Asteraceae, dispersal success is contingent on both propagule mass and dispersal structure (i.e. pappus) size (Matlack, 1987), which are involved in separate, though related, ecological functions (Cappuccino et al., 2002). A large pappus and a light propagule are expected to favour dispersal, whereas a heavy propagule is expected to produce large and more vigorous seedlings (Black, 1957 ; Dolan, 1984), presumably conferring a competitive advantage (environmental maternal effects, Roach et al., 1987). Trade-offs between propagule mass and dispersal structure size might therefore be the result of contrasting forms of selection. Previous studies have analysed dispersal trait variation in plants, primarily in Asteraceae (Cody et al., 1996 ; Flann et al., 2002 ; Gravuer et al., 2003 ; McGinley, 1989 ; O'Connell et al., 2001 ; Prince et al., 1995 ; Riba et al., 2005 ; Soons et al., 2002 ; Welham et al., 1998). These studies reported evidence of variation in seed and pappus traits, and in some cases concluded the observed variation was the result of rapid evolutionary processes. However, the majority of these studies were performed from diaspores collected under natural conditions, and the variation may be the result of genetic and environmental effects. Generally, the contribution of phenotypic plasticity to seed dispersal traits is not well understood (Donohue, 1999 ; Olivieri et al., 1985). Riba et al. (2005) used both diaspores from field populations and a common garden to address phenotypic variation in seed dispersal traits. This approach allowed for a separation of genetic-based differences and phenotypic plasticity in response to environmental variation.

In invasive plant species, variation in dispersal traits is expected for range expansion (Darling et al., 2008 ; Holt, 2003). During early naturalization, short-distance dispersal that extends population size incrementally should be favoured by selection because long-dispersed descendants are likely to suffer a strong Allee effect (Allee, 1931 ; Cappuccino, 2004 ; Lewis et al., 1993 ; Stephens et al., 1999). During early colonization, dispersing individuals are exploiting new areas and are the primary progenitors to the subsequent generations. The individuals and resulting populations at the invasion front will face selection for traits conferring effective dispersal and an increase in dispersal ability during invasion is expected (Holt, 2003 ; Travis et al., 2002). At an invasion front, however, population densities are frequently low, and reproduction in small (or sparse) populations may be inhibited, with the result that population growth rates exhibit positive density dependence at low densities, the basis of the Allee effect (Allee, 1931 ; Taylor et al., 2005).

Dispersal evolution during range expansion has been mainly approached through predictive modelling (e.g. Holt, 2003 ; Phillips et al., 2008 ; Ronce et al., 2005 ; Travis et al., 2002). Travis and Dytham's model (2002), which focussed on alien species invasion from a single introduction source, indicated that the best dispersers were selected during invasion. Therefore, selection may result in a more rapid expansion than expected under evolutionary stasis. But when the Allee effect was considered in the model, results showed that invasion was slowed due to a decrease in selection for dispersal and diaspore survival outside the current range. Clearly, this approach lacks the empirical validation required to fully understand the properties inherent in invasive plant species. To our knowledge, no empirical studies on seed dispersal trait variation along an invasion route have been published (but see Phillips et al., 2008 for an example in animal dispersal). This may be due to several constraints in methodology: 1) the relationship between dispersal and diaspore traits must be assessed, 2) the invasion history and ideally, the particular invasion route must be well documented, and 3) diaspore traits should be analysed from field collections and those grown under controlled conditions (Riba et al., 2005).

In southern France, *Senecio inaequidens* DC. (Asteraceae; African ragwort) is particularly well suited to serve as a model species to study the evolutionary history of invasive plants. The species has a well documented invasion history, including evidence of a single introduction site (Ernst, 1998 ; Guillerm et al., 1990). In the present study, we analysed dispersal-related traits from 837 diaspores representing 99 *S. inaequidens* field and common garden individuals, to ask the following question: How do dispersal-related traits vary along an invasion route? We tested the hypothesis that dispersal capacity increases with range expansion and looked for an Allee effect among field populations.

Material and methods

Study organism

S. inaequidens is a short-lived perennial native to South Africa and Lesotho, with a life span of 5-10 years (Brunel, 2003 ; Ernst, 1998 ; Lafuma et al., 2003). The species typically reaches 1 m in height, with numerous stems bearing yellow capitula. Individuals can produce up to 1500 flower heads during the flowering period, and each capitulum bears approximately one hundred achenes (Lopez-Garcia et al., 2005). In France, flowering occurs from April through December. *S. inaequidens* is pollinated by many different generalist insects, including Hymenoptera, Lepidoptera and Diptera (Ernst, 1998 ; Vanparys et al., 2008). Harland (1965) reported the species as self-incompatible (1955), but some individuals have been observed exhibiting partial self-fertility (Lopez-Garcia et al., 2005). Achenes are mainly wind dispersed (Monty et al., 2008).

S. inaequidens was accidentally introduced to Europe in the late 19th - early 20th centuries where only tetraploids are reported. In its native range, the species occurs as two co-existing cytotypes (Lafuma et al., 2003). Several introductions occurred in Europe, each tied to wool importation (e.g. Ernst, 1998 ; Lousley, 1961). For several decades, specimens were only documented in the vicinity of European wool-processing centres (Ernst, 1998): Hannover (D) in 1889 and Bremen (D) in 1886; Edinburgh (UK) in 1928; Verona (I) in 1947 (Pignatti, 1982); Liège (B) in 1892 (Verloove, 2006); and Mazamet (F) in 1936 (Guillerm et al., 1990). In southern France, Guillerm et al. (1990) documented a progressive colonization of the species, and found that for decades, it was only reported in the vicinity of the wool-processing centre of Mazamet. It was not until after 1950 that it steadily expanded throughout areas geographically removed from existing wool industrial centres. By the 1970s, the species had colonized areas of South-western France well within the first introduction region and was considered an agricultural weed. In the early 1980s, the species reached the Mediterranean coast from Mazamet. Its expansion continued south from the mid- to late-1980s, and was subsequently reported from the Iberian Peninsula (Casasayas Fornell, 1990). The species also colonized the Oriental Pyrenean, and was first recorded in Nohèdes (altitude ca. 800 m) in 1987 (Cottrel et al., 1998). This uphill colonization continued (Cottrel et al., 1998), and the species is presently distributed at elevations reaching 1700 m, well within the Pyrenean. Figure 1 was adapted from Guillerm et al. (1990), and includes additional occurrences from the literature (Aseginolaza et al., 1984 ; Cottrel et al., 1998 ; Garcia-Serrano et al., 2004). It depicts the chronology of *S. inaequidens* range expansion in southern France, from the wool-processing centre of Mazamet. To date, *S. inaequidens* remains absent from several areas in central France, indicating that invasion fronts from Mazamet and other introduction sites (Belgium, Italy) have not yet converged (Monty, unpublished).

Due to a particularly well-documented invasion history, *S. inaequidens* has been used as a model species in several evolutionary studies (Bossdorf et al., 2008 ; Monty et al., 2009). Most notably, a common garden study revealed that *S. inaequidens* experienced clinal genetic differentiation in growth traits during its invasion in southern France (Monty et al., 2009).

Population location at increasing distances from the source

Based on the *S. inaequidens* expansion chronology in southern France (Fig. 1), we selected 10 *S. inaequidens* populations at increasing distance from the initial introduction site. Populations were located along the invasion route (Fig. 1), along the Mediterranean coast, and into the Pyrenean towards northern Spain. The source population was located at an abandoned wool-processing factory in Mazamet, at the site where the species was first recorded. For each population, the expansion distance (km) from the source was calculated as the shortest distance along a road using http://www.viamichelin.fr. Road distance was more relevant than air distance because *S. inaequidens* is known to follow roads and railways during colonization (Ernst, 1998).

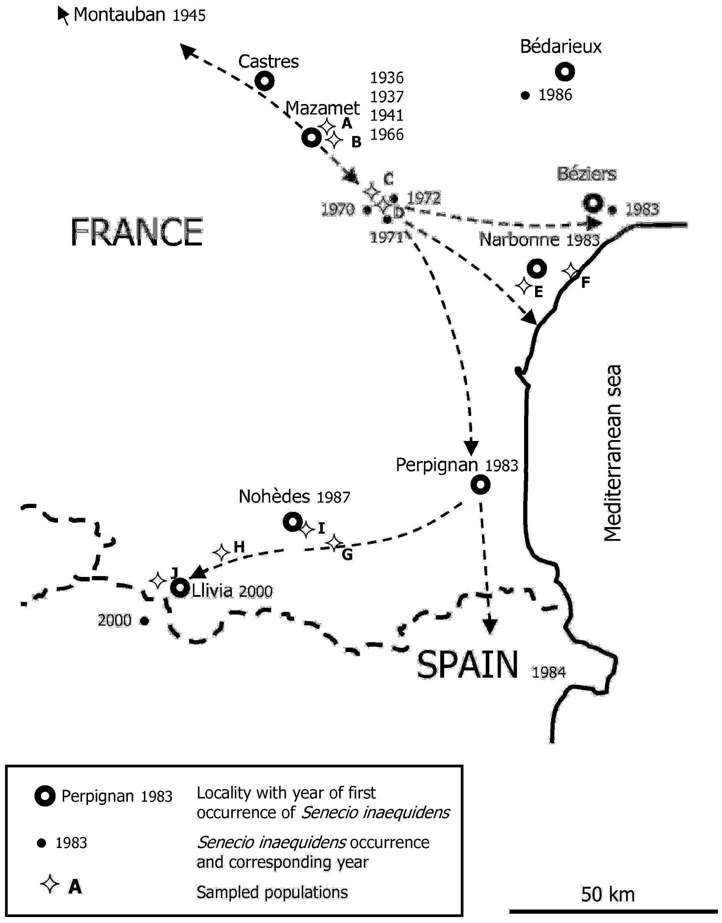

Figure 1 Progressive expansion chronology of *Senecio inaequidens* DC. in southern France from the wool-processing centre of Mazamet, and sampled populations (A to J). First occurrence at the indicated localities are given where available. Adapted from (Guillerm et al., 1990).

Diaspore origin

We compared diaspores collected in the field and from a common garden experiment. In November 2005, we randomly collected seeds from 10 individuals per population for a common garden experiment. The seeds were sown in a randomized block design on an open field in Gembloux, central Belgium (see details in Monty et al., 2009). Free-pollinated capitula were collected from common garden plants in summer-early autumn 2006. We collected one capitulum per plant individual on three to six individuals per population. The total number of individuals sampled was 45. In November 2007, we randomly selected five to six individuals per field population (the individuals were assumed to be different from those sampled in 2005), and collected one capitulum per plant. The total number of individuals sampled was 54. All capitula were stored together at room temperature in pierced boxes until the diaspores were measured in September 2008. The number of viable diaspores (NVD), the number of non-developed diaspores (NAD) and the total number of diaspores (ND) were determined for each capitulum. Non-developed diaspores are typically flat and whitish, and easy to distinguish from viable ones.

Table 1. Means and coefficients of variation (%) for diaspore traits of the different *Senecio inaequidens* DC. populations (A to J), under field and garden conditions. N represents the number of plant individuals per population.

Diaspore trait	Common garden										
		A	B	D	C	E	F	G	H	I	J	All populations
AM: Achene mass (mg)	Mean	0.347	0.334	0.395	0.359	0.332	0.365	0.307	0.332	0.295	0.333	0.337
	CV (%)	16.10	23.23	14.38	24.88	13.81	18.68	11.34	7.15	28.31	6.62	17.04
PD: Pappus diameter (mm)	Mean	9.23	8.51	10.17	9.82	9.88	9.69	10.48	10.07	9.80	9.95	9.75
	CV (%)	3.80	5.95	12.94	12.47	8.94	7.06	7.29	12.65	12.99	8.27	10.22
PL: Pappus length (mm)	Mean	5.44	5.34	5.99	5.86	5.73	5.89	6.16	5.97	6.17	5.98	5.85
	CV (%)	4.24	6.06	7.83	5.05	4.51	6.36	10.40	7.31	4.75	8.11	7.98
MAR: Plume loading (10^{-3}.kg.m^{-2})	Mean	5.25	5.89	5.12	5.18	4.43	4.99	3.62	4.35	4.02	4.39	4.69
	CV (%)	20.72	17.45	36.24	52.96	19.48	13.58	19.25	22.62	36.55	18.26	28.41
	N	5	5	3	4	3	4	6	5	4	6	45
	Natural populations										
		A	B	D	C	E	F	G	H	I	J	All populations
AM: Achene mass (mg)	Mean	0.330	0.296	0.343	0.313	0.283	0.353	0.335	0.318	0.373	0.352	0.329
	CV (%)	13.21	19.33	15.70	11.75	21.70	13.73	12.47	5.51	14.69	12.38	15.59
PD: Pappus diameter (mm)	Mean	9.37	9.72	10.18	10.74	9.40	9.64	9.39	10.12	10.00	9.11	9.79
	CV (%)	8.47	13.46	11.02	7.64	16.22	14.91	3.59	14.47	10.03	19.42	12.37
PL: Pappus length (mm)	Mean	5.30	5.47	5.48	5.98	5.21	5.14	5.91	6.16	5.79	5.48	5.59
	CV (%)	3.96	12.41	10.47	6.02	16.72	21.57	5.52	9.74	5.71	17.67	12.28
MAR: Plume loading (10^{-3}.kg.m^{-2})	Mean	4.89	4.19	4.37	3.54	4.22	5.02	4.90	4.17	4.89	5.91	4.58
	CV (%)	19.49	28.37	30.66	22.00	25.59	20.60	15.43	26.39	21.69	38.46	27.71
	N	5	5	6	6	6	5	5	5	6	5	54

Diaspore traits

Dispersal capability of wind-dispersed diaspores can be successfully predicted from diaspore terminal velocity, which can be accurately estimated from morphological characteristics (Augspurger et al., 1987 ; Cody et al., 1996 ; Gravuer et al., 2003 ; Matlack, 1987 ; Morse et al., 1985 ; Platt et al., 1977 ; Sheldon et al., 1973). A recent study indicated that terminal velocity and dispersal of *S. inaequidens* diaspores could be predicted using a single trait parameter: the square root of the plume loading (Monty et al., 2008). Plume loading is defined as the ratio of the diaspore mass to the area of the horizontal projection of the pappus (Augspurger et al., 1987 ; Matlack, 1987). The lighter the diaspore and the larger the pappus, the lower the plume loading. Low plume loading induces low terminal velocity and therefore, increases dispersal distance.

Seven to 10 diaspores per capitulum were measured with a total sample size of N = 375 from the common garden and N = 462 from field populations. For each diaspore, we measured : pappus length (PL, mm) and pappus diameter (PD, mm) to the nearest 0.01 mm using an electronic digital calliper. After removing the pappus, the diaspores were weighed to the nearest 0.001 mg. Plume loading (or mass-area ratio, MAR, kg.m^{-2}) was calculated as

$$MAR = \frac{4*AM}{\pi*PD^2}$$

where AM is the achene mass (mg). PL and PD are required to quantify pappus size, even if PL is not used to determine MAR. PD might indeed be affected by the relative humidity (Sheldon et al., 1973) and the possible dislocation of the capitulum during storage.

Data analysis

A two-way ANOVA was used to analyse the variation in diaspore number per capitulum. Growing environment (fixed) and population (random) were assigned as crossed factors; and ND, NVD, NAD, and the ratio NAD/ND were assigned as responses. In order to test for the presence of an Allee effect in natural populations, a correlation between the NAD/ND ratios to road distance were calculated. An Allee effect would be indicated by a correlation between an increase in the proportion of non-developed achenes with increasing geographic distance, presumably the result of reduced pollination and/or increased incompatible crossings between relatives at the invasion front. AM, PL, PD and MAR, measured on each diaspore, were averaged per plant prior to statistical analyses. Diaspores from the same plant were indeed not independent and the number of measured achenes per plant varied. The variation in diaspore traits was ascertained by ANCOVA. The covariate was ND, as if more achenes were present on a capitulum, a reduction in pappus size might be expected (due to a compressing effect). The growing environment, i.e. common garden and natural populations, was a fixed factor. The factor population was random and the two factors were crossed. In the case of a significant interaction between factors, a one-way ANCOVA was performed with the factor population, for each growing environment. In order to detect any clinal variation in diaspore traits along the invasion route, we calculated Pearson's correlation coefficient between the population means for each trait and the distance to the source population (for each growing environment). Corresponding linear regressions were graphically generated to aid in interpretation of the results. Statistical analysis was executed using MINITAB ver. 15.1.1.0 (MINITAB Inc., State College, Pennsylvania).

Results

Variation in diaspore number and viability

ANOVA on the number of diaspores per capitulum, including the number of non-developed, viable, and the sum; and the proportion of non-developed achenes showed no significant differences with respect to the growing environment (NAD: $F_{1,9} = 0.13$, $P = 0.730$; NVD: $F_{1,9} = 0.81$, $P = 0.392$; ND: $F_{1,9} = 0.63$, $P = 0.448$ NAD/ND: $F_{1,9} = 0.20$, $P = 0.664$) or the population (NAD: $F_{9,79} = 0.69$, $P = 0.707$; NVD: $F_{9,79} = 0.89$, $P = 0.565$; ND: $F_{9,79} = 1.82$, $P = 0.193$; NAD/ND: $F_{9,79} = 0.67$, $P = 0.718$). Furthermore, a significant interaction between the factors was not detected (NAD: $F_{9,79} = 1.27$, $P = 0.266$; NVD: $F_{9,79} = 0.73$, $P = 0.683$; ND: $F_{9,79} = 0.61$, $P = 0.783$; NAD/ND: $F_{9,79} = 1.02$, $P = 0.429$). In field populations, a correlation between capitulum traits and distance to the source population revealed no significant trend (NAD: $r = -0.089$, $P = 0.806$; NVD: $r = 0.245$, $P = 0.496$; ND: $r = 0.253$, $P = 0.481$; NAD/ND: $r = -0.126$, $P = 0.729$). In particular, the proportion of non-developed achenes (NAD/ND) did not increase with increasing distance, which would be expected if an Allee effect had reduced the number of successful crossings at the invasion front.

Table 2. Results of ANCOVA performed on diaspore traits. The covariate was the number of diaspores per capitulum (ND). The two factors, growing environment (fixed) and population (random), were crossed. Measurements on each of the 837 diaspores were averaged per plant individual (N=99) prior to statistical analysis. AM: achene mass; PD: pappus diameter; PL: pappus length; MAR: plume loading. Significant results are in bold.

	AM			PD		PL		MAR	
Source of variation	*df*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
ND	1	0.6	0.44	1.35	0.248	1.36	0.247	2.11	0.15
Growing environment	1	0.55	0.478	0.001	0.967	6.53	**0.031**	0.06	0.818
Population	9	0.7	0.7	1.08	0.455	2.76	0.072	0.43	0.886
Growing environment x population interaction	9	1.48	0.17	1.16	0.331	0.82	0.603	2.18	**0.032**
Error	78								

Diaspore trait variation

Diaspore traits were not significantly different between natural and controlled conditions, with the exception of PL, which showed lower values in the field populations (Tables 1, 2). No significant population effect was indicated. However, a significant growing environment x population interaction was revealed for MAR, suggesting different patterns of variation in the common garden relative to the field. One-way ANCOVAs applied to each growing environment revealed no significant differences in field ($F_{9,43}$ = 1.84, P = 0.088) and common garden ($F_{9,34}$ = 1.29, P = 0.278) populations.

Pearson's correlation coefficients provided additional information regarding variation in diaspore traits. In the common garden, PD, PL and MAR were significantly correlated to road distance (Table 3, Fig. 2(a), (b) and (c)). Both PD and PL increased with increasing distance, i.e. plants found further from the source produced diaspores with a larger pappus. As a result, the diaspores presented lower plume loading and therefore better dispersal capacities. The trends observed in the common garden were not observed in field populations (Table 3, Fig. 2).

Table 3. Pearson's correlation coefficients of diaspore traits against the distance to the source population, and corresponding P values, in the two growing conditions. AM: achene mass; PD: pappus diameter; PL: pappus length; MAR: plume loading. Significant results are in bold.

Trait	Common garden		Natural populations	
	r	P	r	P
AM	-0.555	0.096	0.47	0.17
PD	**0.668**	**0.035**	-0.196	0.587
PL	**0.813**	**0.004**	0.429	0.216
MAR	**-0.876**	**0.001**	0.473	0.167

Discussion

Variation in diaspore number and viability

No clear pattern of variation was revealed in the total number of diaspores per capitulum over the invasion range of *S. inaequidens* sampled in this study. The number of non-developed and viable achenes in field populations showed no reduction in viability towards the invasion front. These results indicated that populations at the range margins do not suffer from an Allee effect. In other words, populations at the upper altitudinal invasion limit were sufficiently connected to the preceding migrating populations to maintain adequate sexual reproduction. This area had been colonized for at least seven years when sampled. It is possible that a more recent invasion front may have yielded different results, but finding the most newly established populations is not always possible for practical reasons. Travis and Dytham (2002) suggested that in the absence of an Allee effect, high rates of dispersal should be favoured by selection during invasions.

Clinal trends under common garden conditions

Previous analyses of intraspecific variation in dispersal traits have revealed increased dispersal potential in putatively more recently established plant populations (Olivieri et al., 1983 ; Peroni, 1994). However, very few studies of large-scale geographic variation in dispersal traits have been reported. Cwynar and MacDonald (1987) found increased dispersal potential in populations at the migration front associated with post-glacial range expansion.

Monty et al. (2008) demonstrated that in *S. inaequidens*, plume loading (MAR) was integral in terminal velocity. It was therefore a suitable trait to assess dispersal capacity of diaspores or plant individuals. Even though rare long-distance dispersal are not only determined by terminal velocity (Nathan, 2006 ; Nathan et al., 2008), terminal velocity was indeed repeatedly recognized as a key biotic determinants of dispersal (e.g. Gravuer et al., 2003 ; Monty et al., 2008 ; Nathan et al., 2005). In the present study, we found little differentiation in MAR among populations. However, this trait showed clinal variation in the common garden. Under controlled environmental conditions, plants originating from populations located further from the primary introduction site produced diaspores with a larger pappus (both in diameter and length), resulting in lower MAR and a higher dispersal potential. Because it was observed in a common garden, this variation among populations was, at least partially, under genetic control (Clausen et al., 1940). In addition, the predictive model of Travis and Dytham (2002) was congruent to these results. MAR reduction is primarily the result of an increase in pappus size, and not to variation in achene mass. It is important to note that pappus dimensions are essentially associated with dispersal ability, whereas achene mass is likely related to seedling fitness (Black, 1957 ; Dolan, 1984). The costs of the production of the pappus is therefore a cost of dispersal only, whereas the cost related to the seed provisioning are likely to have a direct beneficial effect on descendant seedling. Former studies that addressed the relationship between dispersal capacity and seedling fitness found a trade-off between these two fitness traits, i.e. increased achene mass confers higher growth rates, but carries a cost in terms of dispersal ability (Cappuccino et al., 2002 ; Meyer et al., 2001 ; Píco et al., 2003).

In a recent study, we demonstrated that *S. inaequidens* exhibited clinal differentiation in growth traits during invasion in southern France (Monty et al., 2009). Specifically, plant height at initial flowering and maximum height decreased in mountainous populations as elevation and distance from Mazamet increased. In terms of dispersal, this phenotype elicits a negative influence on the effective seed rain through a reduction in mean release height (Monty et al., 2008). Independent of environmental conditions, an antagonistic relationship between the following attributes may operate: a reduction in mean release height; and an increase in dispersal potential with an elevation gain and distance from the source population.

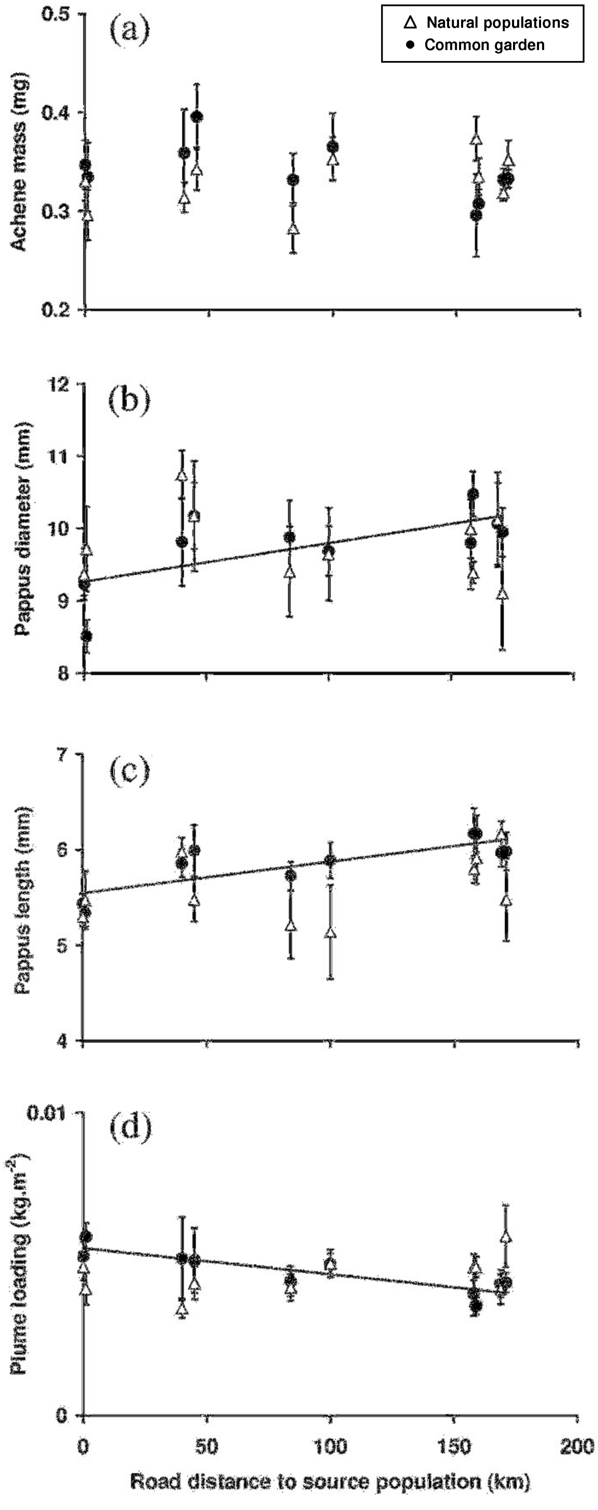

Figure 2 Population mean diaspore traits with standard error against road distance to the source population (km), for each field and garden condition. (a): achene mass(AM, mg); (b): pappus diameter (PD, mm); (c): pappus length (PL) and (d): plume loading (MAR, kg.m^{-2}). Solid lines represent significant regressions for the common garden data.

Comparison between field and controlled conditions

A comparison of field and common garden diaspores was meaningful for several reasons. It may be argued that inferring a genetic trend in the common garden experiment is not well supported, however it is noteworthy that no similar pattern was detected in the field populations. Field environmental conditions masked the genetic trend inferred from phenotypic traits in the common garden. Populations at the highest altitudinal migration front exhibited a high potential for dispersal, but the phenotypes were not characteristic of better dispersers. Few published studies comparing the levels of phenotypic variation under natural vs. controlled conditions for dispersal-related traits are available (but see Riba et al., 2005). Venable and Burquez (1989) found little difference in phenotypic variation between natural and controlled conditions for several plant traits in *Heterosperma pinnatum* (Asteraceae). Riba et al. (2005) reported a similar pattern, with comparable phenotypic variation in both growing environments. Our results also suggested that the levels of phenotypic variation are similar under natural vs. controlled conditions (see CV in Table 1). A decrease in phenotypic variation might be expected under controlled conditions, due to suppression of the environmental sources of variation. However, some authors have argued that controlled conditions might increase differentiation among populations (Mitchell-Olds et al., 1986). Phenotypic variation in the field may not reflect the evolutionary potential of a species because of environmental effects. Alternatively, phenotypic variation measured in common gardens might not be associated with the levels of genetic variation actually present in natural populations, because of genotype x environment interactions (due to e.g. phenotypic plasticity). The present study showed a pattern of variation presumably dictated by growing conditions. The phenotypic differences observed in the field and common garden populations are most likely attributable to phenotypic plasticity. The distance-to-the-source study transect also followed an altitudinal gradient. Therefore, the traits measured in the field may be climatically influenced. Soil nutrient levels may also be partly responsible for the differences observed between field and common garden data. In a study of maternal effects in *S. inaequidens*, Monty et al. (accepted) suggested that a plastic response to environmental conditions, at least in some populations, explained variation in seed mass.

Most studies on the evolution of dispersal infer dispersal ability from field collected diaspores (e.g. Cody et al., 1996 ; Gravuer et al., 2003), and consider the observed patterns of variation as genetically-based. Riba et al. (2005) revealed comparable differentiation patterns among populations in both controlled and natural conditions, lending support to these previous phenotypic studies. In the present study, we found no clear pattern of variation in the field but a significant one under controlled conditions. These results indicated that the study of seed dispersal evolution under controlled environmental conditions might lead to a misinterpretation of results, especially if the field environmental conditions are varied, which can enhance phenotypic plasticity.

Implications for invasion

Our study transect represented the colonization progression of the invasive *S. inaequidens* in southern France. During its range expansion towards the high Pyrenean, the species appears to have experienced slight selection in favour of effective dispersal traits. Based on the inferred genetic trend, a more rapid invasion should result by selection for better dispersers. Phenotypic differentiation was however low, and we did not detect better dispersing phenotypes in the field, most likely due to a plastic response to environmental conditions. In a more homogeneous invasion region than sampled in this study, plastic differences among phenotypes should be reduced and the invasion front progression enhanced by selection on dispersal (i.e. pappus) traits. This scenario would be congruent with recent models (e.g. Travis et al., 2002). Alternatively, the inclusion of phenotypic plasticity in modelling could improve dispersal predictions.

Conclusion

This study is among the first to generate empirical evidence of dispersal trait evolution during invasion. We focussed on a short spatial scale to ensure a well documented, gradual, colonization history. The hypothesis that plants further from the primary introduction produce achenes better suited to dispersal was weakly supported, and selection for a larger pappus was not highly favoured during invasion. The phenotypic trend observed under controlled conditions was not detected in the field, suggesting that plasticity due to environmental conditions was more important than genetic differentiation.

More attention should be paid to studies on dispersal evolution during plant invasion. In particular, the consequences of dispersal capabilities on the invasion pattern and on forecasting and monitoring invasive plant migration should be addressed, and at a broader geographic scale. However, current hypotheses still require further empirical validation. In order to elucidate the genetic patterns and trends of dispersal, studies should include an assessment of phenotypic variation under both controlled and field conditions.

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