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DIPLOMARBEIT

The effects of herbivory and competition on *Senecio inaequidens* DC. (Asteraceae), an invasive alien plant

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Abstract

Since the end of the 19th century, overall per-capita mobility of humans has increased significantly, leading to increased rates in human-mediated transportation of animal and plant species. The rapid spread of alien organisms, however, may lead to quick and unpredictable changes in ecosystems.

Senecio inaequidens DC. (Asteraceae) is an invasive alien plant from South Africa that was first introduced to Europe 100 years ago and is characterized by an exceptionally fast rate of spread; it contains pyrrolizidine alkaloids that are toxic to invertebrates, livestock and humans.

In the study presented here, field, laboratory and greenhouse experiments on the biology of *Senecio inaequidens* were conducted, in order to find out if and how herbivory and plant competition influence growth, survival and reproduction of this plant. Fully factorial or split-plot designs were used in order to answer this question. Vertebrate herbivores (rabbits) as well as native invertebrate herbivores (molluscs and two oligophagous insect herbivores) were used in the field experiments; the insect herbivores studied in detail were *Longitarsus jacobaeae* Waterhouse (Coleoptera: Chrysomelidae) and *Tyria jacobaeae* (Lepidoptera: Arctiidae). Multiple- and no-choice feeding preference experiments with these two insect herbivores were conducted. All experiments were performed at Imperial College, Silwood Park, about 30 km west of London (UK).

Growth and fecundity of *Senecio inaequidens* were significantly affected by interspecific competition, both in the greenhouse and in the field experiments. Rabbits only showed a significant effect when a closed vegetation cover was present at the same time. On artificially disturbed plots, *Senecio inaequidens* showed high capability to overcompensate for clipping of the above-ground parts by these herbivores. The shoots produced after clipping were not eaten by the rabbits any more, and the plants reached the flowering and fruit production stage within the same growth period.

There was a significant negative relationship between mollusc damage to leaves, and number of capitulae produced. Different ecotypes of *Senecio inaequidens* showed different amounts of herbivore damage.

One of the most remarkable results of this study was that *Longitarsus jacobaeae* freely colonized *Senecio inaequidens*, and that this plant was also accepted as a food source in laboratory two-way feeding preference experiments. In contrast, it could be clearly shown that *Tyria jacobaeae* does not accept *Senecio inaequidens* as a host plant.

Host switching from indigenous to invasive alien plant species in oligophagous insect herbivores may be more common than generally thought. The study presented here gives first experimental support for this assertion.

From the results of the experiments on rabbit grazing and plant competition, it is likely that *Senecio inaequidens* will start to colonize heavily grazed or disturbed grassland ecosystems in the near future; because of the toxic compounds this plant contains, it would be advisable to design a preventive management programme and to inform the public about the consequences that might be associated with the invasion of this plant.

Zusammenfassung

Die während des letzten Jahrhunders stark gestiegene Mobilität von Teilen der Weltbevölkerung hat dazu geführt, daß sich Tier- und Pflanzenarten über weitaus größere Distanzen ausbreiten können, als ihnen dies unter normalen Umständen möglich wäre. Die Verschleppung und Einbürgerung von Tier- und Pflanzenarten kann zu raschen und unvorhersehbaren Veränderungen in Ökosystemen führen.

Senecio inaequidens DC. (Asteraceae) ist ein vor ca. hundert Jahren aus Südafrika eingeschleppter Neophyt, der durch eine besonders hohe Ausbreitungsgeschwindigkeit charakterisiert ist; die Pflanze enthält Pyrrolizidin-Alkaloide, die giftig für Wirbellose, Weidevieh und Menschen sind.

Im Rahmen der vorliegenden Arbeit wurden Experimente zur Biologie dieses Neophyten durchgeführt, mit dem Ziel, Prognosen über den zukünftigen Verlauf der Invasion in Abhängigkeit verschiedener Faktoren machen zu können. Hierzu wurden Gewächshaus-, Labor- und Freilandexperimente durchgeführt.

Die Auswirkungen von Herbivorie und interspezifischer Konkurrenz auf Wachstum und Reproduktion von *Senecio inaequidens* wurden in faktoriellen Experimenten und in sogenannten ‘split plot’ Experimenten untersucht. Als Herbivoren dienten Kaninchen, Schnecken und zwei auf eine andere *Senecio*-Art spezialisierte phytophage Insekten (*Longitarsus jacobaeae* Waterhouse, Coleoptera: Chrysomelidae und *Tyria jacobaeae* L., Lepidoptera: Arctiidae). Um zu testen, ob einheimische Herbivoren den Neophyten *Senecio inaequidens* als sekundäre Futterpflanze annehmen, wurden Fütterungsexperimente unter kontrollierten Laborbedingungen durchgeführt. Alle Untersuchungen fanden am Imperial College (Großbritannien) auf einem Forschungsgelände 30 km westlich von London statt.

Wachstum und Reproduktion von *Senecio inaequidens* wurden sowohl im Gewächshaus, als auch im Freiland signifikant durch interspezifische Konkurrenz beeinflußt. Kaninchen hatten nur dann einen signifikanten Effekt, wenn gleichzeitig eine geschlossene Vegetationsdecke vorhanden war. Bei gestörter Vegetationsdecke trat Regenerationswachstum an von Kaninchen dekapitierten *Senecio inaequidens* – Pflanzen auf, und die neu ausgetriebenen oberirdischen Organe wurden nicht mehr von den Kaninchen gefressen. Pflanzen, die auf diese Weise neu ausgetrieben waren, kamen noch im selben Jahr zur Blüte und Fruchtreife.

Je mehr Schneckenfraß an den Blättern auftrat, desto geringer war die Anzahl der produzierten Blütenköpfe. Verschiedene Ökotypen von *Senecio inaequidens* wurden unterschiedlich stark von *Longitarsus* und Molluscen angenommen. Besonders bemerkenswert war, daß *Longitarsus jacobaeae* das Schmalblättrige Greiskraut von selbst zu besiedeln begann und dieses auch in Fraßtests unter Laborbedingungen als Futterquelle akzeptierte. Dagegen konnte klar gezeigt werden, daß *Tyria jacobaeae* diese Greiskraut-Art nicht als Futterpflanze annimmt.

Futterpflanzen-Wechsel von einheimischen zu eingeschleppten Pflanzenarten könnte bei spezialisierten phytophagen Insekten häufiger auftreten als bisher angenommen. Die vorliegende Arbeit liefert hierfür am Beispiel von *Senecio inaequidens* einen ersten Beleg.

Wie aus den Ergebnissen dieser Arbeit geschlussfolgert werden kann, ist es wahrscheinlich, daß *Senecio inaequidens* in naher Zukunft verstärkt gestörte und stark beweidete Graslandökosysteme besiedeln wird. Aufgrund der toxischen Inhaltsstoffe dieser Pflanze ist daher möglicherweise ein präventives Managementprogramm und die Aufklärung der Bevölkerung ratsam.

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Declaration

Hiermit versichere ich, dass ich die vorliegende Diplomarbeit selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe.

Ort, Datum	Unterschrift des Verfassers

„Nowadays we live in a very explosive world,
and while we may not know
where or when the next outburst will be,
we might hope to find ways of stopping it
or at any rate damping down its force.“

Charles S.Elton 1958, p.15

Preface

My first encounter with an invasive plant was during my community service near the beautiful Lake Starnberg in Southern Germany, in summer 1996. We were equipped with spates, raincoats, and brushcutters with circular saw blades – fighting against dense stands of tree-like Giant Hogweed (*Heracleum mantegazzianum* Somme. & Lev.) introduced from the Caucasus, which is probably one of the best-known alien plants in Europe. Exposure to the sap of this plant causes phytophotodermatitis (Lagey *et al.* 1995) and results in swelling, blisters and eruptions of affected sites.

Some people may think that biological invasions are nothing to worry about – but in many areas of the world, invasive organisms are already causing serious ecological and economical problems, and there is increasing evidence that biological invasions can in fact be regarded as one of the most important components of human-caused global change.

Yet, surprisingly little is known about the biology of some invasive species. Without this knowledge, however, and especially without experimental support from carefully designed and replicated experiments, assumptions on the possible impact of a specific invading organism may be misleading.

In this study, I will focus on a species which has only recently arrived in Europe, the Narrow-leaved Ragwort (*Senecio inaequidens* DC). An up-to-date summary of current knowlegde about the biology of this species will be provided, and I hope that at least some of the experimental results will be of help for further studies on this invasive plant.

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1 Introduction

1.1 Preliminary remarks

The nomenclature of species in this work is according to Wisskirchen & Haeupler 1998 and Schaefer 2000. As far as possible, characters of non-English languages (e.g. German umlauts, French accented letters) have been overtaken properly, but especially within the reference list there were restrictions due to the software used. Footnotes will be presented on the bottom of each page or on one of the following pages.

1.2 Why study biological invasions?

1.2.1 The borderless world

There can be no doubt that the human population has grown to a dangerous size (e.g. UN 2001, Bernstein 2001) and there is still no evidence for a decline in the near future (but see Lutz *et al.* 2001 and Smil 1999 for long-term predictions). And with this growth in population size, there is more need for resources, be it in terms of spatial, biotic or abiotic resources. Consequently, as man is altering the resource distribution and composition on earth, there is growing evidence for large environmental changes to take place, namely changes in climate, biotic productivity, water resources, atmospheric chemistry, and ecological systems - commonly referred to as ‚global change' (see Grime 1997 and Grime 1997; Vitousek, Mooney *et al.* 1997 for a general overview).

Since the end of the 19th century, major inventions like the invention of the automobile or the aircraft have led to an exponential increase in per-capita mobility of humans. Schafer & Victor 1999 estimate the worldwide traffic volume to have risen from $6 \cdot 10^{12}$ person kilometres in 1960, to $28 \cdot 10^{12}$ pkm in 1990. Generally speaking, it can be stated that, for all regions of the world, there is a linear correlation between per-capita gross domestic product and per-capita traffic volume. This means that the more industrialized a nation becomes, the more demand for high-speed transportations there will be.

This ever increasing far-distance transport of people and their goods, however, has lead to a breakdown of biogeographic barriers – different types of organisms are brought into regions where they would normally never occur naturally.

Organisms that have been introduced to a given area **outside their native range** by means of **human-mediated transportation**, are commonly called ‘alien' or ‘exotic' organisms (Keane & Crawley 2002; Richardson *et al.* 2000; Sax & Brown 2000); in the case of plants, it can be stated that

(1) if they fullfill the ‘invasion criterion' (Crawley 1997a), they are called ‘naturalized' alien plant species (see chapter 1.2.3.1); and
(2) if they are able to reproduce in natural habitats, they are called ‘invasive' alien plant species[1].

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[1] M.J.Crawley, personal communication, August 2002.

As Mooney 1999 points out, the resulting biological "scramble of species" may have "serious consequences for the future course of evolution and the number of species that will populate the earth". The major consequences discussed in context with biological invasions are for example[2]:

- altered species composition and structure of existing communities (e.g. Kowarik 1990)
- altered ecosystem-level rates of ressource supply, altered trophic structure, and altered disturbance regime of the invaded areas (D'Antonio & Vitousek 1992)
- reduced abundance and sometimes even total displacement of native species – e.g. by means of competitive suppression, altered disease incidence, trophic interactions, or changes in abiotic conditions (e.g. Tilman & Lehman 2001)
- loss of biodiversity (Vitousek, D'Antonio *et al.* 1997, cited in Keane & Crawley 2002)
- economical consequences, especially when invasive organisms affect agricultural ecosystems (e.g. Barbier 2001; Perrings *et al.* 2000)

Yet, surprisingly little is known about which organisms will become successful invaders, where and when invasions will occur, and what effects they may have. It seems that with the increase of speed in our global transportation systems, we have lost control over the consequences, and the "bombardment of every country by foreign species" (Elton 1958) will grow more and more intense. Thus, it is necessary to find quick and reliable ways to spot, predict and manage biological invasions.

1.2.2 Biological invasions: concepts and definitions

Every biological species originates in a single location and expands its range through migration or dispersal[3]. This range expansion is called biological invasion – one species invades an area in which it previously did not occur[4]. Climatic, geographic or topographic barriers as well as biotic and abiotic factors limit the spatial and temporal distribution of a species, and may hence form barriers that prevent biological invasions.

Biological invasion *per se* is a process as fundamental for biological systems as Brownian movement[5] for quantum mechanics. As Crawley 1997a, p. 617 ff. points out, all species that are persistent in a specific habitat pass the invasion criterion:

$$\frac{dN}{dt} > 0$$

This means that all species possess the ability to increase their population size when their population densities (N) are low (Crawley 1997a); hence, every attempt to search for specific traits of "invasive" and "non-invasive" species will lead to wrong conclusions. However, it is of course possible to make assumptions on

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[2] see e.g. Sakai *et al.* 2001 for a detailed review

[3] Local short-distance movements shall not be considered here. See Cain *et al.* 2000 for aspects of long-distance dispersal.

[4] In a strict sense, terms like "invasive species" or "alien species" are value-laden and should be replaced by more rational terms (Trudgill 2001); however, throughout the course of this thesis, they shall be used because they are commonly accepted terms in invasion biology.

[5] as described, for example, in Einstein 1985

the "degree of invasiveness" of a certain species under given environmental conditions, but this will always be a function of abiotic and biotic conditions, time, and interactions between these factors.

Despite all these theoretical considerations, there can be no doubt that human-mediated long-distance dispersal of species has led to an increase in dispersal and migration rates in organisms, and that this increase will result in changes in biological communities. Biological invasions are "a significant component of human-caused global change" (Dukes & Mooney 1999; Lövei 1997; Vitousek, D'Antonio *et al.* 1997), and it is therefore necessary to clearly define terms and definitions used in this context.

- **Alien** organisms have been introduced to a given area outside their native range by means of human-mediated transportation (Keane & Crawley 2002). For plants, a dispersal distance of >100 km is the minimum requirement for this term to be applied (Richardson *et al.* 2000)
- **Naturalized** organisms are alien organisms that reproduce consistently, and sustain populations over many life cycles without direct intervention by humans (Richardson *et al.* 2000)
- **Invasive** organisms shall be defined here[6] as naturalized organisms that produce reproductive offspring usually at considerable distances from the parent organisms (Rejmanek 2000; Richardson *et al.* 2000); for plants reproducing by means of seeds or other propagules, a spread of >100m in less than 50 years shall be used as a criterion (Richardson *et al.* 2000)

1.2.3 Invasive plants

1.2.3.1 General overview

The global extent of invasions by terrestrial plants is difficult to estimate (see Lonsdale 1999 for an overview), and it is even difficult to generalize on taxonomic patterns; however, it can be stated that the families Amaranthaceae, Brassicaceae, Chenopodiaceae, Fabaceae, Hydrocharitaceae, Papaveraceae, Poaceae and Polygonaceae show exceptionally high numbers of invasive species (Rejmanek 2000). The total number of introduced species is highest for the floras of Australia and New Zealand[7] (Heywood 1989). Some commonly accepted generalizations (closely following Rejmanek 2000) on invasive plants are

- the probability of invasion success increases with initial population size and with the number of introduction attempts
- the spread of many alien plant species is highly dependent on human activity (e.g. dispersal by human-mediated transportation)
- the increased abundance of invasive alien plant species outside their native range is due to a decrease in regulation by herbivores and other natural enemies (**enemy release hypothesis**, Keane & Crawley 2002; Sax & Brown 2000)

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[6] the terms 'invasive' (and 'invasive alien') shall be used here only for alien (exotic) organisms that pass the invasion criterion in given habitats *outside their native range*

[7] smaller oceanic islands like Hawaii are a special case and shall not be considered here

- plant communities with high species diversity are more resistant to invasion (**diversity resistance hypothesis**; Kennedy *et al.* 2002)
- small genome size, small seed size, high leaf area ratio and high relative growth rate of seedlings may be "an ultimate determinant of plant invasiveness" (Rejmanek 2000)
- the size of the native geographical range[8] of an herbaceous species may predict invasiveness (Keane, personal communication)
- alien species belonging to exotic genera are more likely to be invasive than those with native congeners
- plant species depending on non-specific mutualisms are more likely to overcome abiotic and biotic barriers in new enviroments
- there are often lag phases between introduction and first spontaneous spread of an alien plant (Kowarik 1995b)

The different stages of biological invasions (survival in transport, establishment in areas outside the native range, lag period, and spread in non-native areas), as well as further characteristics have been summarized by various authors, e.g. Sakai *et al.* 2001. Abbott 1992 mentions that, in case of plant invasions, interspecific hybridizations between native and exotic species may play a major role in the evolution of new plant taxa.

1.2.3.2 Important invasive alien plants in Europe

In the Central European context, it has been common to classify alien plants as "neophytes" and "archaeophytes", depending on their date of first spontaneous appearance (after or before 1492, respectively). There are more detailed classification systems, which are fully covered in Kowarik 1999.

Up to now, about 12,000 ferns and vascular plants have been imported to Central Europe, less than 5% of which having established permanently (Kowarik 1999). For Britain, Crawley 1997a states about 20,000 introduced vascular plants. Neophytes regarded as exceptionally important are for example[9] (largely based on Crawley 1997a and Kowarik 1995a):

- *Heracleum mantegazzianum* Sommier & Levier (Apiaceae)
- *Impatiens glandulifera* Royle (Balsaminaceae)
- *Solidago canadensis* L. (Asteraceae)
- *Helianthus tuberosus* L. (Asteraceae)
- *Reynoutria japonica* Houtt. (Polygonaceae) (Böhmer *et al.* 2001)
- *Spartina anglica* C.E.Hubbard (Poaceae)
- *Senecio inaequidens* DC. (Asteraceae), the species dealt with in this thesis (**Plate 1 a, 7 b**)

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[8] see Sax & Brown 2000 for more characteristics of plant species with large geographical range sizes

[9] woody species not taken into account here

1.3 Herbivory and plant competition

1.3.1 Herbivory

Herbivory is an antagonistic relationship (+/-) between plants and animals (Strauss & Zangerl 2002). Herbivores are animals that feed exclusively on living plant tissues (Crawley 1983), e.g. grazers, phytophages, granivores, frugivores or sapsuckers.

Herbivores can be classified according to their mode of feeding (e.g. sucking, stripping, mining), the tissues aten (granivores, frugivores etc.), the host plant spectrum (specialists, generalists – see below) as well as their phylogenetic position (invertebrate, vertebrate, insect herbivores etc) - see Strauss & Zangerl 2002 for details. The effects of herbivores on the performance and population dynamics of a plant can vary greatly. Sometimes whole plants are killed immediately (e.g. seed and seedling herbivory). Other herbivores may not directly cause plant mortality and instead even increase plant fecundity, as is the case for plants that are able to overcompensate for herbivory (Crawley 1997b). It needs to be stated that even low herbivore densities may have significant effects on plant performance and fecundity, if the dispersal rate and searching efficiency of the animal are high (Harper 1977, cited in Whittaker 1978). It is furthermore important to note that several herbivores may also be involved in pathogen transmission, e.g. aphids that may transmit plant viruses.

For the purpose of this study, the following definitions regarding the degree of host specificity in herbivores shall be used:

- **generalist herbivores** shall be defined as herbivores that feed on many different plant species
- **specialist (oligophagous) herbivores** feed on only a few plant species, usually of the same genus
- **monophagous** species are those that feed exclusively on one single host plant species.

However, even within one plant species, host races may alter the performance of monophagous herbivores (Wink & Legal 2001).

1.3.2 Plant competition

Plant competition is an antagonistic relationship (-/-) between individual plants of one or different plant species. Competition generally changes or stabilizes the composition of mixtures (Silvertown & Charlesworth 2001).

There are several ways to define plant competition:

(1) **Mechanistic definition**: neighbouring plant species compete for the same resource (e.g. water or light)
(2) **Demographic definition**: the net reproductive rate of one or both species is lowered

Competition can be intra- or interspecific, and in some cases it may furthermore be necessary to differentiate between above- and belowground competition (McPhee & Aarssen 2001). In this study, the focus shall be on aboveground competition.

As stated by Watkinson 1997, when trying to measure the effects of plant competition, it is important not only to measure final biomass or growth, but also to take into account the survival and fecundity of plants in mixtures. Another important point to notice is that density-dependent effects can be important in regulating the number and sizes of individuals in plant populations.

In the study presented here, competition levels (0 and 1) will be varied by total removal of the vegetation cover; in a strict sense, this could be regarded a disturbance treatment, but it shall be referrerd to as 'competition' treatment from this point onward, as long as not stated otherwise.

1.4 The special case of *Senecio inaequidens* DC

1.4.1 The main reasons for focussing on this species

Senecio inaequidens offers several unique opportunities as a study object:

- its actual rate of spread in Europe is exceptionally high[10]
- it has close relatives in Europe, and native specialist herbivores might well accept this species, despite the predictions of the 'enemy release hypothesis'[11]
- it contains toxic compounds[12] and might become a 'problem plant[13]' if starting to invade pasturelands and agricultural fields in the near future
- very little is known about its biology
- there are no experimental data on wether or not *S.inaequidens* can invade grassland ecosystems
- without these data, no decisions can be made on how to manage ecological or economical impacts that the invasion of *S.inaequidens* might have

1.4.2 Phylogenetic position[14]

The genus *Senecio* L. (Asteraceae) comprises about 1000-3000 species and has been subdivided into approximately 150 sections (Pelser *et al.* 2002). According to these authors, the genus is presumably "paraphyletic or even polyphyletic". About 180 *Senecio* species can be found in South Africa (Hilliard 1977).

Senecio inaequidens DC belongs to the section Fruticulosi DC and the tribe Senecioneae within the family Asteraceae (Clapham *et al.* 1987). The type specimen of *Senecio inaequidens* DC was collected in South Africa by Drège (Nr.5879, Herbarium G-DC; Hilliard 1977) and described as *S.inaequidens* by de Candolle 1837.

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[10] Böhmer *et al.* 2001

[11] Keane & Crawley 2002

[12] see Biological characteristics of *Senecio inaequidens*

[13] sensu Crawley 1997a

[14] the term 'phylogenetic' is used here rather than 'systematic' or 'taxonomic'; D.L.J.Quicke, Silwood Park, personal communication.

Synonyms are:

- *S.lautus* auct. non Soland ex Willdenow (Stace 1997)
- *Senecio burchellii* DC. p.p. (Hilliard 1977)

Some of the most often used common names include:

- narrow-leaved ragwort, canary Weed, Molteno disease *Senecio*, Burchell *Senecio* (in English-speaking countries; Bromilow 1995)
- séneçon du Cap (France; Troussel 1998)
- Schmalblättriges Greiskraut, Schmalblättriges Kreuzkraut, Gleiskraut, Ungeradzähniges Greiskraut (German-speaking countries)
- senecione sudafricano (Italy)
- "la escondilla" (Colombia; Herrera 2000)
- Geelopslag (South Africa; Bromilow 1995)

In Europe, *S.inaequidens* has presumably been confused with other *Senecio* species (according to Böhmer *et al.* 2001, Werner *et al.* 1991, and Kuhbier 1977), such as:

- *S.lautus* (Soland ex Forster) A.Richard
- *S.lautus* Forster f. ex Willdenow
- *S.harveianus* Mac Owan
- *S.vimineus* Harvey non DC.
- *S.reclinatus* L.f.
- *S.linifolius* L.
- *S.paniculatus* Berg.
- *S.douglasii* DC.
- *S.burchellii* DC.
- *S.carnulentis* DC., and
- *S.fasciculatus* ssp. *minor* Schlecht.

Several authors, including Radford *et al.* 2000, Radford & Cousens 2000, and Sindel *et al.* 1998 have discussed the phylogenetic of this species and especially its relationship to *Senecio madagascariensis* Poir that has been introduced to Australia from South Africa. A molecular phylogeny of *Senecio* sect. *Jacobaea* (Mill) Dumort including some data on the taxonomic position of *Senecio inaequidens* has recently been published by Pelser *et al.* 2002. Unfortunately, these authors included only *S.inaequidens* out of sect. Fruticulosi into their cladogram for Senecioneae, and further data on members of the Fruticulosi section would be needed to resolve the phylogenetic relationships of *S.inaequidens*.

In a recent yet unpublished phylogenetic analysis, W. Kadereit[15] places *S.inaequidens* together with *S.cryphiactis* and *S.abruptus* into a South African clade, including the Mediterranean *Senecio malacitanus*. This clade again is included into a larger clade with Mediterranean and North American *Senecio* species. On the other hand, Radford *et al.* 2000 conclude from isozyme and morphological studies (including scanning electron micrograph analyses of achene surface morphology) that *S.inaequidens* belongs to a complex of closely related species from South Africa and Madagascar, including *S. madagascariensis* Poir, *S.skirrhodon* DC, *S.burchellii* DC and *S.pellucidus* DC. Stefan Neser[16] names *Senecio harveianus* MacOwan, *S.burchellii* DC, *S.skirrhodon* DC, *S.inaequidens* DC and *S.madagascariensis* Poir as belonging to a complex that has been called the "*S.madagascariensis*/*S.inaequidens* complex" by Scott *et al.* 1998.

1.4.3 Country of origin and natural habitats

Senecio inaequidens is native[17] to South Africa. In the following section, data on the distribution and natural habitats of this species and closely related members of the "*S.madagascariensis* complex" will be given, according to Adolphi 1997, Hilliard 1977 and personal communication with Dr.S.Neser[18] and O.Bossdorf[19]. It is likely that hybridizations between *Senecio inaequidens* and other members of this complex have occurred, and many of the data given below may have to be revised and confirmed by additional observations, including biochemical traits and DNA fingerprinting.

The natural habitats in South Africa lie in the high-altitude so-called 'Highveld' areas (c. 1400 m – 2850 m above sea level, Hilliard 1977) in Freestate, Lesotho, Natal, Gauteng, as well as in the Northwest Province, Mpumalanga and the Northern Province of South Africa. Hilliard 1977 describes the original habitats of *S.inaequidens* as follows:

"Its natural habitat in Natal is among rock outcrops on steep, moist, grassy mountain slopes and along rocky watercourses, but it often becomes a weed along roadsides or firebreaks or in trampled or otherwise disturbed areas."

Adolphi 1997 has found *S.inaequidens* on roadsides in the Featherbed Peninsula near Knysa, and in fynbos vegetation on the eastern side of the False Bay in South Africa, more or less close to the sea. Bromilow 1995 reports on weed occurrences of *S.inaequidens* in South Africa in wheat lands and other crop fields, gardens, roadsides and waste places, "mainly in the Cape especially in the Ceres, Middelburg and Prieska areas".

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[15] E-mail correspondence, 09.05.2002

[16] e-mail correspondence, 31.01.2002, Plant Protection Research Institute, National Department of Agriculture, Pretoria, ZA

[17] wether or not it can be regarded as being endemic to the Cape floral kingdom needs to be discussed in the future

[18] E-mail correspondence, 31.01.2002, Plant Protection Research Institute, National Department of Agriculture, Pretoria, ZA

[19] E-mail correspondence, 4.4.2002, UFZ Centre for Environmental Research Leipzig-Halle, Halle, Germany

According to Neser[20], herbarium records from the National Botanic Institute (South Africa) indicate that

- *S. inaequidens* DC and the closely related *S.burchellii* DC have a rather similar distribution "from the South West Cape Province diagonally across to near the North of the country, with a few records from Southern Namibia and adjacent areas";
- *S.burchellii* was "relatively often" collected in the Western Cape area in the vicinity of Cape Town, whereas *S.inaequidens* records are "almost absent" in that part;
- *S. madagascariensis* POIR has been collected mainly near and along the Southeast coast from the Southern Cape area through KwaZulu-Natal with a few inland occurrences in Mpumalanga Province, in Gauteng, and also in the North of Northern Province of South Africa;
- *S.skirrhodon* is recorded as occurring only locally on the coast of the Eastern Cape and Mpumalanga Provinces;
- *S.inaequidens*, S.*burchellii* and *S.madagascariensis* have also been collected in Pretoria.

1.4.4 Invasion history

1.4.4.1 World (excluding Eurasia)

Due to the still unresolved phylogenetic position of *Senecio inaequidens* – and especially its unclear relationships with *S.lautus* (Soland ex Forster) A.Richard and *S.madagascariensis* Poir. (see section 1.4.2, p. 6) – there are still no reliable data on the distribution and the timing of arrival of *S.inaequidens* in areas outside Eurasia.

However, there is growing evidence that populations of *S.inaequidens* might already have established in parts of South America, Australia and New Zealand. Kuhbier 1977 and Stengl 1982 report on occurrences of *S.inaequidens* in Argentina and South Australia. Further support for occurrences in these countries comes from internet sources[21]. Asmus 1988 (cited in Wieners 1994) furthermore reports on possible occurrences in New Zealand.

In Colombia, *Senecio inaequidens* already seems to occur in agricultural ecosystems. Herrera 2000 and 2002 (unpublished) reports on successful biological control of *Senecio inaequidens* in Colombia, using the pyralid moth *Homoeosoma oconequensis* DYAR (Lepidoptera: Pyralidae). Records of *Senecio inaequidens* from Colombia are also supported by data presented by Robinson *et al.* 2002. In summary, there are indications that *S.inaequidens* or closely related species out of the '*S.madagascariensis* complex' already occur in

- (South) Australia
- New Zealand
- Argentina
- Colombia

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[20] E-mail correspondence, 31.01.2002, Plant Protection Research Institute, National Department of Agriculture, Pretoria, ZA

[21] http://www.school.za/projects/ptw/description.htm

Other parts of South America, especially in the Andean region, as well as regions with mediterranean or temperate climate, are likely to be invaded in the future.

1.4.4.2 Eurasia

S.inaequidens has been introduced to Europe together with wool transports from South Africa (Schmitz & Werner 2000). The earliest documented occurrences have been summarized by Ernst 1998 and Kuhbier 1977; the first European herbarium specimen have been collected around 1896 in Bremen (North Germany) near wool washeries. At all locations *S.inaequidens* has first been recorded from the vicinity of wool washeries or other wool-processing factories.

In the initial phase of spread, there have been at least five dispersal centres located at Mazamet (Southern France), Calais (Northern France), Verona (Italy), Liege (Belgium) and Bremen (Germany) - see Ernst 1998,Werner *et al.* 1991 and Kuhbier 1977 for details. Since the 1970s, *S.inaequidens* has been spreading quickly throughout parts of Central, Western and Southern Europe. The rapid spread of *S.inaequidens* has been explained by its ability to migrate along motorways and railway tracks, and this process has been well documented by Griese 1998 and 1996.

A detailed summary describing the whole process of invasion of *S.inaequidens* in Central Europe (with focus on Germany) has been published by Böhmer *et al.* 2001.

At the moment (August 2002), *Senecio inaequidens* has been recorded from

- Spain (especially Catalonia, see for example Vicens 1996)
- France ("Montpellier", Troussel 1998; "Mazamet", Gaida & Schneider-Gaida 1999; "dunes near Calais", De Langhe *et al.* 1973, cited in Kuhbier 1977)
- Italy ("Verona", Pignatti 1982, cited in Brandes 1999; "widespread in the Northeast", Bicchi *et al.* 1985; "Lago di Garda", collected by Stengl 1982; at high altitudes > 1000m (Kuhbier 1977))
- Denmark (Henker 1996, cited in Schmitz & Werner 2000)
- Belgium (cited in Ernst 1998)
- The Netherlands (Ernst 1998)
- Luxembourg (Colling & Reichling 1996, cited in Schmitz & Werner 2000)
- Switzerland (Schmitz & Werner 2000)
- Austria (Polatschek 1984, cited in Schmitz & Werner 2000)
- Poland ("Kattowice", Ernst 1997, unpublished, cited in Ernst 1998)
- Norway (Often 1997, cited in Schmitz & Werner 2000)
- Finland[22]
- Great Britain ("Dover", "R[iver] Tweed", "S[outh] Scotland", Clapham *et al.* 1987).

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[22] http://www.helsinki.fi/lehdet/uh/299b.htm

1.4.4.3 Germany

A large amount of data has been published describing the history of introduction and the dynamics of spread of *S.inaequidens* in Germany. Detailed summaries can be found in Radkowitsch 1997 and Werner 2000. The initial phase of spread seems to have started from two dispersal centres, one in Northern Germany (Bremen; Kuhbier 1977) and one in Western Germany (Aachen, Cologne; Radkowitsch 1997). According to Werner *et al.* 1991, the city of Hannover has recently become a major new dispersal centre for *S.inaequidens*. Clearly, North Rhine Westphalia and Lower Saxony show the highest number of records per square. There seems to be a strong correlation between the density of major transport pathways (motorways, railway tracks etc), and the current distribution of this species.

For Germany, the easternmost occurrences are in Eastern Saxony (Niederschlesischer Oberlausitzkreis, near the river Lausitzer Neiße, Kartenblatt (TK25): 4655 Rothenburg[23] (Oberlausitz), and on the island of Rügen (Mecklenburg-Vorpommern, Kartenblatt (TK25): 1447 Saßnitz[24]; Litterski & Berg 2000).

1.4.4.4 United Kingdom

For the UK, occurrences of *S.inaequidens* have been recorded from Scotland (Edinburgh), and also from some counties in the South, especially Kent.

In Berkshire, where the experiments for this study were performed, there are up to now two locations at which *S.inaequidens* has been found[25]:

a) East Berkshire: as an escape from the Botanic Gardens in Whiteknights Park [7371] in 1988 (Herbarium RNG in Reading).

b) West Berkshire: at Abingdon [49] in 1917 (Plant Sciences Herbarium OXF, Oxford).

1.4.5 Biological characteristics

1.4.5.1 Morphology and life form

According to Clapham *et al.* 1987 and Hilliard 1977, members of the genus *Senecio* L. may be characterised by the naked, epaleous receptacle, the smooth pappus consisting only of simple hairs, and the uniseriate involucral bracts. The ray florets are female, whereas the disc florets are hermaphrodite.

a) general morphology

Senecio inaequidens is a subglabrous, erect or often spreading chamaephytic perennial of up to 80-100 cm height[26], often forming stems branching from a more or less woody base (in contrast, *S.madagascariensis* forms stems that are "often simple below", Hilliard 1977). The up to 50 (own observations) 1st order branches are often branching again several times, making the plants appear bushy. Basal branches with contact to the soil

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[23] Datenbank Gefäßpflanzen am Bundesamt für Naturschutz und Zentralstelle für Phytodiversität, Germany, distribution map from 12/1999; "TK" means topographical map with scale 1:25,000

[24] see [23]

[25] M.Crawley, e-mail correspondence, 3rd September 2002

[26] Under greenhouse conditions, a maximum height of 210 has been recorded (own observations)

surface are capable of producing adventitious routs; branches or parts of the stem that have been bent over and/or wounded are also capable of forming adventitious roots.

b) leaf morphology

The leaves produced under high light intensities and moderate to low soil water content are approximately 2.5 cm long and 1-2(-7) mm wide (Clapham *et al.* 1987, Adolphi 1997), sessile, (mostly) linear with more or less entire or slightly irregular denticulate margins and with an auriculate, half-amplexicaulous base.

Leaf morphology is highly variable, presumably depending on light intensity, soil water potential, relative humidity and plant age (own observations). In low light intensity, especially under altered UV regime and greenhouse conditions, individuals of the same ecotype differ significantly in leaf morphology from the description given above; they produce leaves that are often up to 15 mm broad and more than 15 cm long (**Plate 10 a**), and even serratulate forms with teeth of about 10 mm length can be found (own observations; see **Plate 7 b**). In the leaf axils, *S.inaequidens* forms tufts of lanceolate axillary leaves that may grow out to form additional axillary shoots. The leaves are amphistomatic (Wieners 1994).

c) capitula morphology and structure

The flower heads (**Plate 1 a**) measure 2-2.5 cm in diameter (Clapham *et al.* 1987). There are (7-)13(-15) clear yellow ("canary yellow", Hilliard 1977) ray-florets, each 5-8(-10)mm long (Stace 1997), approximately 70 clear yellow disk-florets (de Candolle 1837) and 10-20 short outer involucral bracts about (4-)5(-7) mm long (Hilliard 1977). The involucral bracts are keeled, with 1-3 nerves (Hilliard 1977) and fimbriate margins (Clapham *et al.* 1987). *S.inaequidens* shows only few calyculus bracts, which are often dark-tipped, non-overlapping and "much shorter than the involucre" (Hilliard 1977). The involucre itself is campanulate in shape.

d) achene morphology

The achenes are cylindrical, 2-2.5 mm long (Hilliard 1977) and pubescent between the ribs (Hilliard 1977, de Candolle 1837). Achene surface micromorphology is used for classification .

e) chromosome number

The base chromosome number is 2n=40 (Clapham *et al.* 1987).

1.4.5.2 Reproductive biology

a) pollination

S.inaequidens is mostly pollinated by

- Hymenoptera (Apidae, especially *Apis mellifica* and *Bombus* spec.) and
- Diptera (especially Syrphidae; **Plate 1 a**).

Other flower-visiting (but not necessarily pollinating) insects include

- Coleoptera (presumably pollen-feeding),
- Heteroptera (mostly achene-feeding or predatory) and
- Lepidoptera (e.g. *Pieris brassicae* L., photograph in Wieners 1994).

Although a research group at Montpellier (France; Prof. Isabelle Olivieri) is currently working on pollination biology and reproductive biology of *S.inaequidens*, there are up to now no data available on wether this species shows self-incompatibility (as is the case e.g. for *S.squalidus* L. - see Hiscock 2000), or not.

b) dispersal and germination

Ernst (1998) found 26 ±5 capitula in small plants (n=50), and 230 ±45 (n=50) in tall, at least 2-year-old plants. Basilowski 1993 (cited in Werner 2000) found 1590 ±1537 capitulae per plant (range: 0 to 6084). All achenes in one capitulum ripen at the same day. Achene development takes approximately 17-35 days from fertilization until achene release; the achenes have a high wind dispersal ability, resulting from low settling velocities of approximately 0.3-0.4 ms-1 in calm atmosphere (Ernst 1998). However, Ernst 1998 was not able to find large dispersal distances in his experiments with three experimental plants (in which the furthest distance recorded was 7.6 m). There are approximately 50-80 (Ernst 1998), sometimes up to 94 ±16.4 achenes per capitulum (Basilowski 1993, cited in Werner 2000).

According to Basilowski, the trichomes on the ribs of the achenes secrete a mucilage when in contact with water, which presumably serves as an adhesive to the soil substrate.

The temperature optimum for germination (which only occurs under daylight conditions) is approximately 20 °C, and a two-week period with temperatures of –23°C has lead to a 50% reduction in the number of viable achenes (Wieners 1994). A storage time of less than three years at room temperature did not affect seed viability, but longer storage times lead to a reduction to only about 10% viable seeds (Wieners, 1994).

c) flowering time

In greenhouse experiments performed by Ernst 1998, plants have started flowering 79 days after sowing, independent of the temperature regime. In Europe, flowering may start as soon as May and last until December (Ernst 1998), whereas in its native range in South Africa, *S.inaequidens* flowers “mainly from October to February but can be found in flower in any month” (Hilliard 1977).

For Central Europe, there seem to be two main flowering periods – one from mid-July to mid-August, and one from mid September to mid October (Ernst 1998), and some authors have claimed to have observed a shift in flowering time towards an adaptation to lower temperatures (mainly, a shift from late [August] to early [May] start of the flowering period – Ernst 1998).

However, it should be noted that *S.inaequidens* occurs in high altitudes in its native range, and therefore may already have pre-adaptations to temperatures below 0°C (Wieners 1994, p.8).

1.4.5.3 Herbivore spectrum and pathogens

(1) In South Africa

In the natural habitats of *Senecio inaequidens* in South Africa, a wide variety of insects can be found developing externally on roots, leaves, stems, flowers and seeds of this species. According to S.Neser[27], various stem- and flower-head borers and several different Diptera species developing in the capitulae have been recorded. Bossdorf[28] has recorded the frequency of feeding holes of capitula-feeding insects, but these data are still unpublished. The most recent overview dealing with insect herbivores in the '*Senecio madagascariensis* complex' has been presented in an unpublished project report (Marohasy 1991, see Marohasy 1989 for a short version). It remains unclear which proportion of the specimens found in the native range are specialist or generalist herbivores, and there are up to now no data on the relative abundances of members of different herbivores feeding on *Senecio inaequidens* and related species in the natural habitats in South Africa.

Bromilow 1995 writes that, in its native range in South Africa, "grazing animals [i.e. large vertebrate herbivores] find *Senecio* [*inaequidens*] unpalatable and will usually only eat the plants by accident".

(2) In Europe

In Central European populations, Ernst (1998) observed "achene consumption by granivorous birds" and reports on "seed-predating heteropterans, e.g. *Nysius senecionis* and *Strictopleurus punctatonervosus*" and *Tyria jacobaeae* (Lepidoptera: Arctiidae) as "biological means which may contribute in the long term to the regulation of the population size" of *Senecio inaequidens*.

Werner 2000 reports on sheep feeding on flower head buds and flowering capitulae. Wether other large vertebrate herbivores feed on *S.inaequidens*, is still an unresolved question.

Mazel & Garrigue 2000 found endophytic larvae of a pterophorid moth (*Platyptilla farfarella* Zeller, Lepidoptera: Pterophoridae) on *S.inaequidens*, which had up to then only been recorded from annual *Senecio* species. They also conducted some feeding trials with *Tyria jacobaeae*, which will be discussed later.

The most important study dealing with phytophagous insects on *Senecio inaequidens* has recently been published by Schmitz & Werner 2000. They found "sixty-two phytophagous arthropod species from 6 orders, 10 families and at least 48 genera" on *S.inaequidens*.

Schmitz and Werner confirmed larval development for at least 25 species colonizing *S.inaequidens*, six of which were endophagous. The most important results of their study are:

- there are **at least three monophagous** (i.e. *Senecio*-specific) phytophagous insects that have been found on *S.inaequidens*, including *Tyria jacobaeae* (Lepidoptera: Arctiidae; **Plate 2 a,b; 3a; 7a**)
- there are **at least eight oligophagous** species, including three species of *Longitarsus* (Coleoptera: Chrysomelidae)

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[27] E-mail correspondence, 31.01.2002, Plant Protection Research Institute, National Department of Agriculture, Pretoria, ZA)

[28] E-mail correspondence, 4.4.2002, UFZ Centre for Environmental Research Leipzig-Halle, Halle, Germany

Based on these data, it was decided to use *Tyria jacobaeae* and *Longitarsus jacobaeae* (**Plate 1 b**) as herbivores in this study, as at least *Tyria* had already been found colonizing European *S.inaequidens* populations.

The only pathogens that have been found infecting *S.inaequidens* up to now are two rust fungi (Basidiomycota, Teliomycetes: Uredinales), *Puccinia lagenophorae* Cooke and *Coleosporium senecionis* Fr. ex J. Kickx f. (probably wrongly classified as *C.senecionis* (Pers.) J.Kickx) (Schmitz & Werner 2000).

(3) New World

Herrera 2002 (unpublished) conducted some experiments on biological control of *S.inaequidens* in Colombia. He successfully used larvae of an **monophagous pyralid moth**, *Homoeosoma oconequensis* Dyar (Lepidoptera: Pyralidae), a species native to Peru and presumably also to Colombia[29]. The author writes that – depending on the habitat studied - about 10 to 50 per cent of the capitulae of *S.inaequidens* have been colonized by this species within a two-week observation time.

1.4.5.4 Physiology, biochemistry and secondary metabolites

(1) Stress physiology

A thorough observation dealing with drought resistance, stomatal conductivity and germination parameters of *S.inaequidens* (in comparison with *S.jacobaea*) has been conducted by Wieners 1994. *S.inaequidens* shows high rates of transpiration and high stomatal gas exchange rates. Under drought stress, transpiration rates are lowered significantly to a level of approximately 5% of the normal rate, i.e. only cuticular transpiration occurs if soil water potential or mean leaf temperatures are high.

(2) Pyrrolizidine alkaloids

The focus in biochemical research in *S.inaequidens* has been on pyrrolizidine alkaloids. They are colourless, optically active alkamine esters of hydroxylated 1-methylpyrrolizidines, and share a common so-called pyrroline structure (Marquardt & Schaefer 1997). Pyrrolizidine alkaloids play a significant role in plant-herbivore interactions in that they serve as feeding deterrents for most herbivores (Hartmann 1999).

As various studies have shown, pyrrolizidine alkaloids and their N-oxides have hepatotoxic (veno-occlusive liver disease), carcinogenic, teratogenic and possibly even pneumotoxic and immunotoxic effects on humans and some vertebrates (e.g. Rubiolo *et al.* 1992, Stewart & Steenkamp 2001, Prakash *et al.* 1999). Even mutagenic or genotoxic effects to some insects and yeast have been reported (Naumann, Hartmann et al 2002). According to Stewart & Steenkamp 2001, pyrrolizidine alkaloids can be found mainly in members of the Fabaceae (*Crotalaria*), Boraginaceae (*Heliotropium*, *Symphytum*, *Cynoglossum*, *Amsinckia*, *Echium*) and Asteraceae (Senecioneae, Eupatorieae). In total, over 13 families with about 300 plant species have been found to contain pyrrolizidine alkaloids (Prakash *et al.* 1999).

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[29] e-mail correspndence with Kevin R.Tuck, Collections Management Division, Entomology Department
The Natural History Museum, Cromwell Road, London SW7 5BD, UK

Pyrrolizidine alkaloids from *Senecio burchellii*, a species often regarded as synonymous to S.*inaequidens*, have already been found in contaminated flour and lead to death of humans in at least two cases (Stewart & Steenkamp 2001).

The most extensive studies on pyrrolizidine alkaloids in *Senecio inaequidens* have been performed by Bicchi *et al.* 1985, and, with a focus on mutagenicity, by Rubiolo *et al.* 1992. Using capillary gas chromatography and mass spectrometry, Bicchi and colleagues identified senecivermine, senecionine, integerrimine and retrorsine in *S.inaequidens*. Stengl 1982 comes to similar conclusions, using thin layer chromatography, IR spectroscopy, and ^{1}H and ^{13}C NMR spectroscopy.

Ernst 1998 states that the major difference in secondary metabolites between *S.inaequidens* and, for example, *S.jacobaea* and *S.sylvaticus* is the high proportion of retrorsine that can be found in *S.inaequidens*.

It has been suggested that senecionine N-oxide is synthetizised as a backbone structure in the root; after that, it is subsequently transported to other plant organs, where it is chemically modified (Naumann *et al.* 2002; Hartmann & Dierich 1998).

Pierson *et al.* 1977 write that rabbits show a low absorption of the pyrrolizidine alkaloids of *Senecio jacobaea* after ingestion, and rabbits seem to be resistant to chronic *Senecio* intoxication.

1.4.5.5 Plant sociology

As there is still a lack of available data on sociological characteristics of *Senecio inaequidens*, in the following section the focus will be on observations from Germany.

According to Oberdorfer 1994, at locations in Germany *Senecio inaequidens* may occur in ruderal habitats on soils with more or less low water content, and can be found in plant sociological units like Artemisio-Tanacetum (Dauco-Melilotion), Agropyretalia, Convolvulotalia and Arrhenateretalia.

The most extensive study on plant communities in which *S.inaequidens* occurs has been published by Werner *et al.* 1991. They found *S.inaequidens* in the following major groups of vegetation types[30] (nomenclature follows Schubert & Vent 1990):

- Dauco-Melilotion Görs 1966
- Sisymbrietalia J.Tx.1961 em. Görs 1966 and Chenopodietea Oberd. 1957 em. Lohm., J. et R.Tx. 1961 (Sisymbrion Tx., Lohm. et Prsg. 1950)
- Arrhenaterion elatioris Br.-Bl 1925
- Sedo-Scleranthetea Br.-Bl. 1955
- Polygonion avicularis Br.-Nl. 1931
- Convolvulo arvensis-Agropyrion repentis Görs 1966 and Agropyro-Rumicion Nordh. 1940
- *Calamagrostis epigejos* communities

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[30] Werner *et al.* 1991 did not give details on what their nomenclature of plant communities was based on, but it is assumed that they referred to Schubert & Vent 1990 or a similar reference book.

- Sambuco-Salicion caprae Tx. et Neum. 1950
- moist areas, including populations of *Molinia caerulea* (Molinietalia W.Koch 1926), and members of the Bidentetea tripartitae (Br.-Bl. ex Tx. 1943) and Phragmitetea Tx ex Prsg 1942
- other species-poor habitats; recultivated brown coal slagheaps, and ruderal coastal habitats
- wind throw areas in forests

In a more recent publication on this topic, Werner 2000 mentions a number of new occurrences of *S.inaequidens* in different plant communities.

Several authors (e.g. Brandes 1999, but also Werner *et al.* 1991) have mentioned that *S.inaequidens* is – at least in some areas- already invading fallows, pasturelands or vineyards, and this might become a significant problem from an economical point of view (see section 1.4.7 below). This tendency is meanwhile supported also by photographical documents from Colombia.

1.4.6 Possible ecological impact

It is still unclear wether *S.inaequidens* may have any effect on the species composition of native plant communities. However, there is some evidence that dense populations of narrow-leaved ragwort are already colonizing rock vegetation, crevices and rock terraces in the Rhenish Slate Mountains and the Southern Eifel region in Germany[31], and that this might alter the structure of *Oedipoda* spp. (Insecta: Orthoptera, Caelifera) habitats.

1.4.7 Possible economical impact

Poisoning of humans and livestock by members of the genus *Senecio* has frequently been recorded. As stated by Stewart & Steenkamp 2001, the most common form of human poisoning is "bread poisoning", i.e. the ingestion of grain accidentally contaminated with pyrrolizidine-containing weeds. Pyrrolizidine alkaloids have furthermore been found in milk (Stewart & Steenkamp 2001, Marquardt & Schaefer 1997), honey (Deinzer *et al.* 1977), and in traditional medicines prepared from *Senecio*. Even an uptake through the skin has been recorded (Stewart & Steenkamp 2001).

According to Prakash *et al.* 1999, large outbreaks of human poisoning have occurred "through contamination of wheat crops in Afghanistan, India and the former USSR". Cattle poisoning has especially been recorded in the USA and in Australia. In Central Queensland, 226 cattle of 10 herds died between 1988 and 1992 (Noble *et al.* 1994), equalling a death rate of 8% (ranging from 2 to 58%), after access to large stands of *Senecio lautus*, a close relative of *S.inaequidens*.

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[31] mentioned in an internet text document provided by the German National Government, www.global-society-dialogue.org/dgsk/anfrage.pdf

1.5 Current research and the objectives of this study

1.5.1 Overview

Although a large amount of literature has been published up to now on various aspects of the biology of *Senecio inaequidens*[32], most of the key questions have not been answered yet. These unresolved questions are, for example:

- how is *Senecio inaequidens* affected by native herbivores?
- how does *S.inaequidens* respond to competition with other plant species?
- can native specialist herbivores perform host-switching, i.e. will they accept *S.inaequidens* as an alternative food source?
- what might the future look like? Will *S.inaequidens* stay a "railway and road alien" (Ernst 1998), or will it – due to its toxic compounds - become a 'problem weed' affecting livestock populations and crop production?

In this study, the focus is on the effect of herbivores native to Europe (United Kingdom), rather than using herbivores from South Africa (as would be the case in a study on classical biological control)[33].

The experiments for this study have been performed at Imperial College, Silwood Park, Ascot, United Kingdom.

1.5.2 Hypotheses

- *Senecio inaequidens* is capable of invading grassland ecosystems
- Herbivores and interspecific plant competitors affect the population dynamics of *Senecio inaequidens*
- Oligophagous insect herbivores of *Senecio jacobaea* can perform host-switching and accept *Senecio inaequidens* for feeding and reproduction
- Vertebrate herbivores do not feed on *Senecio inaequidens*

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[32] for an overview, see Böhmer *et al.* 2001

[33] see Simberloff & Stiling 1996 for risks associated with biological control

2 Material and Methods

2.1 Living organisms and how they were obtained

2.1.1 Plants

2.1.1.1 *Senecio inaequidens* DC. (Asteraceae)

Seeds of *Senecio inaequidens* were obtained by collecting fresh achenes in ruderal habitats around Rostock (Germany, Mecklenburg-Vorpommern), from commercial suppliers, non-commercial plant breeders, and from Botanical Gardens throughout Europe.

Seeds from the Rostock locations were harvested by detaching whole ripe capitulae and fruits (achenes with pappus) from *Senecio inaequidens* plants. They were transported in small plastic bags and transferred to DIN C3 paper envelopes for drying and storage at room temperature. After drying, the harvested capitulae were cleaned in a sieving process with brass test sieves. and stored after that at room temperature (ca. 22 °C). Seeds from commercial and non-commercial suppliers were stored at room temperature before sowing.

The *Senecio inaequidens* achenes (with pappus removed) were either sown out on filter paper following the procedures given in section 2.2.2 (page 23), or directly on multi-purpose compost (Levington Multi-Purpose Compost, „Premium Blend“ with N,P,K and Trace Elements, containing MBI600 *Bacillus subtilis* strain; Levington Ltd, Paper Mill Lane, Bramford, Ipswich, 1P8 4BZ, United Kingdom) in standard seed trays (376x232x58 mm), containers or pots of different sizes.

The achenes for all of the experiments were sown out on the following dates:

- **germination trials, preliminary feeding trials and pollination experiments:** 29th January 2002 (sown out in petri dishes, and transferred to greenhouses between 4th and 22nd February 2002)
- **greenhouse herbivory/competition experiment:** 9th, 12th and 15th April 2002 (two ecotypes: "Kew Gardens" and "Rostock, Germany, harvested 1.11.2001")
- **field herbivory/competition experiment:** 28th May 2002 (three ecotypes: "Montpellier, France", "Rostock, Germany, harvested 1.11.2001", and "Konstanz, Germany")
- **additional experiments:** 24th April 2002 ("Kew Gardens" ecotype, to obtain a reference culture)

It needs to be noted, that an established culture of *Festuca rubra* was needed for the greenhouse competition experiment prior to transplantation of the *S.inaequidens* seedlings (see p.27). This explains why *S.inaequidens* achenes for this experiment were sown out in April and not directly by the beginning of February.

Seedlings from the germination tests were transferred to seed trays filled with multi-purpose compost after four weeks and grown up under greenhouse conditions. During the first days after transplantation, the seed trays were covered with a transparent plastic lid to reduce evapotranspiration.

All other achenes were directly sown on multi-purpose compost in large container pots and either transferred to the pots used for the greenhouse competition experiment, or to the field plots (see sections 2.2.4 and 2.2.5, p. 27 ff).

2.1.1.2 *Festuca rubra* L. ssp.*rubra* (Poaceae)

Festuca rubra caryopses were obtained from a commercial supplier (Herbiseed, Twyford, UK). For a germination test, the caryopses were sown out in petri dishes on 29th January 2002
For the competition experiment, the calculated number of caryopses per container pot was directly sown out on multi-purpose compost in the greenhouses, on 5th March 2002 (**Plate 5 a; 6 b**).
The amount of caryopses needed for the competition experiment was determined using high-precision digital scales. The mean caryopse weight was taken to be 0.79 mg (Thompson & Grime 1983). As the average proportion of germinated seed was 57% (n=2), 5.00 g caryopses were sown out per container pot to give an estimated density of 1 seedling per cm², or 5.000 seedlings per pot, the surface area for each pot being roughly 0.20 m². Although the *F. rubra* caryopses had been obtained from a reliable commercial supplier, they were contaminated with *Poa trivialis* L.(determination verified by M.J. Crawley); as *P. trivialis* germinated in approximately equal and low numbers in every pot, it was not removed.

2.1.2 Herbivores

2.1.2.1 *Tyria jacobaeae* L. (Lepidoptera: Arctiidae)

2.1.2.1.1 Biology of the species

Senecio jacobaea and related *Senecio* species are considered the main host plants of the larvae (**Plate 2 a**) of this arctiid moth (Tinney *et al.* 1998; Cameron 1935; but see also Wink & Legal 2001). It has been successfully used for biological control of *Senecio jacobaea*, e.g. in North America and Australia (McLaren *et al.* 2000; McEvoy & Rudd 1993; McEvoy *et al.* 1993; James *et al.* 1992; McEvoy & Cox 1991), yet no one has up to now tried to test wether *Tyria jacobaeae* larvae accept *Senecio inaequidens* as food source, and how this might affect the population dynamics of *S.inaequidens*. Only Ernst 1998 mentions the occurrence of *Tyria jacobaeae* on *Senecio inaequidens*, but in a recent e-mail correspondence[34] he writes that, due to a "tremenduous increase" in the population size of *S.jacobaea*, *S.inaequidens* was (at least in 2002) "not attacked by *T. jacobaeae* [in 2002], obviously a preference for *S. jacobaea* is present".
The adults of *Tyria jacobaeae* (cinnabar moth) are aposematically coloured (Wink & Legal 2001; **Plate 2 b**); due to the uptake and sequestration of pyrrolizidine alkaloids from its host plant (Hartmann *et al.* 1999), the cinnabar moth is chemically protected against (vertebrate) predators (Hartmann 1999). The adults generally select the host plant, where they lay egg batches on the underside of leaves; larvae pass through five instars, each of which lasts about 5-6 days, the 5th stadium more than 10-11 days. An excellent description of the biology of *T.jacobaeae* can be found in Cameron 1935.

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[34] W.H.O.Ernst, e-mail correspondence, 12th August 2002

2.1.2.1.2 Experiments

Tyria jacobaeae larvae were primarily obtained in three different ways:

- by collecting adult females and making them lay eggs
- by collecting egg batches from *Senecio jacobaea* in the field
- by directly collecting caterpillars in the field (**Plate 2 a**)

In order to obtain first instar larvae that could be raised directly on *Senecio inaequidens*, two female and two male adults of *Tyria jacobaeae* (**Plate 2 b**) were caught for mating and oviposition trials on the 2nd of June 2002 at Nursery Field (Silwood Park) using a butterfly net. They were transferred to a 1x1m insect cage containing three several-week-old *Senecio inaequidens* plants (Kew ecotype) as well as three *Senecio jacobaea* plants of approximately the same age and size class, each planted in a standard plastic tray in multi-purpose compost. As the egg batches were found attached to the insect gaze rather than to the host plants, and therefore dried out quickly, this trial was stopped. Similar trials should be repeated under field conditions in bigger cages in the future.

Further egg batches were collected in the field – including the leaf lamina of the host plant, *Senecio jacobaea* - at Nash's slope and Nash's field, Silwood Park, on 11th, 12th and 17th June 2002. They were transferred to standard petri dishes and incubated at room temperature on damp filter paper. After hatching, the first instar larvae were transferred to 15 x 10 cm plastic boxes of approximately 8 cm height with a holed lid and on a capillary mat that was kept moist all the time. They were raised in a CT room at 18 °C and a 18:6 hour light:dark cycle. Fresh *Senecio inaequidens* leaves and flower heads were provided as food source and exchanged every two to five days. The leaves were cut from rooted *Senecio inaequidens* stem cuttings that had been cultivated in an extra large 50 cm container pot under standard JIF greenhouse conditions. 2nd, 3rd and the following instars were transferred to potted *Senecio inaequidens* plants on 24th of June and prevented from escape with 60 cm acrylic plastic tubes and insect netting. The larvae raised in this way were used for feeding trials under controlled laboratory conditions.

For the herbivory experiments in the greenhouses and in the field, for which a total of about 500 final instar caterpillars were needed, it was necessary to collect these directly from their host plants, *Senecio jacobaea*, in the field. Collections were made on 9th July 2002 for the greenhouse experiments, and on 17th (plot 2) and 24th (plot 1) July 2002 for the field experiments. Further 5th instar larvae were collected in the field for two-way choice feeding trials.

2.1.2.2 *Longitarsus jacobaeae* Waterhouse (Coleoptera: Chrysomelidae)

2.1.2.2.1 Biology

Like *Tyria jacobaeae*, *Longitarsus jacobaeae* (ragwort flea beetle; **Plate 1 b**) is generally regarded highly host specific (Syrett 1985; Frick 1970), feeding preferably on *Senecio jacobaea.* Only *Longitarsus dorsalis* F., *L.succineus*

Foudr. and *L. ochroleucus* Marsh. have been found colonizing *Senecio inaequidens* up to now (Schmitz & Werner 2000), and it has never been tested wether *Longitarsus jacobaeae* is capable of performing host switching and accepting *S.inaequidens* as host plant.

L.jacobaeae has been used as a biological control agent against *Senecio jacobaea*, mostly in combination with the cinnabar moth (McEvoy & Rudd 1993; McEvoy *et al.* 1993; Windig 1993; James *et al.* 1992; McEvoy & Cox 1991; Frick 1970).

The larvae feed on the roots of the host plants and pass through two instars (Frick 1970) before pupating in the soil near the plants (own observations). Adults feed on the leaves of their host plants, producing characteristical 'shotholes' in the leaf lamina (see **Plate 9 a**).

There are at least two closely related *Longitarsus* species feeding on *Senecio jacobaea* at Silwood Park, *L.jacobaeae* Waterhouse and *L. flavicornis* Stephens; as these are only distinguishable by their micromorphology, no distinction between these species will be made in the following sections; instead, they will be referred to as *Longitarsus jacobaeae* Waterhouse. However, specimens were conserved in specimen tubes in order to facilitate further taxonomic considerations.

2.1.2.2.2 Experiments

Longitarsus jacobaeae adults for feeding trials were obtained in three different ways:

- by collecting adults in the field from *S.jacobaea* in April 2002 (for no-choice feeding trials on whole greenhouse-grown *S.inaequidens* plants)
- by collecting adults in the field from *S.jacobaea* in August 2002 (for laboratory two-way choice feeding trials)
- through allowing free colonization of transplanted *S.inaequidens* seedlings in the field

For preliminary feeding trials, adults of *Longitarsus jacobaeae* were collected on 12th and 21st April 2002 using a battery-driven pooter. Roots and root crowns of *Senecio jacobaea* at Rookery Slope (Silwood Park) were dug out and searched for larvae and pupae on 18th and 19th June. Larvae and pupae were kept in a CT room at 20 °C and a 18>6 h light/dark cycle, until the first adults emerged. Due to free colonization of transplanted *Senecio inaequidens* seedlings in the field plots on 1st of July and the following weeks, the cultivation was stopped.

2.1.2.3 *Oryctolagus cuniculus* L. (Mammalia: Lagomorpha) and molluscs

Due to the high abundance of this species at Silwood Park, **rabbits** could be used directly for herbivory field experiments. They were either excluded or included using rabbit fences (see chapter 2.2.5).

The rabbit density at the locations studied can only be estimated indirectly, and there are only rough estimates of several hundred individuals being present in an area of about 300x300 m. The grazing intensity at these locations is so high, that excrete pellets can be found scattered over the ground, the grasses being cut down to about 2 cm leaf lamina length, and in some places (especially near warrens) the soil surface is disturbed.

Molluscs (mostly *Arion* spp. and *Cepaea hortensis* O.F. Müller) were already present in the experimental plots (field experiment) and it was therefore possible to exclude them experimentally using mollusc pellets (Metaldehyde).

2.2 Experimental design

2.2.1 Overview

Two main experiments were conducted during this study: One **greenhouse experiment** under controlled conditions, and one **field experiment** to test wether similar results could be obtained under 'real world' environmental fluctuations. These observations were made in order to find out if and how *Senecio inaequidens* is affected by the presence or absence of vertebrate and invertebrate herbivores, and by competing plant species (i.e., by **herbivory** and **competition**).

In addition, germination tests, pollination trials, observations on natural populations, as well as a test for vesicular-arbuscular mycorrhiza were performed with *Senecio inaequidens*, and leaf pieces of *S.inaequidens* were offered to insect herbivores under controlled laboratory conditions.

2.2.2 Germination tests

In order to decide which design for the competition experiments would be most suitable, germination tests were conducted in a CT room (constant temperature 15 °C, 16 hours additional lighting, 8 hours dark period). Achenes of *Senecio inaequidens* and caryopses of *Festuca rubra*, as well as some additional plant species not included into the final experiment, were treated as follows (they shall be referred to as "seeds" in the following section):

- Seeds were soaked in a 3,5% aqueous solution of a commercial fungicide („Nimrod-T" double-acting systemic fungicide, 62.5 g/l bupirimate, 62.5 g/l triforine [from American Cyanamid Co.], Zeneca Ltd., distributed by Miracle Garden Care Limited, Godalming, Surrey, UK) for 5 minutes before sowing
- 0.05 g Gibberellic acid (88%, BDH Laboratory Supplies, Poole, BH15 1TD, England) were weighed out using high-precision digital scales, dissolved in 2 ml 99.86% w/v ethanol abs. and diluted with 100 ml purified water after full evaporation of the ethanol, resulting in a 0.5 g/l aqueous solution of GA3.
- Seeds were soaked in this GA3 solution for 5 minutes before sowing
- Seeds were either sown on filter paper (Whatman No.1, 9.0 cm, 0.06% ash content , Whatman Ltd., Maidstone, England) or a 4 mm layer of silver sand in standard 9 cm petri dishes (Bibby Sterilin Ltd., Stone, Staffs, UK) . Purified water was used to moisturize the germination medium.
- The petri dishes were sealed using Parafilm „M" ® 5.08 cm laboratory film (American National Can ™, Chicago, IL); the lids were lifted daily for air circulation;

The petri dishes were transferred to an incubator with 12:12 h light/dark cycle (lamp types: Silvania white F8W/W and Osram L8W/23 white) and constant temperature of 22 °C. Due to a malfunction of the incubator (accumulation of condensate, raised levels of average temperatures up to about 30 °C), they were transferred to a CT room at 15 °C constant temperature (day & night) after two days of incubation.

Every day, seedling emergences were recorded, an emergence being defined as the appearance of the first visible tip of the radicula. The filter paper was kept moist at all times, using purified water. In case of infestation with fungi, the filter paper was replaced, seeds were treated according to a fungicide treatment and re-transferred to a new petri dish. After a maximum period of 31 days, when no more seedling emergences occurred, the germination experiments were terminated and the final number of germinated seeds recorded.
Due to the different amount of seeds available, the total number of seeds sown per petri dish was different for every plant species and ecotype (see results in section 3.1, page 39).

2.2.3 Feeding trials

For all experiments that involved a choice between at least two different food plants under replicated laboratory conditions, it was taken care that these met the criteria of multiple-choice feeding preference experiments, as stated, for example, in Peterson & Renaud 1989.

2.2.3.1 Feeding trials with *Longitarsus jacobaeae*

2.2.3.1.1 No-choice feeding preference experiment

The feeding of adult *Longitarsus* beetles was observed in greenhouse experiments on *Senecio inaequidens* plants grown from seed (date of sowing: 29th January 2002). The plants were kept in flat seed trays and were in the flowering stage when the beetles were applied. Sixteen adult beetles were collected on 12th April 2002 as described in section 2.1.2.2.2, p.22. They belonged to the spring generation of *Longitarsus* which lasted from around 10th April until 1st May 2002.
Feeding behaviour was observed visually, and the survival time was noted. Due to a malfunction of the greenhouse temperature regulation system, the no-choice trials had to be stopped.

2.2.3.1.2 Multiple-choice feeding preference experiments

(1) Preliminary test with five plant species

Twelve adults of *Longitarsus jacobaeae* were collected from the rosette leaves of *Senecio jacobaea* plants in the field on August 7th, 2002, using a battery-driven pooter. Prior to the actual feeding experiment, the beetles were kept in a 30x15x10cm plastic box with loosely covered lid and damp filter paper at the base. *Senecio jacobaea* leaves harvested on 7th August were provided as food source. Care was taken that all the leaves did not show any herbivore damage at the point of harvesting, and that they were all of approximately the same age (leaves from young, non-flowering rosettes). The plastic box with the beetles and their food plant was placed in a CT room at 18 °C under constant 18:6 h light for three days, the food source not being replaced during this time.

The feeding trial itself was started on 11th August 2002 at room temperature and under constant artificial lighting. Whereas *S.inaequidens* leaves were cut from plants cultivated in the greenhouse, leaves of all other plant species used were collected in the field. The plant species offered as food were

- *Senecio inaequidens* DC. and *Senecio jacobaea* L.(as representatives of the genus *Senecio*, and containing pyrrolizidine alkaloids)
- *Hieracium murorum* L. (as representative of the Asteraceae, and not containing pyrrolizidine alkaloids)
- *Pentaglottis sempervirens* (L.) Tausch (as representative of the Boraginaceae, presumably containing pyrrolizidine alkaloids) and
- *Plantago lanceolata* L. (as a plant species not closely related to any of the other species chosen, and co-occuring with *S.jacobaea* at several habitats in Silwood Park).

Roughly equal-sized rectangular leaf pieces (approx. 1.5x1.5 cm) were cut from freshly collected leaves[35] of each plant species and moistened with mineral water. Twelve petri dishes (9 cm diameter) with damp filter paper were arranged on a working desk (**Plate 3 b**). For every petri dish, each one leaf-piece of every plant species was randomly assigned to a position in one of five equal-sized sectors, and one beetle was placed in the centre of this arena.

(2) 2-way choice test with *S.inaequidens* and *S.jacobaea*

Twenty-four *Longitarsus* beetles were collected in the field as described above, and starved overnight for 12 hours in a plastic box prior to the feeding trial. Water was supplied in order to allow the beetles to drink as necessary.

Leaf-disks of 6 mm diameter were cut from freshly collected leaves of *S.inaequidens* (from greenhouse plants) and *S.jacobaea* (from plants growing in the field), using a punch (Universal Office Supplies). Each one leaf-disk of each species was offered to one *Longitarsus* beetle in standard 9 cm petri dishes on damp filter paper under constant artificial light on a working desk. In order to exclude observer bias, the leaf disks were randomly labelled "A" or "B" and plant species identity was noted elsewhere (double-blind strategy).

2.2.3.2 Feeding trials with *Tyria jacobaeae*

2.2.3.2.1 No-choice feeding preference experiments

A series of no-choice tests was conducted with whole plants grown in greenhouses.

- on 24th June, several **egg batches** of *Tyria jacobaeae* (collected as described in section 2.1.2.1.2, p.21) were added to each 6-9 potted one-month-old *Senecio inaequidens* plants in cages consisting of clear acetate tubes covered with insect gaze
- **2nd and 3rd instar caterpillars** were collected in the field on 21st June and immediately transferred to a 50x50 cm insect cage consisting out of a metal frame covered with gaze and with nine *S.inaequidens* (sowing date: 28th May) in a single pot in the centre

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[35] young leaves with no visible herbivore damage, as described above

- on 9th July 2002, a total number of **300 caterpillars** were collected from flowering individuals of *Senecio jacobaea* in the field (**Plate 2 a**), and transferred to randomly chosen container pots (see section 2.2.4, p.27)

The 300 caterpillars were divided into three size classes by visual inspection. For ten container pots (50 cm diameter, with clear acetate caging, see **Plate 6 a**), each 30 caterpillars were applied (five of the smallest size class, fifteen of the medium size class, and ten of the biggest size class). The size classes corresponded to the following larval instars of *T.jacobaeae*:

- **size class 1**: 2nd instars (no 1st instars were present)
- **size class 2**: 3rd and 4th instars
- **size class 3**: 5th instars

These instars were easily distinguishable with the experience of previous experiments, especially because it had been possible to observe the transition between the different instars from the egg stage onward under laboratory conditions.

In all no-choice tests, feeding behaviour was documented, the amount ant type of plant tissue eaten was estimated by visual inspection, and the number of surviving caterpillars and plants was noted.

2.2.3.2.2 Multiple choice feeding preference experiments

Three different multiple choice tests were performed:

a) a **preliminary test** with flowering capitulae of five different plant species (n=12)
b) two **two-way choice trials** with flowering capitulae/leaf disks of *Senecio inaequidens* and *S.jacobaea* (n=24)
c) a **free choice test under field conditions**, with five larvae per plant in special enclosures in the field (64 plants in total, equalling 320 caterpillars that were applied)

The **preliminary test** with only n=3 replicates was performed in order to study host-seeking and feeding behaviour of *T.jacobaeae* which had previously been raised on *S.inaequidens*.

A **two-way choice feeding trial** with n=24 replicates was conducted on 20th August 2002. Leaf disks with 6 mm diameter were punched from leaves of *S.inaequidens* (5-months-old greenhouse-grown plants) and *S.jacobaea* (basal rosettes from plants growing in the field at Silwood Park). They were floated in distilled water before being positioned at random in 9 cm petri dishes on moist filter paper. Each two leaf disks per species were applied to one petri dish; they were numbered clock-wise, species identity being noted elsewere in order to reduce observer bias. Similarly, a 2-way choice test with capitulae was performed (**Plate 3 a**).

The **free choice field experiment** was started on 17th July 2002. In each of the two experimental field plots (see section 2.2.5), each two *Tyria* larval enclosures (**Plate 4 b**) measuring about 5x8 metres were erected, using bamboo sticks and clear polythene sheeting (125 µm). Each five final instar larvae were applied to randomly chosen plants within the enclosures. They were free to forage within the enclosures; to give them an equal choice between *Senecio jacobaea* and *S.inaequidens* seedlings, all flowering stems of *S.jacobaea* plants were cut down to the same height as the *S.inaequidens* plants (simulating a depletion of the original host plant, *Senecio jacobaea*). For details on the experimental design see section 2.2.5, p.31.

2.2.4 Greenhouse experiments

In total, six computer-controlled[36] greenhouse chambers (so-called JIF greenhouses[37] located at Silwood Park; **Plate 5 a; 6 b**) were used for the experiments. Two large ones (referred to as block 10/8 and 10/9) were chosen for herbivory/competition studies, whereas four smaller ones (referred to as block 10/1, 10/3, 10/5 and 10/6) were used mainly for pollination experiments. All greenhouse chambers had main and side vents[38], heating, supplementary lighting[39], aluminium screen, tap water supply and three large aluminium benches. To prevent aphids and other small insects from entering, insect gaze was spanned across the window frames.

The temperature regime for all blocks was set to 22.0 °C (day) and 15.0 °C (night). Supplementary lighting (400 W high pressure sodium or mercury) was switched on between 08.00 a.m. and 10.00 p.m. The lights were switched off if natural radiation exceeded 700 W/m^2. Ventilation (corridor and side vents) during daytime was started at temperatures above 25 °C, at nighttime the temperature threshold was 18 °C.

The design for the greenhouse herbivory/competition experiment was as follows:

- n=40 pots (50 cm diameter) were randomly chosen out of a population of 50 pots in total, using a random number generator. The 50 pots had previously been arranged and numbered in the greenhouse compartments 10/8 and 10/9
- each of the 40 pots was randomly assigned one of the following treatments:

Senecio inaequidens (4 individuals per pot)	n = 5 [control]
+ *Festuca*	n = 5
+ *Tyria*	n = 5
+ *Longitarsus*	n = 5
+ *Tyria* + *Longitarsus*	n = 5
+ *Festuca* + *Tyria*	n = 5
+ *Festuca* + *Longitarsus*	n = 5
+ *Festuca* + *Tyria* + *Longitarsus*	n = 5

In this sense, the type of the greenhouse competition experiment was additive (sensu Gibson 2002 and Gibson et al. 1999), with the density of *Senecio inaequidens* held constant, and a varying density of *Festuca rubra* (**Plate 5 b**)

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[36] Van Viet Automation Ltd., Computer GPK 2000

[37] Cambridge Glasshouse Company, Wallington Business Park, 236 Main Road, Newport, Brough, East Yorkshire, HU15 2RH

[38] electric drive 100 Nm/3.1 U; Lock Antriebstechnik, D-88521 Ertingen/Germany

[39] Philips Belgium HPI-T Plus 400W & SON-T AGRO 400

As the cultivation and propagation of enough *Longitarsus* individuals proved too difficult, the design was collapsed to n=10 treatments involving *Festuca* and *Tyria.*, n=10 treatments involving only *Festuca*, and n=10 treatments involving a combination of both:

Senecio inaequidens (4 individuals per pot)	n = 10 [control]
+ *Festuca*	n = 10
+ *Tyria*	n = 10
+ *Festuca* + *Tyria*	n = 10

For the herbivory /competition studies, large plastic container pots (46 cm maximum inside diameter; 43 l Heavy Duty Container Pots, 36 cm depth, LBS Horticulture Ltd., Cottontree, Nr.Colne, Lancs., UK) were used. The arrangement of the experimental pots in one of the greenhouse compartments is shown in **Figure 1.** The containers were placed on capillary matting underlain with clear polythene sheeting (gauge 125 µm) in order to keep the base of the pots moist at all times.

The container pots were filled with multi-purpose compost between 17th February and 4th March 2002 and watered as necessary with tap water in order to achieve an equilibrium of microflora and a homogenous substrate moisture prior to sowing of the seeds.

Unfortunately, the compost was contaminated with fungus gnats ('Trauermücken", Diptera: Sciaridae) , which – due to root- and shoot feeding of the larvae - proved to be a problem when the first seedlings emerged. Each 14 yellow sticky traps (Levington Horticulture Ltd., Paper Mill Lane, Bramford, Ipswich, Suffolk IP8 4BZ, UK) were installed in each of the greenhouse compartments 10/8 and 10/9 on 8th May 2002 in order to reduce the population density of these flies.

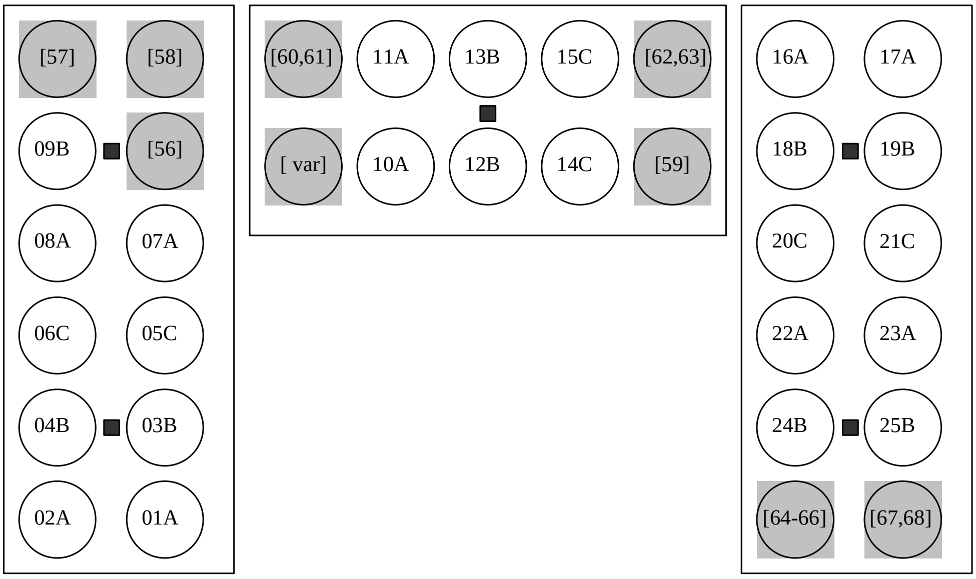

Figure 1

Arrangement of container pots in greenhouse 10/8. Two large benches with space for 12 pots and one smaller bench with space for 10 pots. Pots are continuously numbered and assigned A, B or C according to their posi-tion under one of the light bulbs (indicated by small black quadrates). Variable space [var] or additional pots for various sowing experiments [56-68] indicated by grey filled circles.

The time sequence of treatment applications was as follows:

- on 5th March 2002, *Festuca rubra* was sown out in 20 randomly assigned pots
- on 9th, 12th and 15th April 2002, achenes of two *S.inaequidens* ecotypes ("Kew Gardens" and "Rostock, Germany, harvested 1.11.2001") were sown out; the differences in the sowing dates were due to the fact that the previously sown achenes did not show sufficient germination, and additional achenes had to be sown
- on 9th May 2002, each four of the seedlings were transplanted into each one of the randomly chosen 50 cm pots; the seedlings that had to be transplanted into high-competition pots (with Festuca rubra) were transplanted into small holes driven through the grass cover with a 1 cm diameter bamboo stick, and holes of similar depth were created in the no-competition pots; all the seedlings were heavily watered immediately after transplantation
- on 9th July 2002, herbivores were added (see section 2.2.3.2.1, p.25).

The positions of the pots relative to the supplementary lighting were changed at a four-week basis according to a randomized rotational scheme (ABC,BCA,CAB and so on) in order to reduce variation in morphological parameters due to light intensity variation on the greenhouse benches.

As there was variation in seedling size, each of the 40 pots received

- one 'large' seedling (from 9th April 2002)
- two or three 'small' seedlings (from 15th April 2002) and
- one out of 20 seedlings of the 'Kew' ecotype

It was decided at random wether a pot received a 'Kew' seedling or not; if not, a 'small' seedling was transplanted into that pot instead, so that all of the pots had (roughly speaking) one large and three small seedlings in it. Measurements of plant growth later in the year showed that these initial size differences did not have any significant effects, which justified the design used. Importantly, to reduce unexplained variation, for every position in each pot every seedling was chosen at random out of a population of each 20 seedlings per size class.

pot number	treatment	plant sizes	pot number	treatment	plant sizes
2	SFT	N	29	SFT	N
5	S	K	31	SF	K
7	SF	N	32	SFT	N
8	SFT	N	33	ST	K
10	SF	K	34	SFT	N
11	SF	N	36	SF	N
12	S	K	37	ST	N
13	SF	K	38	S	K
14	S	K	41	S	K
15	S	K	42	ST	N
17	SF	N	43	ST	N
19	SFT	N	44	ST	K
20	ST	N	48	ST	N
21	ST	K	49	S	K
23	SFT	N	50	S	K
24	SF	N	51	SFT	N
25	SF	K	52	SF	K
26	SF	K	53	S	K
27	SFT	N	54	S	K
28	SFT	N	55	ST	K

(pot numbers refer to the figures given above; treatments: S=*Senecio inaequidens*, F=*Festuca rubra*, T=*Tyria jacobaeae*; plant sizes: N="normal", i.e. three small and one large seedling, K="with Kew", i.e. two small, one large and one 'Kew' seedling)

Water was supplied throughout the whole experiment (until the 30th August 2002) using a watering hose trolley with adjustable trigger nozzle and rose. Each pot received the same amount of tap water by assuring equal watering time for every pot. In order to prevent differences due to a higher evapotranspiration in the pots that already had established plants in them at the beginning of the experiment, all pots were watered until the substrate was saturated with water, and water was running off from the drainage holes in the base of the pots. Additionally, it was taken care that the 'competition' and 'no competition' pots did not differ too much in overall weight after watering.

Acetate insect cages (**Plate 6 a**) were erected around all the container pots in order to include *Tyria jacobaeae* caterpillars that were to be applied to the pots later in the year. The cages were built between 29th May and 6th June 2002 using acetate sheet 0.250 mm (1220x850mm) (Film Sales Ltd., 145 Nathan Way, West Thamesmead Business Park, London, SE28 0BE, England).

Throughout the whole course of the experiment, temperature regime, lighting etc. was computer-controlled. On 15th August 2002, the additional lighting had to be shut down because the *S.inaequidens* plants had grown so tall that there was the risk of fire if they had touched the lamps. This did not affect the overall experiment, because plants had already reached their final height and produced ripe capitulae.

The response variables recorded were height, stem diameter, number of branches and number of capitulae. See section 2.2.5.4, page 35 for detailed descriptions of how these variables were measured.

2.2.5 Field experiments

2.2.5.1 Overview

The field experiments were designed to answer the following questions:

1. how does plant competition affect growth and reproduction of *Senecio inaequidens* under field conditions?
2. do vertebrate herbivores (rabbits) feed on *S.inaequidens* - and if so, which damage do they make?
3. do invertebrate herbivores (*Longitarsus*, *Tyria*, molluscs) accept *S.inaequidens* as a food source under field conditions?
4. will the field experiments lead to similar conclusions as the greenhouse experiments?

In order to answer these questions, the effects of several main factors on *S.inaequidens* were studied in a complex split-plot[40] field experiment with two large experimental blocks (refferred to as "plot 1" and "plot 2"; **Plate 4 a, b**).

The main factors studied were

- presence/absence of **vertebrate herbivores** (without or with rabbit fences)
- presence/absence of **plant comptitors** (uncultivated vs. cultivated vegetation cover)

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[40] experimental design, in which different treatments are applied to plots of different sizes; for a detailed introduction see e.g. Crawley 2002

- presence/absence of **insect herbivores** (without or with insecticide[41] application)
- presence/absence of **molluscs** (without or with molluscicide[42] application)

2.2.5.2 Description of the field plots

The field experiments were carried out at Silwood Park, Southeast England, 28 miles west of London (National Grid Rreference 41/944691; longitude 0°35'W, latitude 51°25'N[43]; elevation about 68 m). The average annual rainfall at Silwood Park is 653 mm with little seasonal pattern (Edwards *et al.* 2000) the daily temperature average being 6.2°C -15.3°C. The dominant soil type is free-draining, low base status (pH is 5) nitrogen, phosphorus and base deficient sandy soil[44].

By the beginning of April 2002, the locations for the two large blocks were chosen. It was taken care that the two blocks showed similar characteristics in terms of

- overall physiognomy of the vegetation cover
- having an inclination of zero
- having a similar exposition
- showing no major environmental gradients
- not differing markedly in rabbit grazing intensity

On 21st April and 16th May, the two large blocks (**Figure 2 & 3; Plate 4 a,b**) were demarked with hazard warning tape; around 24th May 2002, rabbit fences were built and vegetation cover of the 'disturbance' subplots was removed by using a motor-driven rotovator. The rabbit fences consisted of galvanised hexagonal wire net (hexagon diameter 3cm) of 70 cm height, a 30 cm strip of the mesh wire being bent outward on the base and attached to the ground surface in order to prevent rabbits from digging underneath the fence. The wire was attached to wooden posts of about 10 cm diameter every 4 m (three on the narrow side, and four on the wide side of the plot).

Each of the large plots measured 24 m x 16 m. Plot 1 was located at Nursery Field (Silwood Park); it was stretched out in west-east direction, parallel to a woodland edge bordering the southern boundary of the experimental plot. Plot 2 was located approximately 200 metres north of plot 1 at Pound Hill (Silwood Park); it was also stretched out in west-east direction, and it was bordered on its northern side by a woodland edge (Cheapside Wood, Silwood Park). Both plots were separated by a road leading from Silwood Farm northwestward to the Northwest entrance of Silwood Park. Whereas plot 2 had no inclination, plot 1 showed a very slight inclination towards the southwest; this was assumed to have no influence on the results of the

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[41] see below for a detailed description of the chemicals used; it was assumed that the treatment application did not have a significant *direct* influence on growth and performance of *Senecio inaequidens*, and that there was no interaction between insecticide and molluscicide.

[42] see footnote above.

[43] Edwards *et al.* 2000

[44] data from http://www.nrel.colostate.edu/projects/glide/silwoodpark.html#description

experiment. In plot 2, low disturbance by *Talpa europaea* L. (European mole) was present, the molehills being equally distributed within the subplots.

The 'competition' plots were characterized by acidic mesic grassland vegetation, dominated by the grasses *Agrostis capillaris* L., *Holcus lanatus* L., *Anthoxanthum odoratum* L. and *Festuca rubra* L., and by the herb species *Rumex acetosella* L., *Galium saxatile* L., *Lotus corniculatus* L., *Trifolium repens* L., *Centaurea nigra* L., *Stellaria graminea* L., *Senecio jacobaea* L. and *Cirsium arvense* (L.)Scop.[45]

2.2.5.3 Details on the split-plot design

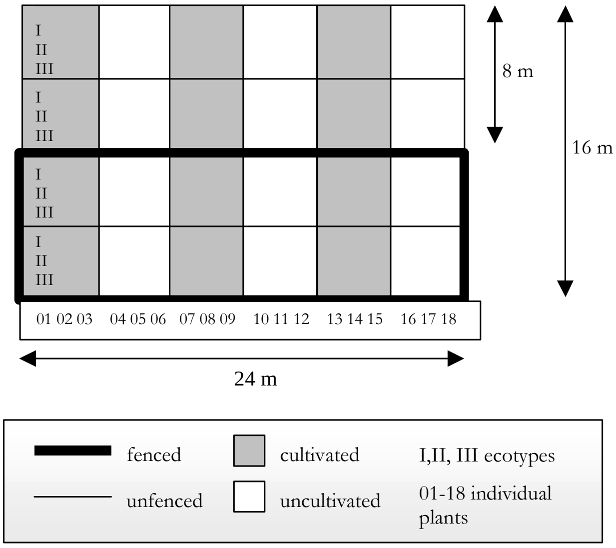

Figure 2

Design for the field experiment at **plot 1**. Each small quadrate measures 4 x 4 m. The total area covered is 16 x 24 m. A fence for rabbit exclusion has been erected in an 8 x 24 m area. *Senecio inaequidens* ecotypes are indicated by roman numbers (I-III), individual plants are numbered from 01-18 in each row. The arrangement of ecotypes was randomised, and individual plants were allocated at random to their positions.

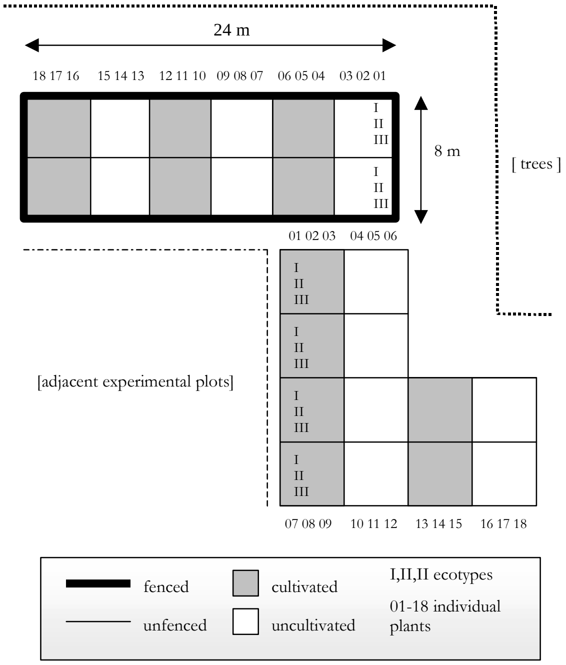

Figure 3

Design for the field experiment at **plot 2**. Each small quadrate measures 4 x 4 m. The total area covered is 16 x 24 m. A fence for rabbit exclusion has been erected in an 8 x 24 m area. Due to restrictions by adjacent experimental plots and trees the shape of the non-fenced area was not rectangular, but L-shaped instead. *Senecio inaequidens* ecotypes are indicated by roman numbers (I-III), individual plants are numbered from 01-18 in each row. The arrangement of ecotypes was randomised, and individual plants were allocated at random to their positions.

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[45] species composition was not analysed in detail; the reader is referred to Edwards *et al.* 2000and especially Edwards & Crawley 1999 for more information.

To give a more detailed overview of the experimental design used, the following table shall be presented.

factor	**nr of levels**	**description of levels**	**plot size**	**replicates in largest block**
block[46]	2	(1) block 1 (2) block 2	24 m x 16 m	-
rabbit grazing	2	(0) no grazing (fenced) (1) grazing (unfenced)	24 m x 8 m	1
plant competition	2	(0) without competition (cultivated) (1) with competition (uncultivated)	8 m x 4 m	3
subplot	3	(1) – (3)	8 m x 8 m	6
ecotype[47]	3	(a) Rostock (Germany, 1.11.01) (b) Montpellier (France), (c) Konstanz (Germany)	24 m x 1 m	4
treatment	4	(w) water (control) (x) insecticide (Dursban[48]) (y) molluscicide (Metaldehyde) (z) insecticide & molluscicide	applied per plant	in fenced part: (w) 36 (x-z) 24

Additionally, the following two treatments were definded:

Tyria herbivory	2	(1) with *Tyria* (within enclosure) (2) without *Tyria* (no enclosure)	8 m x 5 m	2 in fenced part
Longitarsus herbivory	2	(2) with *Longitarsus* (no insecticide) (3) without *Longitarsus* (insecticide)	applied per plant	24 in fenced part

These were not included into the statistical analyses because of two reasons:

(1) *Tyria jacobaeae* did not feed at all on *Senecio inaequidens*

(2) *Longitarsus* colonized *S.inaequidens* on its own, without having to be applied to each plant seperately

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[46] random effect, i.e. the cause of variation is unknown (sensu Crawley 2002)

[47] a random effect, like block

[48] 0,0-diethyl 0-(3,5,6-trichloro-2-pyridinyl)-phosphorothioate ($C_9H_{11}Cl_3NO_3PS$), M=350.6 g/mol; a broadspectrum, chlorinated organophosphate insecticide, acaricide and nematocide, inhibiting the acetylchoolinesterase; Dow AgroSciences LLC; applied directly to individual plants in liquid form mixed with tap water;

The field experiments were laid out as multipe-split-plots with the two large main blocks mentioned above (see **Figure 2 & 3**). Each block was split into two halves (one with rabbit fences and one without), and each half was then divided into three subplots (disturbed and undisturbed vegetation cover; see strips in **Plate 4a** and **b**).

The *Senecio inaequidens* seedlings were sown out in the greenhouse compartments 10/8 and 10/9 on 28th May 2002 (see section 2.1.1.1, p.19, and 2.2.4, p.27). They were transferred to the field plots on 27th June (plot 1) and 1st July (plot 2), respectively. Immediately after transplantation, they were watered using tap water. Three ecotypes of *S.inaequidens* were used (Rostock/Germany, Konstanz/Germany and Montpellier/France); for every ecotype, each 18 seedlings were planted in parallel rows measuring 24 m in length about one metre apart from each other towards all four sides. The different ecotypes were assigned at random to the rows, resulting e.g. in a series of “Rostock, Konstanz, Montpellier, Montpellier, Konstanz, Rostock” per half-plot. In total, plot 1 consisted of twelve rows – so every ecotype row was replicated once in the unfenced, and once in the fenced part.

For each block of three ecotype rows (e.g. “Konstanz, Montpellier, Rostock”), the same randomly calculated sequence of insecticide/molluscicide/water treatments was applied on 16th July (plot 2) and 18th July (plot 1). A fixed amount of mollusc pellets (granular Metaldehyde 6% w/v) was applied directly to the base of every seedling. Insecticide was mixed with tap water, and the seedlings assigned to insecticide treatment were watered with it using a watering can. The seedlings receiving no insecticide were watered with the same amount of tap water. In summary, the treatments were applied as follows:

(2) insecticide treatment: insecticide dissolved in tap water
(3) molluscicide treatment: mollusc pellets + watering with tap water
(4) insecticide & molluscicide treatment: mollusc pellets + insecticide dissolved in tap water
(5) control treatment: only tap water was applied.

The treatments were not applied to the plants in the unfenced subplots, because rabbit grazing intensity had been so severe that almost no plants had survived at the time of application. Insecticide and molluscicide were only applied once in the season.

On 16th, 17th, 23rd and 24th July each two *Tyria* enclosures of about 40 cm height were erected per fenced 8 x 14 m plot (see section 2.2.3.2.2, p.26, and **Plate 4 b**). The horizontal position of the enclosures was not randomized in order to prevent edge effects if the enclosures would have been situated to close to each other. Instead, the position was chosen to be symmetrical, so that each enclosure included two ‘no competition’ and two ‘competition’ plants of each ecotype row.

2.2.5.4 Notes on the response variables used

The morphological parameters measured on *S.inaequidens* were defined as follows:

- **height**: the maximum height of the main axis (in cm) was measured from the soil surface to the position of the apical meristem using a ruler; if the apical meristem had been damaged or the main axis had been clipped by herbivores, the height of the longest observed axis was measured; zero height was given to plants that were dead or could not be found due to being clipped directly above the soil surface

- **stem diameter**: the diameter of the main axis directly at its basis, measured to the nearest mm using a ruler
- **number of branches**: the number of first-order branches originating from the main axis, including the main axis; first-order branches of less than two centimeters length were not counted
- **number of large leaves**: leaves that were of more than 7 centimeters in length
- **number of small leaves**: leaves that were less than 7 centimeters in length
- **number of leaves**: the calculated sum of number of large and small leaves
- **number of holes**: number of 'shotholes' resulting from *Longitarsus* feeding on the leaves (**Plate 9 a**); one hole corresponded to approximately 1mm^2 leaf area eaten. No distinction was made between superficial feeding and 'full' feeding; leaves that showed only slight signs of superficial feeding were treated as undamaged.
- **Number of holes on upper three leaves**: Like 'number of holes', but only the holes on the uppermost three fully expanded leaves was counted; this method was used to test wether insecticide application influenced the feeding damage made by *Longitarsus* beetles
- **number of leaves eaten by molluscs**: Calculated as the equivalent numer of large or small leaves that had been totally removed by molluscs (**Plate 9 b**); the reliability of this measure decreased with plant age, as more and more leaves dried out during the season, and the remainders of only partially eaten dried-out leaves were difficult to measure; the number of dried out leaves was noted separately
- **number of flowers**: the number of capitulae produced at the time of measurement; the numbers of flower head buds and capitulae of all flowering stages were summed up; the number of dried-out or eaten capitulae was noted separately.
- **Herbivore presence**: the presence or absence of molluscs, aphids, *Longitarsus* beetles, *Tyria* larvae and adult Heteroptera was noted

In addition, binary response variables were recorded, such as clipped/not clipped or dead/alive. A total of 70 measurements were made per plant during the observation period, resulting in over 30.000 measurements that were conducted in total.

2.2.6 Additional observations

2.2.6.1 Studies on natural populations

Natural populations of *S.inaequidens* in Germany were visited several times in autumn 2001, and in June 2002. Occurrences of flower-visitors, herbivores and other organisms inhabiting flowering and fruiting individuals of *S.inaequidens* were recorded. A large white cloth was placed under individual plants, and the stems were beaten with a padded heavy club in order to screen for arthropods associated with *S.inaequidens*. Details on the location were noted and notes on plant sociology were made.

2.2.6.2 Pollination experiments

In order to test for self-infertility and to conduct cross-pollination trials with different *Senecio* species, pollination trials were carried out in four separate computer-controlled greenhouse compartments. They were equipped with fens preventing pollen flow between individuals; cross-pollination trials were performed using size one paintbrushes.

2.2.6.3 Vegetative reproduction

Some simple trials with shoot cuttings were carried out in order to test wether *Senecio inaequidens* is able to grow roots from stem fragments. Stem pieces of various lengths were positioned on moist compost or loosely stuck into the substrate, and rooting was observed at an irregular basis.

2.2.6.4 Test for vesicular-arbuscular mycorrhiza

On June 8th 2002, three individuals of *Senecio inaequidens* were collected at Warnemünde-Werft station (Rostock, Germany). The individuals were of equal size, and at least in the second year of their life cycle (indicated by remainders of vegetative parts of the last growth period). The root system was carefully extracted from the soil using a pocketknife. Above- and below-ground parts of the plants were left intact as far as possible, and the plants were transferred to standard compost for cultivation before further observations could be taken in the laboratory. Microscopical observations were kindly performed by Christel Baum (Agrarwissenschaftliche Fakultät, University of Rostock, Germany). Root sections were dyed using Chlorazol Black E.

2.3 Statistical Analysis

Data were analysed using the statistical software package S-Plus 2000 (Release 3, © 1988-2000 MathSoft, Inc.). If not stated otherwise, count data were either square root or log transformed as follows:

a) square root transformation: $x' = \sqrt{x + 0.5}$

b) log transformation: $x' = \ln(x + 1)$

Only significant results will be discussed; non-significant results will only be presented (but not discussed) as far as they are of interest in terms of the analysis of experimental designs, or of other general interest.
As far as not stated otherwise, 1 s.e. (standard error of the mean) will be given as an unreliability measure.

the s.e. is calculated as $s_\mu = \sqrt{\frac{s^2}{n}}$, where s^2 is the variance and n is the sample size.

In hypothesis testing, the probability to commit a Type I error was usually taken to be $p<0.05$ in order to distinguish between significant and non-significant effects.

Significance is indicated as follows:

n.s.	non significant	p>0.05
*	significant	p<0.05
**	significant	p<0.01
***	highly significant	p<0.001

In box plots, central tendency is shown as the median (dividing the box) and the 25% and 75% quantiles, the vertical lines above and below the box indicating observations of each 1.5 interquartile ranges from the top vs. the bottom of the box (Sokal & Rohlf 1995). Outliers (more than three interquartile ranges away from the box) are shown as horizontal bars above and below.

3 Results

3.1 Germination tests

The proportions of germinated achenes in different *Senecio inaequidens* ecotypes were generally lower than expected. Percentages were always calculated from the original data; no statistical analyses will be given on these data, because the germination tests only served as a first orientation, in order to decide which ecotypes would be most suitable for the further experiments.

The highest proportions of germinated seeds were obtained with the 'Konstanz' and one of the 'Rostock' ecotypes (68% and 56% out of a total of 50 and 25 achenes sown, respectively). As the seed portions that had been available at that time were generally very small, no replicates of the petri dishes for these two ecotypes could be made.

Achenes from Rostock Botanical Garden (n=3 petri dishes) as well as those that had been hand-collected on 6th October (n=4) and 1st November 2001 (n=5) showed only five to six per cent germination. *Festuca rubra* (n=2) showed 57 ±11% germination.

Because of the generally low proportions of germinated seeds (achenes), it was decided

a) to sow out seeds from all plant species and ecotypes needed directly on moist compost in the greenhouses and
b) to establish own cultures of *S.inaequidens* plants from which viable achenes could be obtained.

Especially the material from southern France (Montpellier), which arrived in February 2002, but also achenes obtained from Kew Gardens as well as the achenes harvested in the second generation after sowing, showed high proportions of germinating achenes when directly sown out on compost. It was therefore decided to use the following ecotypes of *S.inaequidens* for all the experiments on herbivory and competition:

(1) Kew Gardens (UK), Serial number 0143329, 28th January 2002
(2) Rostock – Warnemünde Werft (Germany), 1st November 2001
(3) Montpellier (France), 11th February 2002
(4) Konstanz Botanical Garden (Germany), 30th January 2002

3.2 Feeding trials

3.2.1 Longitarsus jacobaeae

3.2.1.1 No-choice feeding preference experiment

The sixteen adult *Longitarsus* beetles applied on 12th April started feeding on flowering *Senecio inaequidens* plants in a computer-controlled greenhouse compartment within fifteen minutes after application, preferably sitting on the underside of the leaves. The feeding damage was less intensive than the observed 'shotholes' that *Longitarsus* normally produces in *Senecio jacobaea* leaves. Often only the uppermost (presumably epidermal) layer of the leaves was damaged. Most of the observed time the beetles were not showing feeding behaviour at all, but sitting motionless on stems or the underside of leaves. On 21st April, only three survivors could be found, and on 6th May no more beetles were present. However, these results need to be handled with care, as there were extraordinarily high temperatures in the greenhouse compartment at that time, due to a malfunction of the thermal regulation system.

It can be conluded that adults of *Longitarsus jacobaeae* were feeding freely on *S.inaequidens* for a maximum period of about three weeks under laboratory no-choice conditions.

3.2.1.2 Multiple choice feeding preference experiments

3.2.1.2.1 Statistical analysis

The feeding preference experiments were analysed using a simple t-test on differences - as suggested by Peterson & Renaud 1989 - rather than nonparametric tests.

3.2.1.2.2 Results

The results obtained in the field free-choice experiment were different from the observed situation under laboratory conditions. *Longitarsus* adults colonized *Senecio inaequidens* in the field on their own, and could be found feeding throughout the whole observation period (5th July until at least 6th September 2002) on *S.inaequidens* plants (see **Figure 4**).

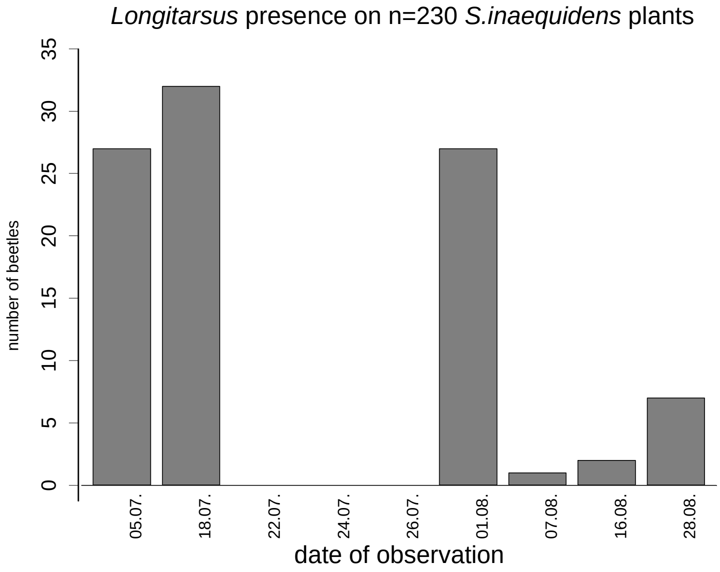

Figure 4

Total number of *Longitarsus* adults present on the 230 *Senecio inaequidens* plants in the fenced part of the two field plots on different observation dates. No individuals could be found on 22nd,24th and 26th July, which is probably due to dry weather conditions at that time. On average, 0-1 individuals were present per plant, rarely up to five. On 18th July, *Longitarsus* adults could be found on 10 per cent of the plants.

On average, 0-1 individuals were present per plant, rarely up to five. At one occasion, mating behaviour of one pair of adult beetles was recorded on *Senecio inaequidens*. Characteristic shotholes could be observed especially on the broad and less rigid leaves that had been developed under greenhouse conditions before transplantation of the seedlings (**Plate 9 a**). With increasing leaf age and altered leaf texture, there was a switch towards superficial feeding, resulting in less severe damage to the leaves. This result is consistent with the observed mode of feeding in the no-choice greenhouse trial on adult flowering *S.inaequidens* plants.

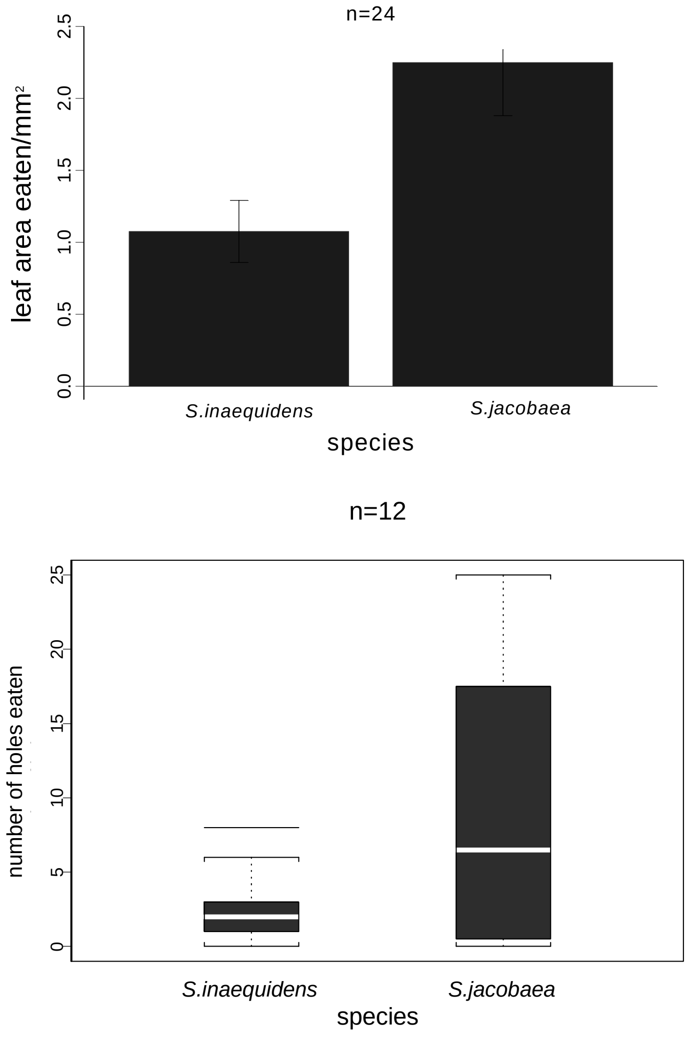

Figure 5

Feeding preferences of *Longitarsus jacobaeae*. (a) Two-way choice trial with *Senecio inaequidens* and *S.jacobaea* leaf disks provided as food source. Leaf area eaten/mm^2 within t=25.0 hours on 13th Aug 2002 (total leaf disk area 7 mm^2), t-test on differences: $t_{1,23}=3.88$, p=0.0008***, $x_{diff}=1.29$ mm^2. Error bars show mean ±1 s.e. (b) Number of holes eaten in a multiple-choice trial with rectangular leaf pieces of three control species, *S. inaequidens* and *S. jacobaea* (t=22 hours, 11th August 2002). Feeding occurred only on the two *Senecio* species; t-test on differences (response square root transformed): $t_{1,11}=2.71$, p=0.02*, $x_{diff} \approx 7$ holes.

In the laboratory multiple-choice feeding trials, the beetles significantly preferred leaf pieces of *Senecio jacobaea* over those of *Senecio inaequidens* (see **Figure 5**).

It can be concluded that

- adults of *Longitarsus jacobaeae* freely colonized *Senecio inaequidens* under field conditions; they were found feeding throughout the whole period of observation (at least three months); they were furthermore found mating on *S.inaequidens* at one occasion
- *Longitarsus* adults significantly preferred *S.jacobaea* leaf pieces over *S.inaequidens* leaf pieces under laboratory conditions.

3.2.2 Tyria jacobaeae

3.2.2.1 Oviposition

The four adults collected on 2nd June survived in the insect cage for seven days. One female laid two egg batches after 4 days, but these were not attached to any of the food plants (*S.inaequidens* and *S.jacobaea*) provided.

3.2.2.2 Development and survival of larvae in no-choice tests

The incubation time for the larvae to hatch after collection of egg batches was 5 ± 1 days (n=7 egg batches). Egg batch size was 49 ±25 (n=7).

40 out of a total of 166 1st instar larvae survived for the first 14 days when *S.inaequidens* was provided as the only food source.

2nd and 3rd instar larvae (n=40) collected in the field from *S.jacobaea* on 18th June were feeding for 16 days on *S.inaequidens* in an insect cage when no other food plant was present. They were passing through the instars and reached the 4th instar stage. After this time, there were no survivors.

4th and 5th instar larvae (n=40) collected in the field from *S.jacobaea* on 1st July were feeding for 17 days on potted *S.inaequidens* plants (Kew ecotype) whithout special caging or enclosure built up (**Plate 7 a**). After this time, there were only three survivors.

5th instar larvae (n=300) collected in the field from *S.jacobaea* on 9th July (**Plate 2 a**) were surviving, but rarely feeding, for 9 days on potted *S.inaequidens* plants. After this time, there were no survivors.

It can be concluded that

- all larval instars of *Tyria jacobaeae* were feeding on leaves of *Senecio inaequidens* under no-choice laboratory conditions; they survived for nine to seventeen days.
- *Tyria jacobaeae* larvae were moulting on *S.inaequidens* and some individuals even reached the final larval stage under no-choice laboratory conditions
- *Tyria jacobaeae* did not survive to pupation when fed on *Senecio inaequidens*

3.2.2.3 Damage to *Senecio inaequidens* in no-choice tests

When the original host plant, *Senecio jacobaea*, was absent, *Tyria jacobaeae* larvae were able to decapitate and kill plants of *Senecio inaequidens* when kept in enclosures where population density of the larvae was artificially high; when allowed to freely forage in the greenhouse, larvae that had directly been raised on *Senecio inaequidens* preferred to stay on the plants to which they had been applied after transfer from the plastic boxes in which they had been raised. Under these circumstances, 4th and 5th instars of *Tyria jacobaeae* were preferably feeding on the capitulae; feeding proceeded - starting at the rayflorets – subsequently towards the center of the capitulum, and often the involucrum itself was left intact. Sometimes plants were completely decapitated, but only if they were younger than four weeks. During the whole two-week observation period, *Tyria* did not reduce the amount of capitulae produced by *S.inaequidens* remarkably, and only an estimated percentage of ten per cent of the capitulae showed feeding damage. As the involucrum was mostly left intact, plants were often able to still produce viable achenes.

3.2.2.4 Multiple choice feeding preference experiments

Laboratory and field experiments lead to similar and clear results. In the preliminary laboratory test with flowering capitulae of five different plant species (n=12), as well as in the two-way choice trial with flowering capitulae of *Senecio inaequidens* and *S.jacobaea* (n=24), *Tyria jacobaeae* larvae did never feed on capitulae of *Senecio inaequidens* before T_{50}[49]. Feeding on *S.inaequidens* only started when 100% of the simultaneously offered *S.jacobaea* capitulum had been consumed, and even then, not all larvae chose to do so. Often, only the rayflorets of *S.inaequidens* were eaten, the diskflorets and the rest of the involucrum being refused. In the five-way choice trial, the average time spent on *S.inaequidens* and each one of the three control plants was the same. The laboratory test with 6 mm leaf disks lead to the same clear result; *S.inaequidens* leaf disks were never eaten until both of the two offered *S.jacobaeae* disks had been consumed.

In the free choice feeding preference experiment under field conditions, *Tyria jacobaeae* could only be found sitting (but not feeding) on *S.inaequidens* on two days within a total observation time of more than two months; *Tyria jacobaeae* larvae were found aggregated on the rosettes and stem bases of the cut-back *Senecio jacobaeae* plants, even if they were growing only 30 cm away from *Senecio inaequidens* within the enclosures.

It can be concluded that

- capitulae and leaf pieces of *Senecio inaequidens* are not accepted as a food source by *Tyria jacobaeae* larvae under laboratory multiple-choice conditions
- under field conditions, three-month-old *Senecio inaequidens* plants are not accepted as a food source by 5th instar larvae of *Tyria jacobaeae*

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[49] defined as the time after which 50% of one of the two offered food types had been consumed; see Jermy *et al.* 1968 for detailed examples.

3.3 Greenhouse experiments

3.3.1 Plant competition

3.3.1.1 Statistical Analysis

The greenhouse competition experiments were analysed using two-sample t-tests (factors: competition/no competition, and greenhouse compartment 1 and 2, respectively). As variances and means were correlated within each of the pots, the overall mean for every response variable was calculated for every pot. Count data (number of capitulae, number of branches) were square root transformed, whereas data on continuous measurements (height, stem diameter) were left untransformed in order to calculate all the parameter estimates.

3.3.1.2 Results

Competition with *Festuca rubra* lead to the death of 38 out of 40 *Senecio inaequidens* plants in ten large container pots within two weeks. However, two individuals survived for four months (until the end of the experiment) and were able to produce capitulae and ripe seeds, and to reach approximately the same height as the plants grown in monoculture (**Fig. 6 c; 8**). All morphological parametres measured were significantly affected by plant competition (**Plate 10 b**), such as the overall number of capitulae produced (**Fig. 6 a**), the number of branches (**Fig. 6 b**), plant height (**Fig. 6 c**) and stem diameter (**Fig. 6 d**).

The high death rate may partly be explained by the high numbers of fungus gnats larvae ('Trauermücken", Diptera: Sciaridae) that were present in the onset of the experiment; these were feeding on the stem bases, leaves and rootstocks of freshly transplanted seedlings; however, fungus gnats larval herbivory was not restricted to the competition pots.

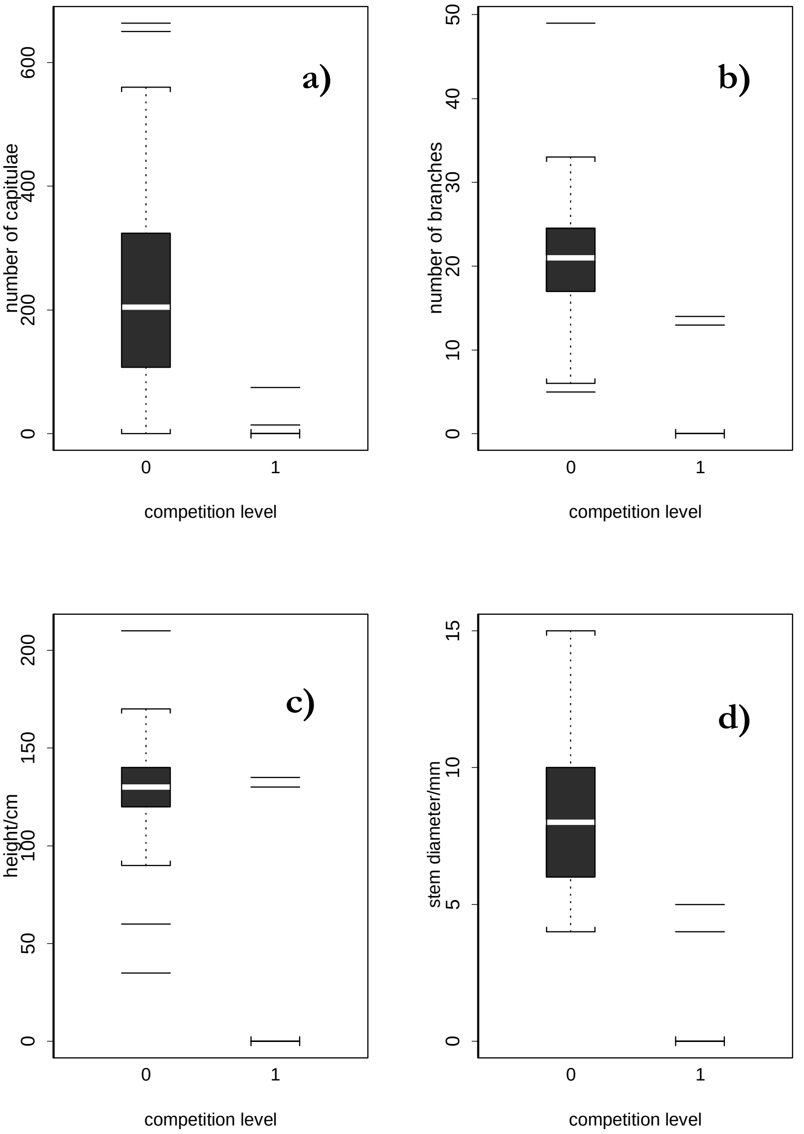

Figure 6

Greenhouse experiment. Effects of competition on different morphological parameters in *Senecio inaequidens* (03rd September 2002); 0=without, 1=with competition; (a)-(d) analysed using t-tests on means for every container pot, (a) and (b) transformation: $\sqrt{x+0.5}$; (a) effect on number of capitulae; $t_{2,38}=23.79$, $p<0.0001$***, back-transformed mean values: 199 and 0.2 capitulae per pot (b) effect on the number of first-order branches; $t_{2,38}=35.62$, $p<0.0001$***, back-transformed mean values: 20 and 0.1 branches per pot (c) effect on plant height/cm; $t_{2,38}=32.74$, $p<0.0001$***, mean values per pot: 130.5 cm and 3.31 cm (d) effect on stem diameter/mm; $t_{2,38}=28.43$, $p<0.0001$***; mean values per pot: 8.3 mm and 0.11 mm.

There were no significant differences in the no-competition pots between the two greenhouse compartments (see **Fig. 7 a-d**)

It can be concluded that

- competition with *Festuca rubra* showed a very strong and highly significant effect on morphology and reproductive ability of *Senecio inaequidens* under greenhouse conditions
- there were no significant differences between the greenhouse compartments

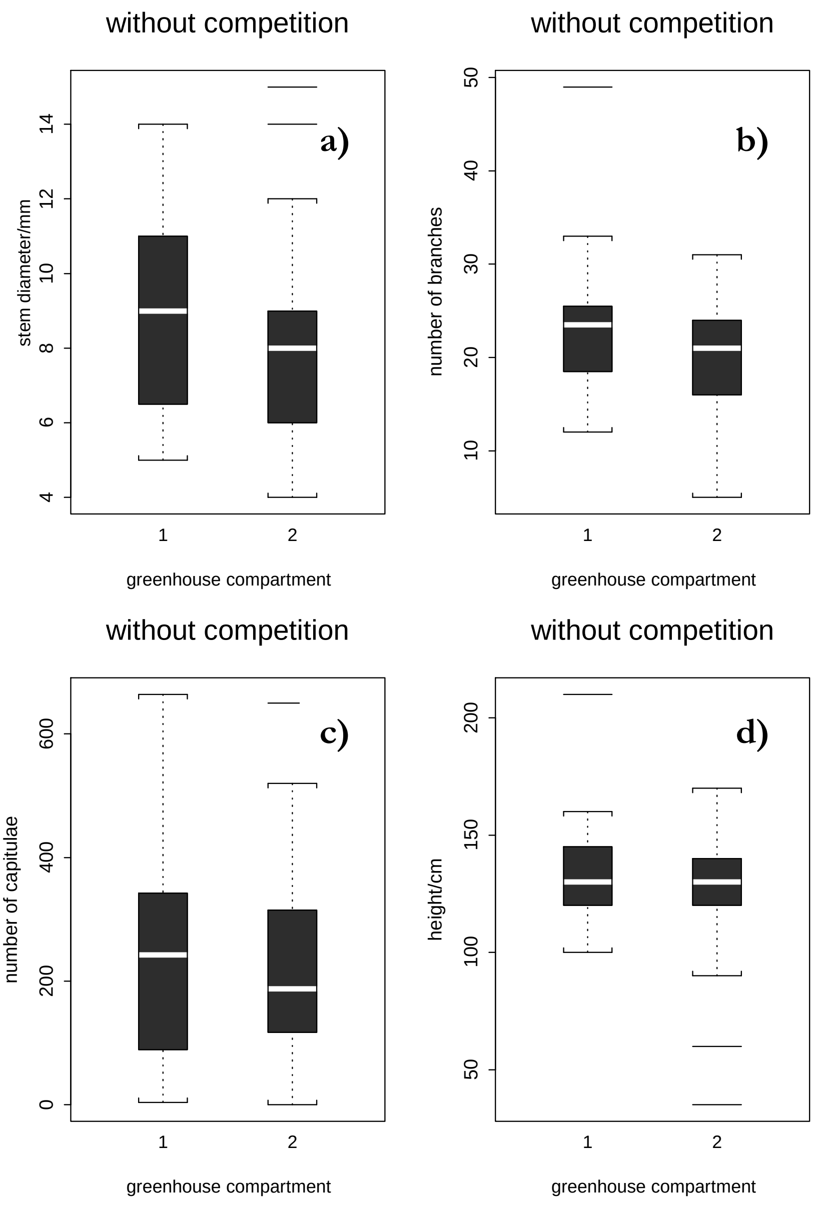

Figure 7

Differences in morphological parameters of *Senecio inaequidens* grown in 2 different greenhouse compartments (3.9.02; only results for "no competition" pots shown); 1, 2=greenhouse compartments 10/8 and 10/9; (a)-(d) analysed using t-tests on means for every pot, transformation in (b), (c): $\sqrt{x+0.5}$; (a) differences in stem diameter/mm; $t_{2,18}$=1,73, p=0.1 (n.s.), mean values per pot: 9 mm and 8 mm (b) differences in number of 1st order branches; $t_{2,18}$=2.09, p=0.0503 (n.s.), back-transformed mean values per pot: 23 and 19 branches (c) effect on number of capitulae; $t_{2,18}$=0.34, p=0.74 (n.s.), back-transformed mean values per pot: 207 and 196 capitulae (d) effect on plant height/cm; $t_{2,18}$=0.62, p=0.54 (n.s.); mean values per pot: 133 and 129 cm.

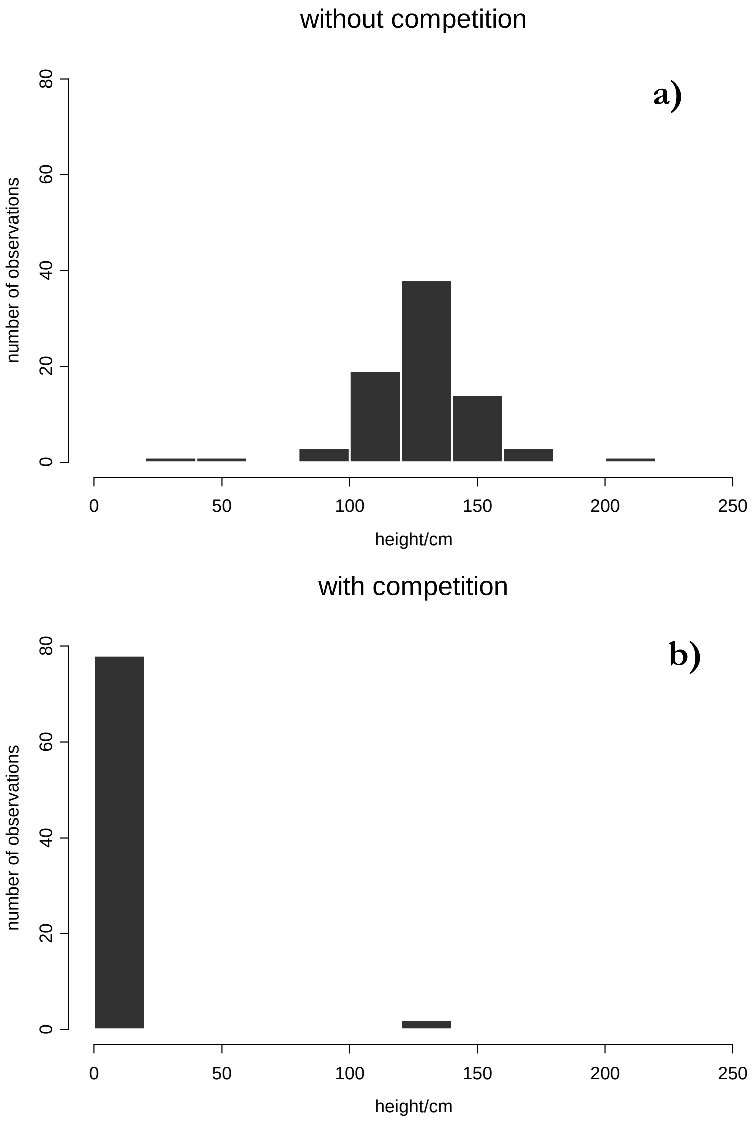

Figure 8

Greenhouse experiments. Histograms showing the frequency distributions of *Senecio inaequidens* plant height/cm as observed on 3rd Sept. 2002 (a) without competition and (b) with competition. With competition, there were only two survivors out of 80; notably, these reached a similar height compared wuth the plants growing without competition.

3.3.2 Herbivory

The greenhouse herbivory experiment with 5th instar larvae of *Tyria jacobaeae* (n=300) added to the acetate cages that had been erected around the container pots (**Plate 6 a**) showed very clear results. Of the 300 larvae added in total to the 10 pots, only five started feeding on flowering *S. inaequidens* plants in two of the ten pots. The rest of the larvae showed extremely high mobility, and did not feed on any of the plants within nine days after application. Even when experimentally applied to *S. inaequidens* seedlings, no feeding occurred. Instead, *Sonchus arvensis* L. plants that had grown accidentally from a field collection of *S.inaequidens* achenes were attacked by some of the larvae, where they were feeding for several days. It can be concluded that

- flowering *Senecio inaequidens* plants were not accepted as a food plant under greenhouse conditions
- *Sonchus arvensis* was even preferred over *S. inaequidens* under these conditions

3.4 Field experiments

3.4.1 Statistical analysis

3.4.1.1 General description

Because treatments were applied to plots of different sizes, the field experiments were analysed using split-plot ANOVA with normal errors. Count data were square-root or log transformed (see p.37); continuous measurements were left untransformed for analysis. Plants with zero height were excluded from the analysis except for the interpretation of the rabbit grazing effects. One split-plot ANOVA table is presented below as an example.

3.4.1.2 Split-plot ANOVA involving rabbit grazing, plant competition, and ecotype effects

In this case, plant height is the response variable, while plot and ecotype are random effects (see Crawley 2002 for a definition), and there is one fixed effect treatment (competition, classified as a categorical variable). In the following analysis, interactions between rabbit grazing, competition and ecotype will be examined. In S-Plus 2000, the model formula looks like this:

```
Height ~ rabbitgrazing*competition * ecotype + Error (plot / rabbitgrazing/subplot)
```

For more details on mixed effects models of this type, see Crawley 2002. The error structure is defined by the 'Error' term in the model formula. It depends on the plot sizes in the split-plot experiment; there are four different plot sizes in this experiment: 'plot' has the largest size, 'rabbitgrazing' the second-largest one, 'subplot' the third largest one, and the individual plant corresponds to the smallest plot size.

In the output, there arefour separate ANOVA tables, one for each plot size. There are two replicates of 'plot', two levels of 'rabbitgrazing', two levels of 'competition' and three levels of 'ecotype'; therefore, the corresponding values for the degrees of freedom are as shown in the ANOVA tables below. 'Competition', 'rabbitgrazing' and 'ecotype' are abbreviated as 'compet', 'rabbitgraz' and 'ecotype', respectively:

Error: plot

	d.f.	Sum of Squares	Mean Squares	F Value	p Value
Residuals	1	94.4537	94.4537		

Error: rabbitgraz within plot

	d.f.	Sum of Squares	Mean Squares	F Value	p Value
rabbitgraz	1	56672.93	56672.93	109.8944	0.06054528
Residuals	1	515.70	515.70		

Error: subplot within (plot/rabbitgraz)

	d.f.	Sum of Squares	Mean Squares	F Value	p Value
Residuals	4	276.0741	69.01852		

Error: Within

	d.f.	Sum of Squares	Mean Squares	F Value	p Value
compet	1	6271.56	6271.565	96.24859	0.0000000***
ecotype	2	58.50	29.250	0.44889	0.6386437
rabbitgraz:compet	1	2542.37	2542.370	39.01731	0.0000000***
rabbitgraz:ecotype	2	44.52	22.259	0.34161	0.7108262
compet:ecotype	2	157.63	78.815	1.20956	0.2993814
rabbitgraz:compet:ecotype	2	177.91	88.954	1.36516	0.2564876
Residuals	414	26976.27	65.160		

(Interactions between factors are indicated by a colon ':' in the column to the left; d.f.=degrees of freedom)

The mean squares are calculated by dividing each value in the sum of squares column by the corresponding degrees of freedom (e.g. 6271.56/1=6271.56). The F-values are then calculated by dividing the treatment variance (mean squares) by the error variance (residual mean squares) at the appropriate plot size, e.g. 6271.56/65.160=96.24859; the resulting F value is then compared with the critical F value of statistical tables for p=5%, the treatment degrees of freedom in the numerator and the residual degrees of freedom in the denominator (in this case: $F_{0.05}(1,\infty)=3.84$).

The p-values in the last column to the right show the probability that the F-values in the neighbouring columns could have arisen by chance alone.

There are two significant p-values (indicated by ***), indicating that plant height was significantly affected by competition, and by a combination of rabbit grazing and competition (see **Fig. 22 d**). Rabbit grazing alone had no significant effect on plant height.

3.4.2 Frequency distributions of plant height in *Senecio inaequidens*

To get a first impression on how overall morphological parameters of *Senecio inaequidens* changed over time during the course of the field experiments, histograms of maximum observed plant height were drawn (**Fig. 9**). Whereas plant height showed a uniformly symmetric frequency distribution of plant height in the beginning of the experiment (**Fig. 9 a**), the patterns in the histograms drawn at later stages of the experiment look much more asymmetric (**Fig. 9 b-d**). The frequency distribution tended to have a positive skew towards the end of the observation period (not visible at the presented x axis scale).

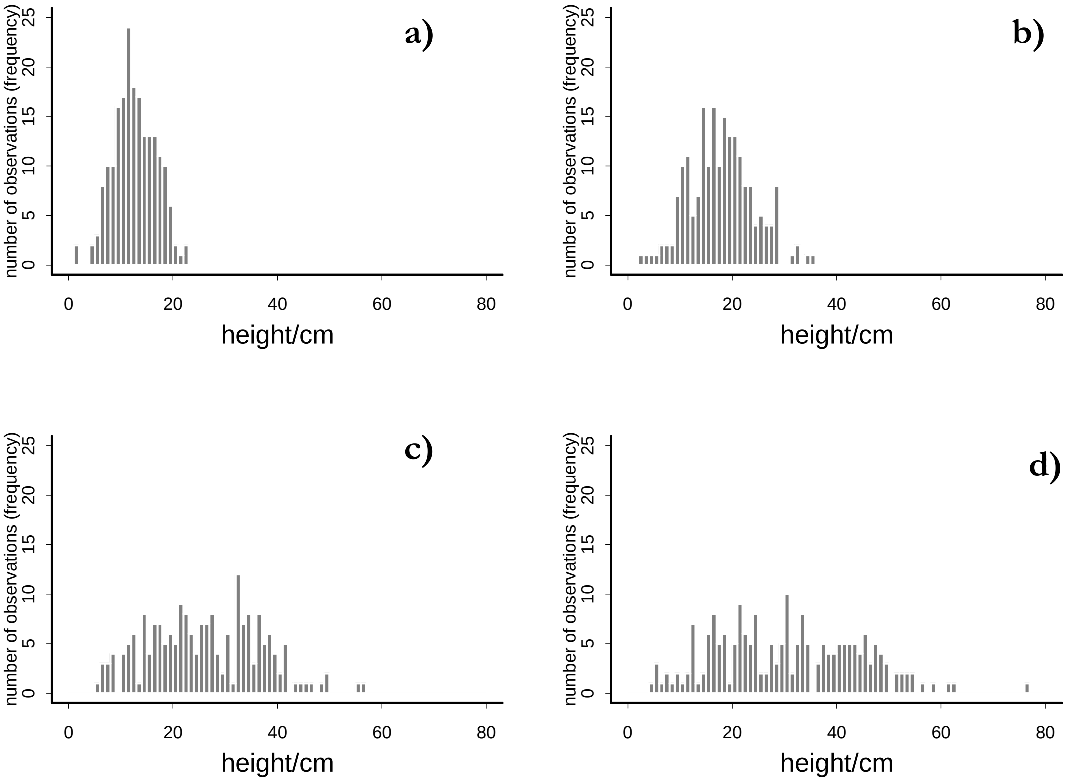

Figure 9

Histograms showing the frequency distributions of plant height/cm in *Senecio inaequidens* on four different times of measurement, (a) 18th July 2002; (b) 1st August 2002; (c) 16th August 2002 and (d) 28th August 2002. Plots grazed by rabbits were excluded.

3.4.3 Differences between the two experimental plots

Whereas maximum plant height of *Senecio inaequidens* and the overall number of capitulae produced did not differ significantly between the two experimental plots (**Fig. 10 a-b**), there were significant differences in the number of branches (**Fig. 10 c**) and in the number of leaves eaten by molluscs (**Fig. 10 d**). Plants in plot two showed lower numbers of branches, and higher levels of mollusc herbivory than plants in plot 1.

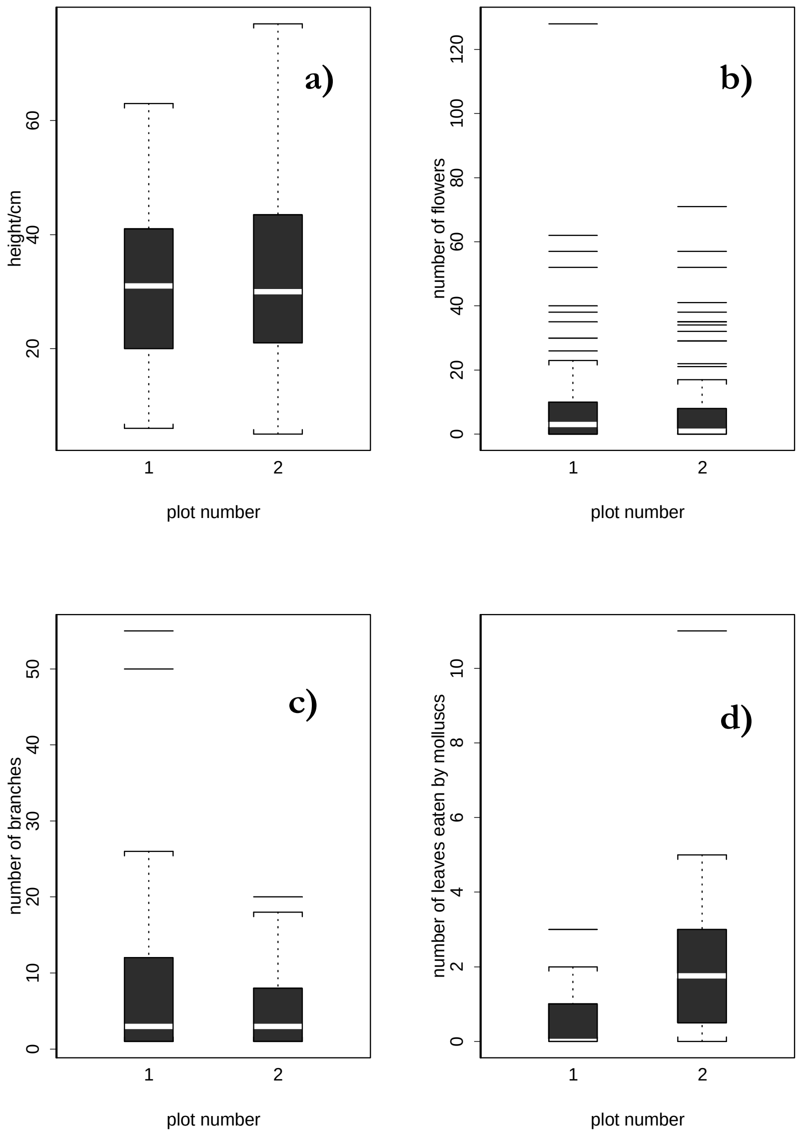

Figure 10

Differences between the two experimental plots in the field experiment (28th August 2002; plots grazed by rabbits excluded). (a) Height/cm of *Senecio inaequidens*; $p=0.76$ (n.s.), $F_{1,208}=0.09$ (b) number of capitulae; $p=0.27$ (n.s.), $F_{1,208}=1.20$ (c) number of first-order branches; $p<0.001$***, $F_{1,208}=13.43$ (d) number of leaves eaten by molluscs; $p<0.0001$***; $F_{1,208}=81.98$; (a)-(d) analysed as split-plot-ANOVA, response transformation in (b) - (d): $\sqrt{x+0.5}$

3.4.4 Differences between ecotypes of *Senecio inaequidens*

As mentioned in section 2.2.5.3 (p.33), three different ecotypes of *Senecio inaequidens* had been used for the field experiment; below, differences between these ecotypes will be described with regard to their statistical significance.

3.4.4.1 Ecotype differences in morphological parameters

Maximum plant height (**Fig. 11 b**), number of first-order branches (**Fig. 11 a**) and number of intact leaves did not differ significantly between the three ecotypes studied (**Fig. 11 a-b**). However, stem diameter (**Fig. 11 d**) and number of capitulae (**Fig. 11 c**) were significantly different between ecotypes; the plants that had been grown from achenes obtained from Montpellier (ecotype 'b' in **Fig. 11 d**) showed a strong positive skew in the frequency distribution of stem diameters (with the median at 0.4 cm, and the mean at 0.35 cm) compared to the other two ecotypes (mean for ecotype a: 0.27 cm; mean for ecotype c: 0.31 cm). In contrast, the number of capitulae produced by ecotype b was generally lower than in ecotypes a and b (**Fig. 11 c**), with zero median number of capitulae. The difference in number of capitulae between ecotypes was even significant when plants that had produced zero flowers had been excluded from the analysis ($F_{2,101}=8.44$, $p=0.0004$***; not shown in the figures).

An additional regression analysis showed that there was a positive relationship between stem diameter and number of capitulae produced, indicating that more capitulae were produced by plants with higher stem diameter (log-transformed response; intercept: 0.24±0.01, $t=23.5$, $p<0.0001$***; slope: 0.054±0.006, $t=9.26$, $p<0.0001$***; residual s.e.: 0.1096 on 214 d.f.; $R^2=0.28$; not shown in the figures).

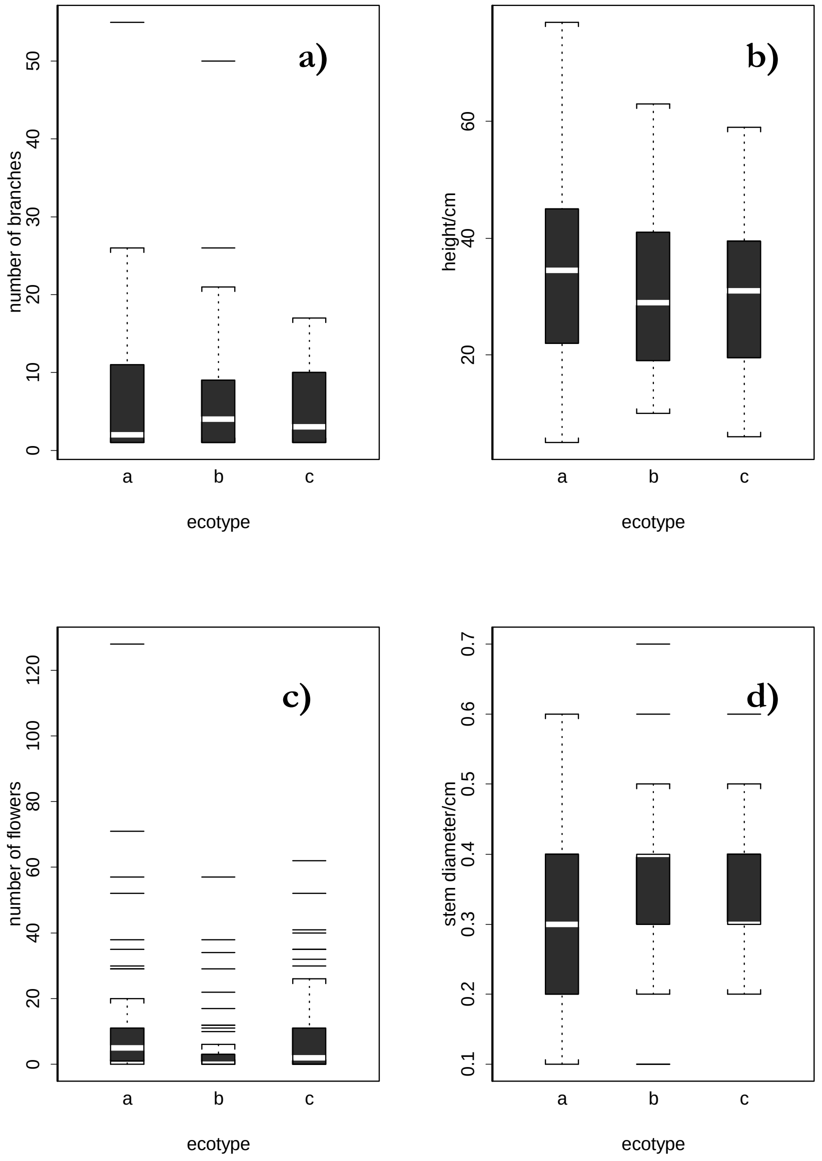

Figure 11

Morphological differences in ecotypes of *Senecio inaequidens* in the field experiment on 28th August 2002; ecotype levels: a=Rostock (Germany, 1.11.2001), b= Montpellier (France), c=Konstanz (Germany); (a) shows differences in the number of first-order branches; $F_{2,208}$=0.54, p=0.59 (n.s.); (b) shows differences in height/cm; $F_{2,208}$=0.32, p=0.72 (n.s.); (c) differences in the number of capitulae produced; $F_{2,208}$=11.51, p<0.0001***; (d) differences in stem diameter/cm; $F_{2,208}$=7.79, p<0.001***; (a)-(d) analysed as split-plot-ANOVA; (a) and (c) response transformation: ln(x+1)

3.4.4.2 Ecotype differences in invertebrate herbivore damage

Mollusc damage, measured as the total number of leaves consumed by molluscs (**Plate 9 b**), was significantly different between ecotypes (**Fig. 12 c**). Ecotypes a and c (from Rostock and Konstanz, respectively) generally showed lower mollusc damage than the plants of the 'Montpellier' ecotype.

The overall number of *Longitarsus* shotholes differed significantly between ecotypes ($F_{1,208}$=8.81, p<0.001***; **Fig. 12 d**), whereas the number of holes on the uppermost three leaves did not differ significantly between ecotypes ($F_{2,58}$=0.19, plants with zero total number of holes excluded; not shown).

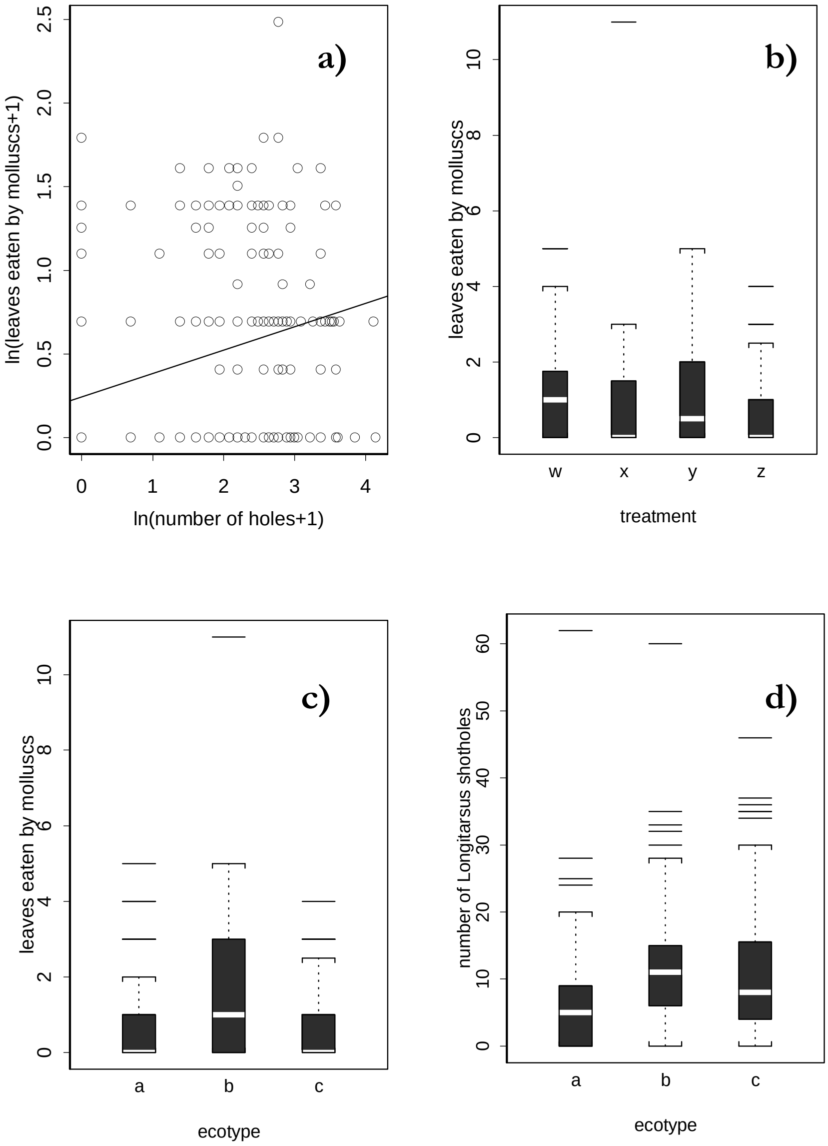

Figure 12

Herbivore damage to *Senecio inaequidens* leaves in the field experiment (28th Aug. 2002; (a) and (b) for height >0); (a) number of holes eaten by *Longitarsus* in relationship to number of leaves eaten by molluscs; linear regression, intercept: 0.27 ±0.077, t=3.26, p<0.01**; slope: 0.15±0.035, t=4.04, p<0.001***, R^2=0.07; (b) mollusc damage in relationship to different treatments: w=water, x=insecticide, y= molluscicide, z=insecticide+molluscicide, $F_{3,174}$ =2.48, p=0.66 (n.s.); (c) mollusc damage in different *S.inaequidens* ecotypes: a=Rostock (Germany, 1.11.2001), b=Montpellier (France), c=Konstanz (Germany); $F_{2,208}$=12.97, p<0.0001***; (d) number of shotholes eaten by *Longitarsus* in different *S.inaequidens* ecotypes (levels see (c)); $F_{2,208}$=8.81, p<0.001***; (b)-(d) analysed as split-plot-ANOVA, response transformation: ln(x+1)

3.4.4.3 Treatment effects

The effects of insecticide and molluscicide application on invertebrate herbivore damage in *Senecio inaequidens* are shown in **Fig. 12 b**, **13** and **14**. It is important to note that none of the treatments had a significant effect on insect or mollusc herbivore damage, which means the differences might have occurred by chance alone. Nevertheless, some of the tendencies that can be seen in the figures shall be described, although they will not be discussed later.

Longitarsus damage on the uppermost three leaves was lower when insecticide or a combination of insecticide and molluscicide had been applied (**Fig. 13**).

Mollusc damage was generally lower when plants had been watered with a combination of insecticide and molluscicide (**Fig. 12 b; Fig. 14**); however, it needs to be noted that most of the mollusc damage had already occurred prior to the application of the molluscicide, so these results need to be handled with care.

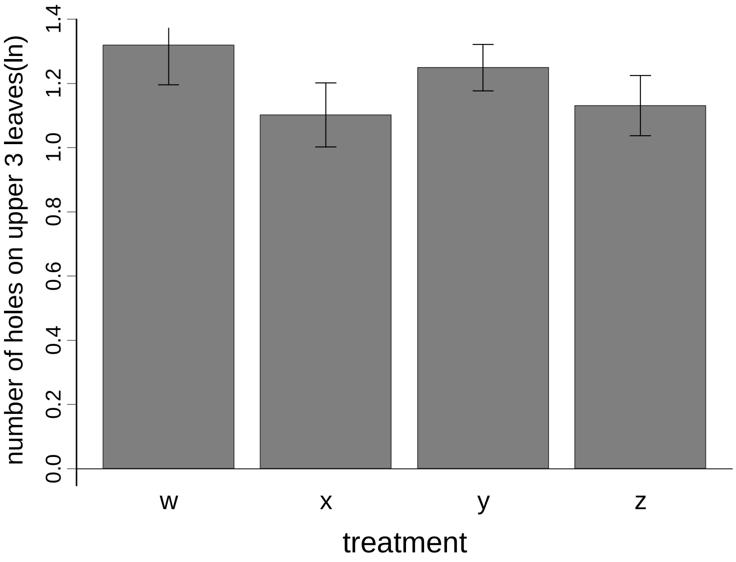

Figure 13

Longitarsus damage to *Senecio inaequidens* in relationship to different treatment applications (field experiment, 28th August 2002). Differences in the number of shotholes (=feeding damage by *Longitarsus*) on upper three leaves are shown (transformation: ln(x+1); plants with zero number of holes excluded); p=0.4 (n.s.), $F_{3,174}$=0.97 (analysed as a split-plot ANOVA with log transformation). Treatment levels: w=water, x=insecticide, y= molluscicide, z=insecticide+molluscicide. Error bars show mean ± 1 s.e.

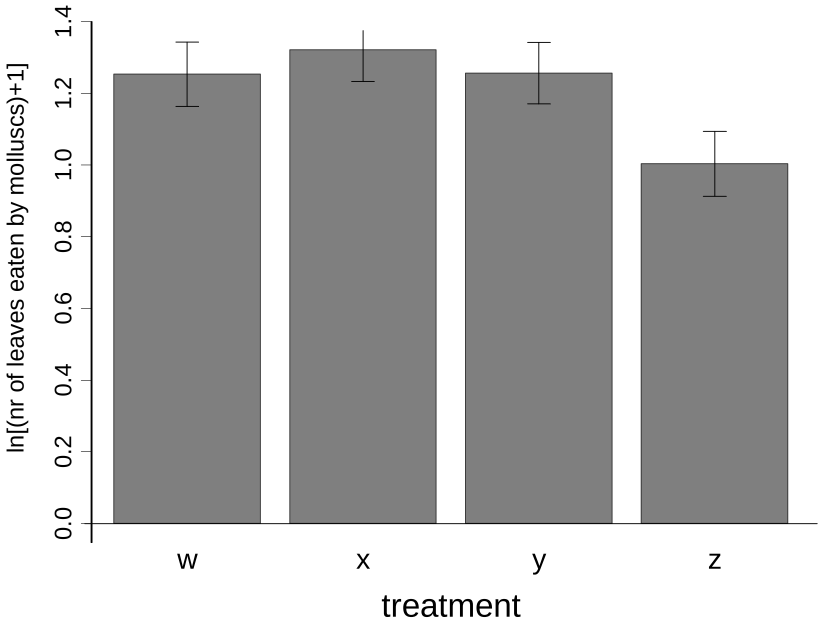

Figure 14

Mollusc damage to *Senecio inaequidens* in relationship to different treatment applications (field experiment, 28th August 2002). Differences in the number of leaves eaten by molluscs are shown (transformation: ln(x+1); plants with zero height excluded); p=0.06 (n.s.), $F_{3,174}$=2.48 (analysed as a split-plot ANOVA with log transformation). Treatment levels: w=water, x=insecticide, y= molluscicide, z=insecticide+molluscicide. Error bars show mean ± 1 s.e.

3.4.5 Direct effects of plant competition

Plant competition (**Plate 10 a**) had highly significant effects on all morphological parameters that had been measured in *Senecio inaequidens* during the observation period. **Fig. 15** gives a first overview, showing that there were highly significant ($p<0.0001$***) effects of plant competition on the number of first-order branches (**Fig. 15a**), number of leaves (**Fig. 15 b**), number of capitulae (**Fig. 15 d**) and stem diameter (**Fig. 15 c**).

The two most important morphological parameters studied are maximum plant height and number of capitulae (as a measurement of reproductive fitness). The changes in these parameters over time are shown in **Fig. 16** and **Fig. 17**. Plants grown without competitors grew taller than those in the uncultivated plots, and the difference between these two groups increased with time (**Fig. 16**). For the overall number of capitulae produced, the effect of competition was even more distinct: Whereas the plants grown under the 'competition' treatment even showed decreasing numbers of capitulae over time, the ones growing without competition exhibited an almost exponential increase in the number of capitulae produced (**Fig. 17**).

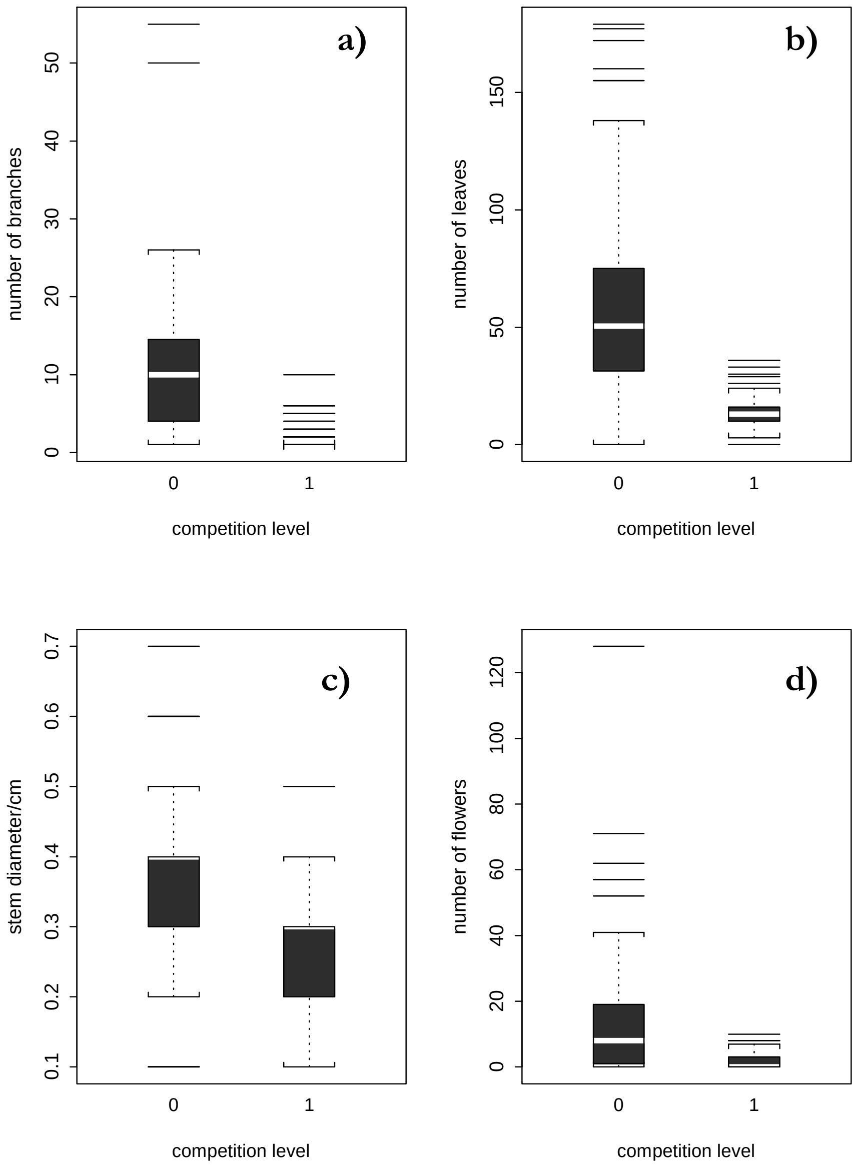

Figure 15

Competition effects on *Senecio inaequidens* in the field experiment on 28th August 2002; competition levels: 0=cultivated, 1= uncultivated; (a) shows the effect on the number of first-order branches; $F_{1,108}=227.00$, $p<0.0001$***; (b) effect on number of leaves; $F_{1,208}=105.41$, $p<0.0001$***; (c) effect on stem diameter/cm; $F_{1,208}=51.29$, $p<0.0001$***; (d) effect on number of flowers; $F_{1,208}=84.18$, $p<0.0001$***; (a)-(d) analysed as split-plot-ANOVA; (a), (b) and (d) response transformation: ln(x+1)

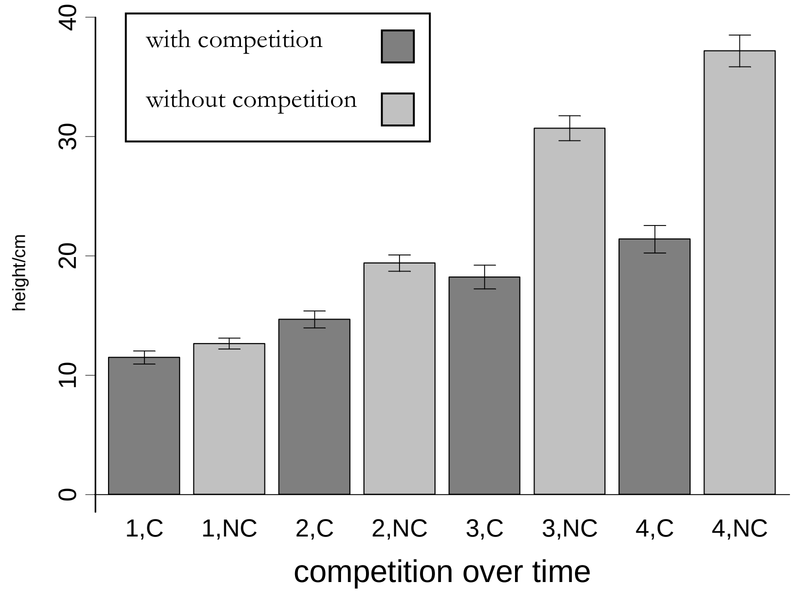

Figure 16

Effects of plant competition on growth of *Senecio inaequidens* in the field experiment at four subsequent observation times (“1”-“4” on the x-axis; “1”=18th July, “2”=1st August, “3”=16th August and “4”=18th August 2002). Treatment levels: C=with competition, NC=no competition; response: plant height/cm. In the “no competition” plots, plant height was significantly increased (28th August: $F_{1,208}$=81.50, p<0.0001***, split-plot ANOVA on “4,C” and “4,NC” data)

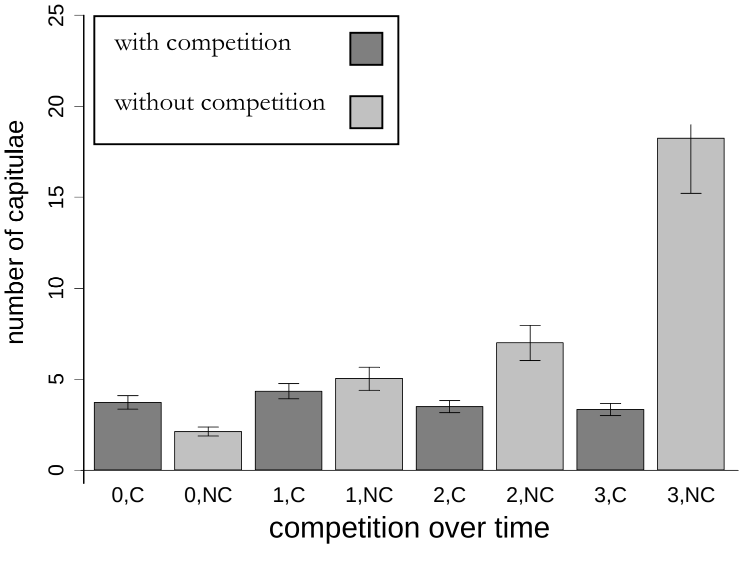

Figure 17

Effects of plant competition on the number of capitulae in *Senecio inaequidens* in the field experiment at three subsequent observation times (“1”-“4” on the x-axis; “1”=7th August, “2”=16th August and “3”=28th August 2002; note that these times were different from the ones in Fig. 7). Treatment levels: C=with competition, NC=no competition; in the “no competition” plots, number of capitulae was significantly higher (28th August: $F_{1,208}$=84.18, p<0.0001***, split-plot ANOVA on “3,C” and “3,NC” data)

3.4.6 Direct effects of herbivory

3.4.6.1 Vertebrate herbivores

Rabbit grazing directly lead to the death of 182 out of 216 plants – i.e. about 84% of the *Senecio inaequidens* plants died through rabbit herbivory (**Fig. 23**).

Plant height of *S.inaequidens* was greatly altered by rabbit grazing, as shown in **Fig. 18** and **Plate 8 a**. Surprisingly, the effect was not significant in a strict sense, but this result is due to the low replication (there were only two grazed plots) and hence the low residual degrees of freedom. Clearly, if there had been more experimental plots, the effect of rabbit grazing would have become significant.

Rabbit herbivory was less intense in plot two compared with plot one, as can be seen in **Fig. 18 b** and **c**. In addition, plants that were grazed by rabbits *and* experienced plant competition, were more likely to die and were less able to form new regrowth shoots (see **Fig. 23** and **24** and especially **plate 8 b**). For details see below.

The only other vertebrate herbivore which was found to be present even inside the fenced plots was roe deer, *Capreolus capreolus* L.[50]. Roe deer herbivory occurred only occasionally, and in total seven out of 108 plants in plot one, and four plants out of 108 in plot two had presumably been clipped by *Capreolus* until the last data collection on 28th August.

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[50] presence of roe deer could be demonstrated by droppings that had been found in both of the fenced plots

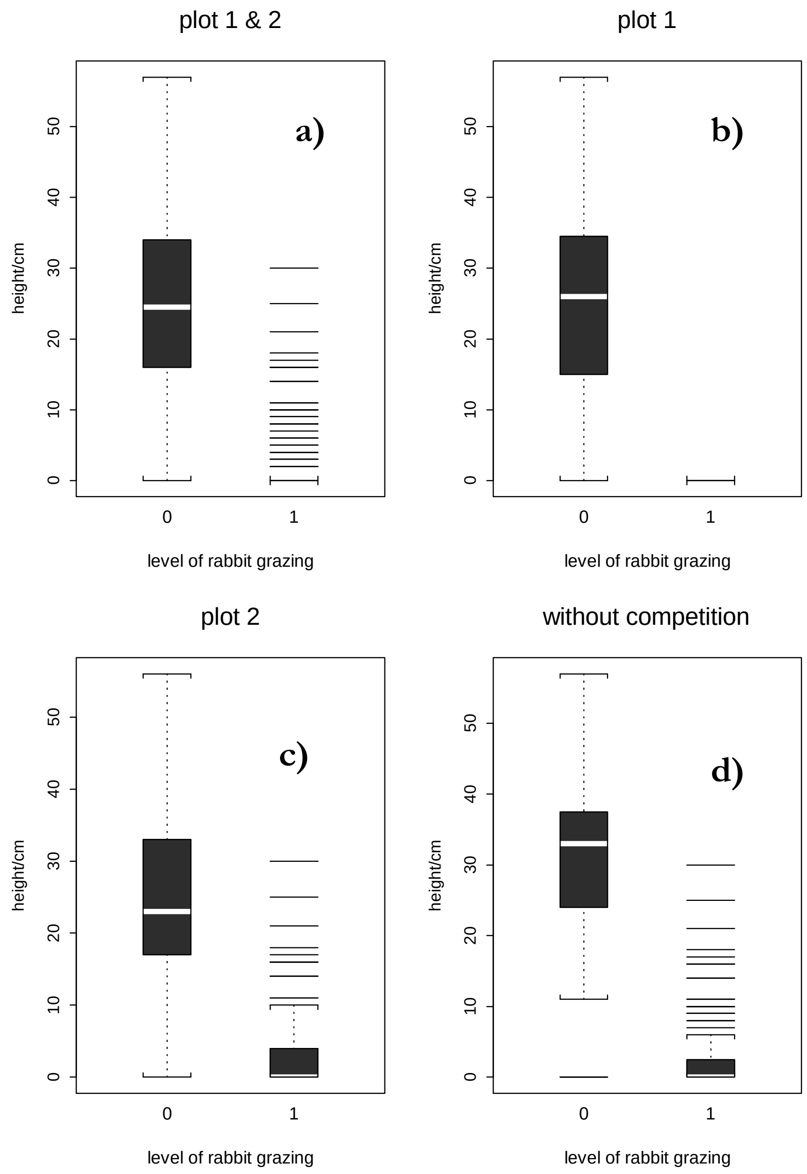

Figure 18

Effects of rabbit grazing on the maximum height/cm of *Senecio inaequidens* in the field experiment (16th August 2002); 0=fenced, 1=unfenced part of the field plots; (a) overall effects without distinction between plot 1 and 2; $F_{1,1}$=109.829, p=0.06 (n.s.); the non-significant p-value is due to the low replication (n=2, residual d.f.=1) (b) effects in plot 1 (no survivors in the unfenced part); (c) effect in plot 2; some plants in the unfenced part show regrowth after clipping; (d) in the cultivated strips (i.e. without competition), plants are more likely to survive than (not shown) with competition; $F_{1,410}$=101.42, p<0.0001*** (a)-(d) analysed as split-plot-ANOVA without response transformation.

3.4.6.2 Effects of invertebrate herbivores

There was a significant negative relationship between number of leaves eaten by molluscs (**Plate 9 b**), and the number of capitulae produced. This means, *Senecio inaequidens* produced fewer capitulae if more leaves had been eaten by molluscs (see **Fig. 19**). However, the regression line in that figure explains only six per cent of the variation in the data (R^2=0.06).

A similar relationship could be found between the number of leaves eaten by molluscs, and the number of branches produced (linear regression on log transformed data; intercept: 1.76±0.082, t=21.56, p<0.0001***; slope: -0.38±0.102, t=3.76, p<0.001***, residual s.e.: 0.8685 on 201 d.f., R^2=0.06573).

About 10 mm^2 of leaf area were eaten by *Longitarsus jacobaeae* per plant (see **Fig. 20**); there was no significant difference in *Longitarsus* herbivory between the plots, and significant differences could only be found between ecotypes (see section 3.4.4.2).

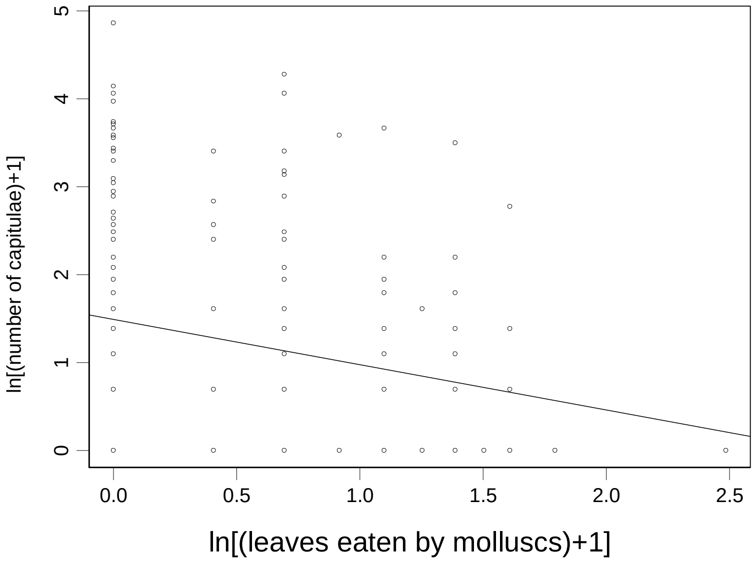

Figure 19

Relationship between the number of leaves eaten by molluscs, and the number of capitulae produced by *Senecio inaequidens* in the field experiment; linear regression (for plants with height>0 cm; data transformation: ln(x+1)), intercept: 1.49 ±0.112, t=13.33, p<0.0001***; slope: -0.51 ± 0.144, t=-3.57, p<0.001***, multiple R^2=0.06, residual s.e.=1.25 (d.f.=214)

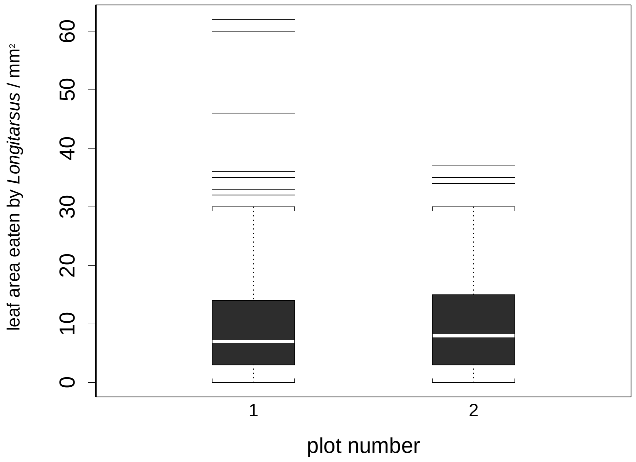

Figure 20

Herbivore damage to *Senecio inaequidens* leaves in the field experiment. Total leaf area eaten by *Longitarsus jacobaeae* per plant is shown for each experimental plot (assuming that one shothole equals the consumption of 1mm^2; split-plot ANOVA on log-transformed data from 18th August 2002, plants with zero height excluded; $F_{1,176}$=0.05, p=0.82 (n.s.))

3.4.7 Interaction effects

Several complex interactions between different factors could be observed, and some of them – especially those involving invertebrate and vertebrate herbivores and plant competition - were significant (see **Fig. 12 a, 21, 22 a**, and **23** for interactions that were analyzed in detail).

3.4.7.1 Interactions involving invertebrate herbivores

Fig. **12 a** shows that there was a significant positive relationship between the number of *Longitarsus* holes found in *Senecio inaequidens* leaves, and the number of leaves eaten by molluscs; however, only seven per cent of the variation in the data are explained by the regression line.

Plant competition significantly affected the number of *S.inaequidens* leaves eaten by molluscs (**Fig. 21**); plants growing in the uncultivated plots experienced significantly more damage to mollusc herbivory than those growing without competition.

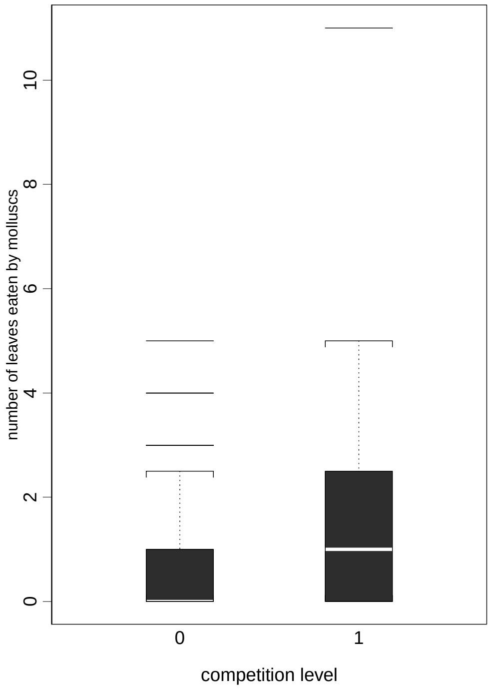

Figure 21

Number of *Senecio inaequidens* leaves eaten by molluscs in relationship to plant competition (0=cultivated, 1=uncultivated) in the field experiment (28th August 2002); $F_{1,208}$=14.28, p<0.001*** (split-plot ANOVA, response transformation ln(x+1)).

3.4.7.2 Interactions involving treatment and ecotype

Fig. **22 a-c** show complex interactions between treatment application, ecotype, and means of morphological parameters of *Senecio inaequidens*. None of these interactions were significant, but nevertheless the results will be described briefly below; they will not be discussed later.

The treatment:ecotype interaction shown in **Fig. 22 a** did not have a significant effect on mean plant height (F1,174=231.81, p=0.06 (n.s.)); however, there were remarkable differences between the ecotypes: whereas ecotype b ('Montpellier') showed a strong positive reaction to insecticide application, ecotypes a and c behaved in opposite direction. All ecotypes exept b showed a positive reaction to molluscicide application, and all ecotypes except a grew taller under a combination of insecticide and molluscicide.

There was also no significant effect of the treatment:ecotype interaction on the number of capitulae produced per plant ($F_{1,174}$=0.72, p=0.62 (n.s.), **Fig. 22 b**); ecotypes a and b produced more capitulae after insecticide application; ecotype a showed a negative reaction to molluscicide application, and all ecotypes except c produced less capitulae under a combination of insecticide and molluscicide.

The last interaction studied in this context is shown in **Fig. 22 c**. Mean number of leaves was highly affected by treatment in ecotype c, being lowest with insecticide application; in contrast, ecotype b reacted highly positively to all treatments compared to the control treatment (w). All these interactions, again, were non significant ($F_{1,174}$=1,10, p=0.36 (n.s.)).

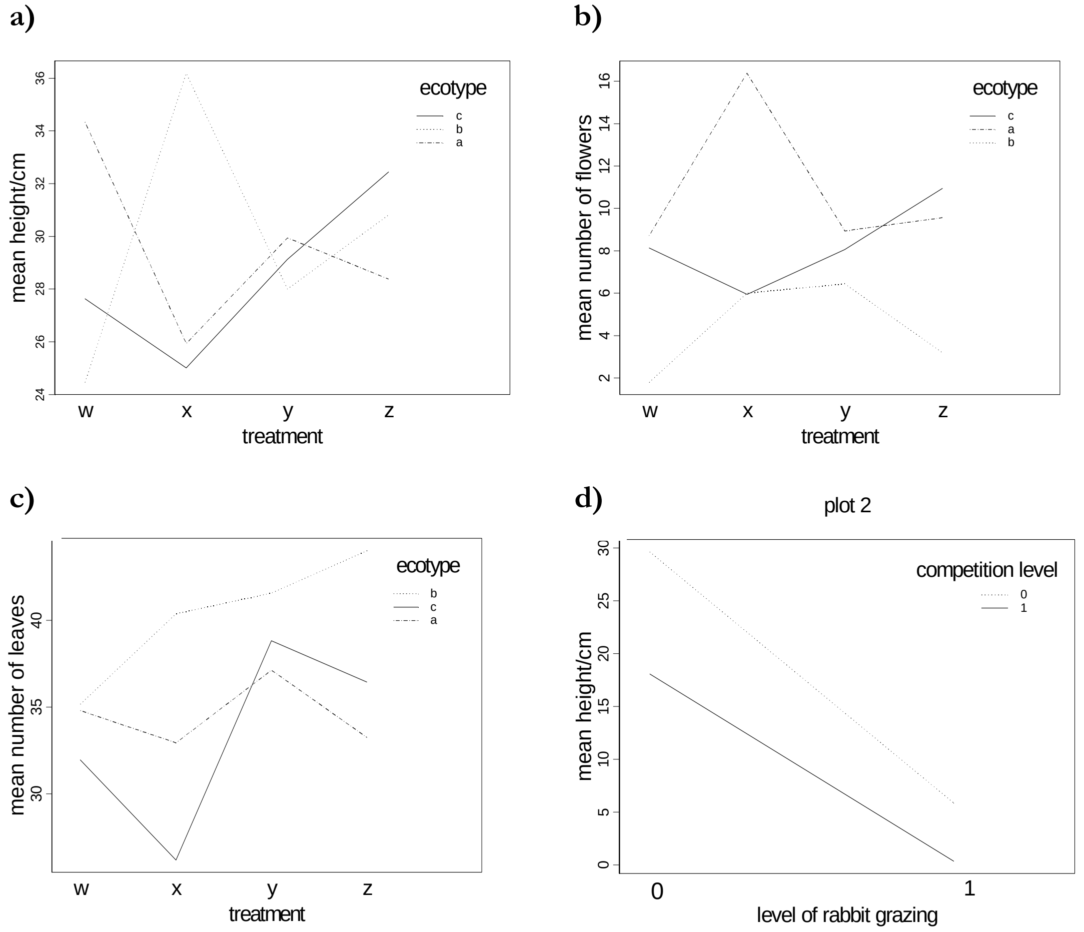

Figure 22

Interaction effects (field plots, 28th August 2002). (a)-(c): Complex interaction effects between different treatments and ecotypes of *Senecio inaequidens* (shown only for plants with height>0 cm) in relationship to (a) mean mean height/cm, (b) mean number of capitulae and (c) mean number of leaves; (d): interaction between level of rabbit grazing and competition level (0=without, 1=with competition or rabbit grazing, respectively); only interactions in (d) were significant; treatment levels: w=water, x=insecticide, y= molluscicide, z=insecticide+molluscicide; ecotypes: a=Rostock (Germany, 1.11.2001), b= Montpellier (France), c=Konstanz (Germany). For details see text.

3.4.7.3 Interactions between rabbit grazing and interspecific plant competition

As already presented in the ANOVA tables above, a highly significant interaction between rabbit grazing and plant competition could be observed where plant height was the response variable. Plants growing without competitors grew taller both with and without rabbit grazing, compared to plants growing in the uncultivated plots (**Fig. 22 d**).

Fig. 23 gives a similar result for the number of dead individuals per treatment. Although these data were not analyzed in detail (a contingency table, calculated for the different plot sizes of a split-plot experiment, would have been the appropriate method), it can be stated that plants growing under a combination of rabbit grazing and plant competition showed the highest proportion of dead individuals ("R+C+", 96%) compared to grazed plants that grew without competition ("R+C-", 72%).

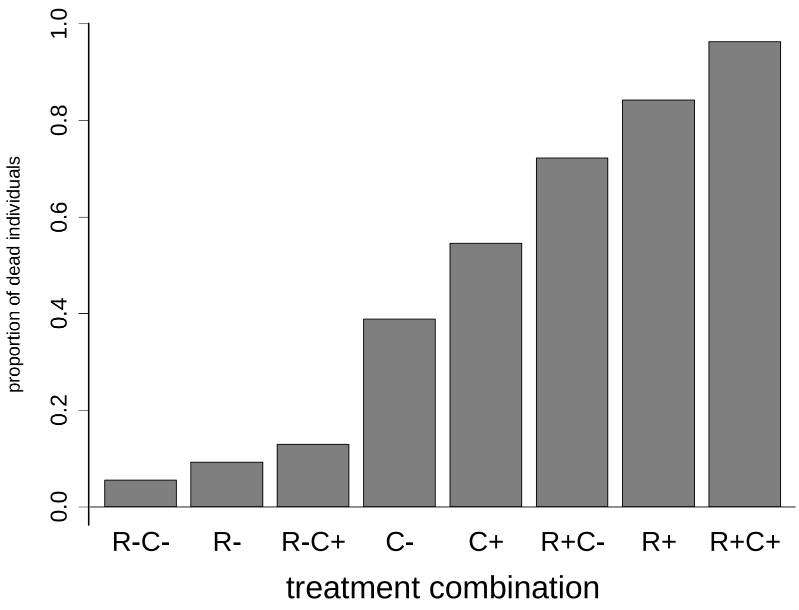

Figure 23

Effects of competition and rabbit herbivory on survival of *Senecio inaequidens* in the field experiment (16th August 2002). Treatment levels: R+/R- with/without rabbit grazing, C+/C- with/without competition. Response: proportion of dead individuals; from left to right, the bars correspond to 6, 20, 14, 84, 118, 78, 182 and 104 dead individuals out of a total of 108, 216, 108, 216, 216, 108, 216 and 108 individuals. A combination of rabbit herbivory and plant competition (R+C+) showed the strongest effect.

3.4.8 Regrowth after clipping

On plot two, *Senecio inaequidens* plants that had been clipped by rabbits showed a high regrowth capability; the shoots produced after clipping were not accepted as food any more by the rabbits, and four out of 108 plants that had initially been clipped by rabbits in plot two even produced capitulae by the end of the experiment.

The high regrowth ability of *Senecio inaequidens* is indicated in **Fig. 24**; plants growing without competition showed significantly longer regrowth shoots ('height') than those with competition. Note the outlier that even reached 30 cm height by 16th August; until the end of the experiment, the regrowth shoots were even taller, but these results were not analysed in detail any more.

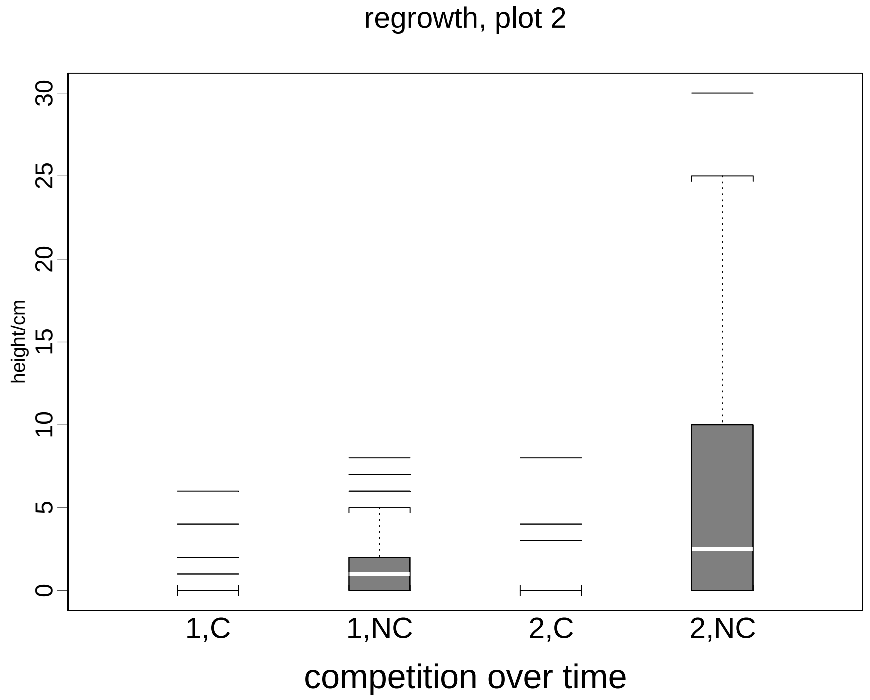

Figure 24

Effects of plant competition on regrowth of *Senecio inaequidens* after having been clipped by rabbits (field experiment, plot 2, unfenced part). Maximum length of the regrowth shoots ("height/cm") is shown as measured at two subsequent observation times (1=24th July and 2=16th August 2002; note the different response variable compared to Fig. 7 and 8). Treatment levels: C=with competition, NC=no competition; in the "no competition" plots, regrowth was significantly higher (16th August: $F_{1,90}$=30.96, p<0.0001***, split-plot ANOVA on "2,C" and "2,NC" data)

3.4.9 Colonization by *Longitarsus jacobaeae*

One of the most remarkable and unexpected results of the field experiments was the free colonization of the *Senecio inaequidens* plants by the specialist herbivore *Longitarsus jacobaeae* (**Plate 1 b**). Colonization started within less than five days after transplantation of the seedlings from the greenhouse to the field plots; it is even assumed that colonization started immediately after transplantation, but all the results were so surprising that the colonization was only spotted for the first time on 5th July (transplantation dates: 27th June and 1st July, respectively). An overview of the total numbers of *Longitarsus* beetles that could be counted on *S.inaequidens* has already been presented in **Fig. 4**. It is remarkable that even plants that had been freshly clipped by rabbits were colonized by *Longitarsus* – the beetles feeding on regrowth leaf laminas of less than two centimeters length.

3.4.10 Colonization by other insect herbivores[51]

Within a few days after *Senecio inaequidens* seedlings had been transplanted into the field, they were already colonized by insect herbivores.

By the end of the field experiment, between August and September 2002, several Heteroptera species could be observed colonizing *Senecio inaequidens*. On 28th August, 13 adults of one unidentified[52] heteropteran species could be counted on the 108 plants in the fenced part of plot one (geometric mean: 0, with a maximum of five individuals on one plant). Some of them seemed to feed on the achenes inside the capitulae; microscopical observation of dissected capitulae (using a stereo microscope) showed no presence of flowerhead-inhabiting insect larvae; nevertheless, some parasitoid wasps could be observed showing typical host-searching behaviour on *Senecio inaequidens*. In some of the plants, holes produced by stem-boring insects could be observed[53]. In many cases, the holes could be found directly at the apical end of the pedicels, leading to bending and drying-out of the capitulae and preventing achene ripening. 37 of these dried-out capitulae could be found on plot one on 28th August, and seven on plot two. Aphids were present in the fenced plots in small colonies on two plants (out of 108 plants in total) throughout the season, without any visible adverse effects on plant performance.

3.5 Additional observations

3.5.1 Studies on natural populations

On 6th October, 24th October, 1st November 2001, and on 8th June 2002, natural populations of *Senecio inaequidens* in Rostock (north-east Germany) were visited in order to collect achenes; on this occasion, a screening for herbivore occurrence and abundance was conducted, and the presence of flowerhead-visiting insects was noted. The only locations where *Senecio inaequidens* could be found in Rostock were:

a) at Warnemünde-Werft railway station
b) at Rostock-Toitenwinkel freight depot (only two several-year-old plants could be found)
c) at Rostock-Südstadt, one single plant growing on an abandoned shunting yard

All habitats were characterized by more or less open vegetation, the most common plants[54] recorded there being *Conyza canadensis* (L.) Cronq. , *Melilotus alba* Medicus, *Hypericum perforatum* L., *Solidago canadensis* L., *Tanacetum vulgare* (L.) Bernh., *Artemisia vulgaris* L., *Epilobium angustifolium* L., *Daucus carota* L., and the grasses *Bromus tectorum* L., *Dactylis glomerata* L. and *Calamagrostis epigejos* (L.) Roth.; whith increasing density of the grass stratum, the population density of *Senecio inaequidens* decreased, and in dense tall-growing stands no more individuals could be found (a very good example for this could be observed at Warnemünde-Werft, where there was a gradient in percentage vegetation cover from the slope of the railway track downward to the

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[51] this section is intended to stimulate future research
[52] samples were taken for future determination.
[53] no inhabitants could be found any more by dissection of pedicels on 28th August
[54] this list does not make any claim to be exhaustive, and is based on own field notes

abandoned shunting yard). In sparse vegetation, seedlings of *S.inaequidens* could be found in June 2002, frequently growing on soils consisting of a mixture of sand and gravels.

Invertebrate herbivores frequently recorded included Heteroptera, green Cicadellidae (Homoptera: Auchenorrhyncha), aphids (only single individuals), and tephritid flies (Diptera: Tephritidae); pollinators observed were especially syrphids (Diptera: Syrphidae). Molluscs could be found very frequently (*Cepaea* sp., Helicidae) resting on the stems during the daytime, but it would still need to be verified wether they do feeding damage at night or not. Very frequently, predatory spiders (mostly non-Araneidae) could be found; all these groups would be worth thorough investigations.

3.5.2 Pollination experiments

The experiments in the pollination chambers clearly showed that *Senecio inaequidens* is capable of producing viable achenes even in total absence of pollinators; it can be stated, therefore, that even single plants arriving at one location are capable of forming initial populations that can form new centres of spread. In the greenhouse experiments, even the second generation of self-pollinated individuals produced ripe seeds in the same year; however, germination rates were not analysed in detail.

3.5.3 Vegetative reproduction

Stem cuttings of *Senecio inaequidens* were capable of producing adventitious roots within three days after having been cut from the mother plant; even when stem fragments were just left lying on bare ground (without sticking them into the substrate), adventitious roots were produced. Main stems that had been damaged or broken by heavy bending often produced a crown of adventitious roots. In the field, stems that had direct contact to the soil surface regularly produced secondary roots, attaching the stem to the ground and supporting the plants in order to enter the phase of upright stem growth; the basis of old first-year stems regularly formed multiple branches that sometimes grew adventitious roots again, so that it was often difficult to decide to which plant the shoots belonged; it can therefore be stated that *S.inaequidens* is able to spread vegetatively, in addition to its high ability for far-distance wind dispersal via achenes.

3.5.4 Test for vesicular-arbuscular mycorrhiza

This test was performed in order to find out wether *Senecio inaequidens* is regularly associated with a mycorrhizal symbiont. Colonization of one of the plants[55] by a vesicular arbuscular type mycorrhizal fungus was estimated to be around 1% of the fine root length at the time when the first new above-ground shoots appeared (6th June 2002), and it is likely that the degree of colonization might increase with ongoing growth of the above-ground plant parts. The whole root surface was furthermore covered with a loose mycelium, which sometimes even occurred endophytic.

All these results raise very interesting questions as to wether there is a mutualistic interaction between below-ground microorganisms and *Senecio inaequidens*, and wether or not these organisms are obligate symbionts of

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[55] from Rostock-Warnemünde Werft, Germany, 6th June 2002

Senecio inaequidens that have been introduced to habitats outside the native range of *Senecio inaequidens*, together with propagules[56]. All these first findings raise highly interesting questions that should be answered in future observations.

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[56] G.Berg, Microbiological Instutite, University of Rostock, Germany.

4 Discussion

4.1 Introductory remarks

As germination tests, pollination experiments, the test for vesicular-arbuscular mycorrhiza and observations on vegetative reproduction only served as preliminary or additional experiments, they shall not be discussed in detail here; instead, the focus will be on the three main experiments conducted in this study, namely

(1) feeding trials
(2) greenhouse experiments and
(3) field experiments.

4.2 Feeding trials

4.2.1 Field and laboratory trials with *Longitarsus jacobaeae*

One of the most unexpected results of this study was that *Longitarsus jacobaeae* freely colonized *Senecio inaequidens* under free choice field conditions. As *Longitarsus jacobaeae* has never been found on *Senecio inaequidens* before (Herrera 2002, 2000; Mazel & Garrigue 2000; Schmitz & Werner 2000; Ernst 1998; Kuhbier 1977), *Senecio inaequidens* can be regarded a new host plant record for *Longitarsus jacobaeae*.

It is unlikely that the presence of *Longitarsus* on *Senecio inaequidens* was due to random dispersal events; of course, this assumption would need to be tested experimentally, but the quick and complete colonization and acceptance of *Senecio inaequidens* as a new food source indicates that further experimental support might not be required.

Although adult feeding damage did not have a significant effect on plant performance, the observed presence of *Longitarsus* on *Senecio inaequidens* is – as stated by Frick 1970 – "important to the behavioural sequence of host selection and plant recognition, because the adults select the plants on which the larvae will feed and develop." The next vegetation period(s) will show wether or not *Longitarsus* larvae will feed on *Senecio inaequidens*, and this would be an interesting starting point for more detailed experimental studies involving feeding trials with immature stages.

The feeding trials conducted in the laboratory showed that *Senecio inaequidens* leaf pieces are accepted as a food source, but that *Senecio jacobaea* is significantly preferred over *Senecio inaequidens* in terms of the surface area removed.

It is very important, however, to distinguish between damage measured in terms of "square millimeters leaf area removed" and damage measured in terms of plant biomass or fitness. It may well be that leaf texture, water content, amount of structural tissue, and leaf nitrogen content between the two *Senecio* species offered were different, and that differences in feeding rates or amounts of leaf area eaten do not reflect preferences properly. In additional tests, it would be advisable to measure consumption rates of the beetles, and to perform trials with filter paper disks and different plant extracts in order to find out about attractants and deterrents, the influence of different pyrrolizidine alkaloid composition and further parameters of special interest.

The points mentioned so far clearly indicate that host plant acceptance cannot be determined by laboratory experiments alone; although the feeding trials under controlled environmental conditions had shown that there was a strong significant preferrence for *Senecio jacobaea* in comparison with *Senecio inaequidens*, the picture seen in the field looked quite different, with flea beetles even colonizing clipped plants that barely had any regrowth foliage at all, despite there being individuals of *Senecio jacobaea* growing in close vicinity. This indicates that host plant selection can by no means solely be based on visual cues, but that instead chemical and olfactorial stimuli must be involved in the acceptance of *Senecio inaequidens* by *Longitarsus jacobaeae*. – an assumption which is also supported by the experimental evidence from the feeding trials with leaf disks, and which has often been described in the literature (see, for example, Way & Ahmad 1983).

4.2.2 Feeding trials with *Tyria jacobaeae*

All experiments conducted with *Tyria jacobaeae* have shown that *Senecio inaequidens* is not accepted as a host plant. This is consistent with results obtained by Mazel & Garrigue 2000 – these authors conducted some feeding trials (with only n=5 larvae) and found that only the rayflorets of the flowerheads were eaten after application of the larvae.

In the study presented here, none of the larval instar stages, neither when directly raised on *Senecio inaequidens*, nor when transferred to *Senecio inaequidens* from the field, survived for longer than seventeen days. Field and laboratory experiments produced very similar results – *Tyria jacobaeae* refused to accept *Senecio inaequidens* even when depletion of its original host plant had been simulated by clipping the flowering stems of *Senecio jacobaea.*

The high mortality rates are definitely not only due to the unsuitability of *Senecio inaequidens* as a host plant; cultivation of *Tyria* proved difficult, at least in conjunction with all the other experiments running at the same time. Nevertheless, all the choice trials conducted have clearly shown that neither capitulae nor leave pieces of *Senecio inaequidens* are accepted as long as the original host plant is present.

Even in no-choice trials, *Tyria* did not have a remarkable influence on fecundity of *Senecio inaequidens* plants that were older than four weeks.

All the results presented are consistent with other studies that have tested host plant acceptance of *Tyria jacobaeae*, especially Tinney *et al.* 1998.

There is only one study, in which *Tyria jacobaeae* egg batches had been deposited on *Senecio inaequidens* under field conditions (Ernst 1998), "despite a well-developed population of *Senecio jacobaea* in the neighbourhood of *Senecio inaequidens*"; although Ernst states in his publication from 1998 that "caterpillars fed freely on the leaves and developed to maturity", personal communication showed that this only seems to have happened by chance, because the population was not stable, and no more observations of this kind could be made since 1998.

To sum up, there is enough evidence to show that *Tyria jacobaeae* is not accepting *Senecio inaequidens* as food source or host plant.

4.3 Greenhouse experiments

The competition experiments performed in the greenhouse basically lead to similar results compared with the field experiments; both experiments together give strong support to reject the null hypothesis that growth and reproduction of *Senecio inaequidens* is not affected by plant competition. All results show clearly that interspecific plant competition significantly reduces growth, survival and fecundity of *Senecio inaequidens*.

As competition mattered both under simplified greenhouse conditions and in complex multi-species interactions in the field, it can be hypothesized that density and not species identity ultimately determines the effect of competition on *Senecio inaequidens*[57].

This conclusion is in contrast to observations performed by Troussel 1998 at University of Montpellier (France), who stated that the effect of competition on *Senecio inaequidens* was dependent on species identity of the competitors[58]; unfortunately, the author did not include monocultures of *Senecio inaequidens*, which complicates direct comparisons to the study presented here.

Although competition with *Festuca* lead to the immediate death of most *Senecio inaequidens* individuals under greenhouse conditions, this result should not be overinterpreted. The difference in the competition levels chosen was very high, and there were only two levels in total; the interaction between interspecific plant competition and seedling herbivory by fungus gnats larvae (Diptera: Sciaridae) in the onset of the experiments may have contributed to higher death rates than would have been obtained in absence of these

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[57] as already noted, the density of *Senecio inaequidens* had been held constant, while density of *Festuca* was varied – which makes it possible to directly assess the effects of competitor density.

[58] in these experiments, mean height of *Senecio inaequidens* (4 plants per pot with 27.5 cm diameter) was significantly lower when grown in competition with *Dactylis glomerata* L. (sown out at a density of 4 g caryopses/m^2), than when *Senecio inaequidens* was grown in competition with various other plants.

herbivores. Nevertheless, it is hypothesized that the overall resulting effect of competition would not have been significantly different without the presence of fungus gnats.

It would have been of interest to vary the density of *Festuca* over a wider range than just two levels, and to determine exactly the point at which competitive exclusion occurs. Again, however, this would not have changed the principal result that *Senecio inaequidens* is less able to survive and reproduce under high competition by *Festuca.*

Notably, time played an improtant role in determining the outcome of the greenhouse competition experiment – if *Festuca rubra* had not been allowed to form a closed vegetation cover prior to addition of *Senecio inaequidens*, it might well have been possible that this would have resulted in stable coexistence of both species. It might furthermore have been possible, that *Senecio inaequidens* shows allelopathic effects on *Festuca rubra* – this would be an interesting question to answer in future observations.

4.4 Field experiments

4.4.1 Differences in morphological parameters, and between experimental plots

It is a commonly observed phenomenon that size distributions in morphological parameters in plant populations change with time, usually towards an asymmetric, positively skewed distribution (Hutchings 1986). The observed overall changes in the height distribution *of Senecio inaequidens* in the field experiments are therefore consistent with theory.

The observed differences between the experimental plots may be due to microclimatic disparities at the two field sites; plot two generally showed a higher formation of dew in the morning and a higher abundance of bryophytes in the grass stratum than plot one. This may be a plausible explanation for the higher mollusc damage found in plot two. However, it has not been tested wether or not the population densities in dominant mollusc species were different between the two plots.

It remains unclear why the mean number of branches was different between the two plots.Yet, a linear regression analysis showed that there was a significant negative relationship between number of leaves eaten by molluscs, and number of branches produced; this may at least partly explain the lower number of branches on plot two.

4.4.2 Differences between ecotypes of *Senecio inaequidens*

The morphological differences between ecotypes were significant in terms of stem diameter and number of capitulae produced. This might be explained by initial size differences that occurred despite the fact that all ecotypes had been sown out on similar dates and had been raised under identical conditions. One plausible explanation might be that there may have been differences in initial germination parameters (but also in seed size, see Crawley 1983) between the three ecotypes. On the other hand, there is no simple explanation for the fact that ecotype b showed the highest mean stem diameter, but the lowest mean number of flowers, because the overall regression analysis would predict the contrary (size-specific fecundity, see Crawley 1983). Therefore, it is hypothesized that the morphological differences between the ecotypes are inherited. It is especially remarkable that it was ecotype b (from Montpellier, France) that differed significantly from the other two (German) ecotypes in the two morphological parameters discussed here.

A similar picture can be drawn from the differences in herbivore damage. Again, ecotype b differed significantly from the other two ecotypes, especially in the number of leaves eaten by molluscs, but also in the number of *Longitarsus* shotholes – indicating that this ecotype suffered higher herbivore damage than the other two.

Of course, all these interpretations are speculative, especially because there are many and complex interactions between the factors studied, and it might well be true that the higher number of leaves eaten by molluscs in ecotype b might have influenced the number of capitulae produced, and that this would explain the inverse relationship between stem diameter and number of capitulae in this ecotype.

It would be most interesting to find out wether the differences in herbivore damage can be attributed to differences in pyrrolizidine alkaloid concentrations or –composition between these three ecotypes. This would be a challenging topic for further studies. Also, it would be interesting to find out if different eco- or chemotypes (possibly even genotypes s.str.) are restricted to different areas in continental Europe, and wether these differences can be attributed to different repeated introductions of *Senecio inaequidens* about one hundred years ago.

4.4.3 Herbivory and competition effects

Especially in the first weeks after transplantation of the seedlings, there was severe damage caused by molluscs, insect herbivores and rabbits. The rabbit fences did indeed protect *Senecio inaequidens* from being eaten, but insecticide and molluscicide treatments were probably applied too late. As the high incidence of invertebrate herbivory was completely unexpected, the whole experimental setup had to be changed quickly, and it was not possible to apply the treatments earlier. Still, the close examination of damage on the youngest three leaves revealed a slight trend that insecticide application may have lead to a reduction in the number of *Longitarsus* shotholes. However, as already mentioned in the results section, these differences may also be explained by chance alone and cannot be attributed to the treatment applications.

The dominant factor influencing growth and fecundity of *Senecio inaequidens* in the field experiments was plant competition. This had already been suggested by Ernst 1998, who found that transplanted seedlings (April 1995) “grew slowly, but died all off during the first dry period in July” under competition with native grassland species in The Netherlands. Additionally, he states that when *Senecio inaequidens* was experimentally sown in grassland plots, no seedlings could be found; his conclusion, therefore, was that *Senecio inaequidens* was "sensitive to competition for light and water". The experiments conducted during the study presented here give further support to these initial assumptions.

Not only was plant competition directly significantly influencing all major morphological parameters studied, including height, stem diameter, number of 1st order branches, number of leaves, and number of capitulae produced, but it was also having significant indirect effects mediated by presence or absence of herbivores.

Joint effects of herbivores and plant competitors are commonly observed in experiments of this kind, and it is often the case that only the interaction between these two factors significantly influences plant fecundity or size distribution (Case & Crawley 2000; Crawley 1997b; Bonser & Reader 1995). This can be readily seen in the effects of rabbit grazing on plant height in *Senecio inaequidens*. Whereas grazing alone was not significant, both competition and the interaction term between rabbit grazing and competition showed significant effects on plant height.

The fact that rabbit grazing did not appear to be significant, despite the obvious differences between the fenced and unfenced plots in the field, is due to the low number of replicates in the split-plot experiment (n=2) and the resulting low residual degrees of freedom (d.f.=1). If there had been more replicates, certainly rabbit grazing would have appeared to be highly significant.

Rabbit grazing was less intense in plot two; this can easily be explained by the fact that plot one lay in close proximity to a rabbit warren, whereas plot two did not. Disturbance created by rabbits in plot one was much more intense than in plot two, and the combined effects of lower disturbance, lower abundance and lower feeding intensity at plot two not only lead to differences in survival, but also in the probability that regrowth could occur after the plants had been clipped by rabbits. Generally speaking, it can be stated that vertebrate herbivores (rabbits and roe deer) did initially feed on *Senecio inaequidens*, but (at least in the case of rabbits) regrowth shoots were not accepted as a food source any more. This may have profound consequences, especially in grazed ecosystems, where *Senecio inaequidens* may have an advantage due to its ability to overcompensate after complete removal of the above-ground plant parts (for a recent review see Agrawal 2000).

Invertebrate herbivores had much less severe effects on morphological parameters of *Senecio inaequidens* – again, this is a well-documented fact in the literature (see, for example, Crawley 1989). As already stated above, mollusc herbivory slightly decreased the number of capitulae produced in *Senecio inaequidens*; this may be due to the fact that overall leaf area was reduced by mollusc herbivory, and less assimilates could be accumulated (lower primary productivity). Zangerl *et al.* 2002 even write that herbivory (in this special case: folivory) may affect photosynthesis even "at considerable distance from the damaged tissue", the indirectly affected areas being on average "six times that of the area directly affected by tissue removal".

Interestingly, mollusc herbivory was more intense in the undisturbed plots – which may be explained by the fact that molluscs sought shelter in dense vegetation and suffered less from drought stress than on the disturbed plots. The slightly positive interaction between overall mollusc damage and *Longitarsus* feeding damage may be confounded by abiotic conditions, such as microclimatic differences between the two plots – as already mentioned above.

4.5 Host-switching in oligophagous insect herbivores

Probably the most exciting result of this study is that *Longitarsus jacobaeae*, a species usually thought to be restricted to *Senecio* jacobaea, performed host-switching to an invasive alien plant. This is one of the rare examples where the 'herbivore release hypothesis' (Keane & Crawley 2002) is not supported by experimental data.

It can be hypothesized that cases like the one of *Senecio inaequidens* and *Longitarsus jacobaeae* are no exception, and that invasive alien plants are colonized and accepted by native oligophagous insect herbivores under the following conditions:

a) the invading plant has relatives, at least of the same genus, in the invaded area
b) the dispersal distance for the herbivore to colonize the invading plant population is low
c) host switching of the native oligophagous herbivore has already occurred between native host plants of different species.

4.6 Susceptibility of grassland ecosystems towards invasion by *Senecio inaequidens*

In a letter from November 2001, D.Brandes (Braunschweig, Germany) wrote that he had "no doubt, that *Senecio inaequidens* can (…) form viable populations in pasturelands." He furthermore reported on occurrences of *Senecio inaequidens* in cattle-grazed limestone pastures in northern Italy (southern ridge of Monte Baldo) and on sandy calcareous grasslands heavily grazed by rabbits within the city of Braunschweig, Germany. As already described in the introduction, there is also strong evidence that *Senecio inaequidens* is already occurring as a pastureland 'weed' in mountaneous areas in the Colombian Andes.

Given these first indications, and given the results from the field experiments conducted at Silwood Park, it is clear that *Senecio inaequidens* is on its way from "railway and road alien" to becoming a species with might sooner or later invade agricultural ecosystems. As *Senecio inaequidens* is favored by low competition (i.e. high disturbance), and capable of overcompensation after total clipping by vertebrate herbivores, it is likely that especially overgrazed pasturelands may in the future be colonized by this species. It is therefore strongly recommended that populations of *Senecio inaequidens* are monitored, and that more research especially on the effects of native oligophagous insect herbivores is conducted.

5 Final conclusions and need for further research

This study has clearly shown that growth, survival and fecundity of *Senecio inaequidens* DC., an invasive alien plant, are significantly affected by the presence or absence of a competing plant species (*Festuca rubra* L., Poaceae) in greenhouse experiments, and by the presence or absence of vegetation cover in field experiments. Under high densities of the competing plant species, *Senecio inaequidens* showed a significant decrease in size and reproductive potential.

Generalist vertebrate herbivores (*Oryctolagus cuniculus* L., Mammalia: Lagomorpha) significantly reduced growth, survival and reproduction of *Senecio inaequidens* in field experiments, when a closed vegetation cover was present, whereas plants growing in plots with reduced vegetation cover were able to survive and form regrowth shoots after rabbit grazing that were not affected by subsequent vertebrate herbivory any more.

Generalist invertebrate herbivores (molluscs, especially of the families Helicidae, Arionidae and others) led to a decrease in reproductive ability of *Senecio inaequidens*, but slug herbivory was restricted to the first weeks after transplantation of *S. inaequidens* seedlings into the field.

A specialist insect herbivore (*Longitarsus jacobaeae* Waterhouse, Coleoptera: Chrysomelidae) with strong host specificity to a different *Senecio* species (*Senecio jacobaea* L.) showed spontaneous host-switching and adult individuals accepted *Senecio inaequidens* as a new host plant in field and laboratory experiments. *Senecio inaequidens* was freely colonized by *Longitarsus jacobaeae*, and feeding as well as mating behaviour on this plant could be observed. The results achieved with *Longitarsus jacobaeae* suggest that resident specialist insect herbivores could reduce the probability of invasion of an alien plant. In contrary to current theory on plant invasions, *Senecio inaequidens* does not seem to benefit from an enemy release.

Larval stages of a second specialist herbivore studied (*Tyria jacobaeae* L.; Lepidoptera: Arctiidae) did not accept *Senecio inaequidens* and showed strong preference for *Senecio jacobaea* in field, laboratory and greenhouse experiments.

There were significant differences between ecotypes of *Senecio inaequidens* in terms of morphological parameters, herbivore damage and reproductive ability. These results point out the importance to include information on the origin of the plant material used in studies like this.

The results obtained in this study are of major importance for the understanding and future prediction of the invasion of *Senecio inaequidens* in Europe. It is now possible to quantify the impact of plant competitors and different kinds of herbivores on this invasive alien plant.

In showing that heavily grazed grassland ecosystems are principally suitable habitats for *Senecio inaequidens*, this study gives a first impression of what habitats might be colonized by *Senecio inaequidens* in the future.

It is highly desirable to conduct further long-term observations on the population dynamics of *Senecio inaequidens* in relationship to different herbivores and plant competitors; in particular, it needs to be tested wether larval stages of *Longitarsus jacobaeae* accept *Senecio inaequidens* as a host plant. A thorough screening especially for capitula-inhabiting, stem-boring and achene feeding insects is also recommended, and the role of fungal pathogens (e.g. rust fungi of the genus *Puccinia*) should be observed in detail and in interaction with herbivory. The role of mycorrhizal symbionts should be determined, and it would certainly be interesting to test for allelopathic effects of *Senecio inaequidens* on neighbouring plants of different species. Large-scale achene sowing experiments under field conditions should be conducted in order to find out about seed limitation, seedling herbivory, and the effects of disturbances on primary establishment of *Senecio inaequidens*.

6 References

Abbott, R.J. (1992) Plant Invasions, Interspecific Hybridization and the Evolution of New Plant Taxa. *Trends in Ecology & Evolution* **7** (12): 401-405.

Adolphi, K. (1997) Anmerkungen zu *Senecio inaequidens* DC. nach einem Aufenthalt in Südafrika. *Floristische Rundbriefe* **31** (2): 162-167.

Agrawal, A.A. (2000) Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. *Trends in Plant Science* **5** (7): 309-313.

Asmus, U. (1988) Das Eindringen von Neophyten in anthropogen geschaffene Standorte und ihre Vergesellschaftung am Beispiel von *Senecio inaequidens* DC. *Flora* **180**: 133-138.

Barbier, E.B. (2001) A note on the economics of biological invasions. *Ecological Economics* **39**: 197-202.

Basilowski, O. (1993) Zur Reproduktionsbiologie von *Senecio inaequidens* DC. [diploma thesis], Universitaet Bochum, Bochum.

Bernstein, S. (2001) *Weltbevölkerungsbericht 2001. Bevölkerung und Umwelt.* Deutsche Stiftung Weltbevölkerung (DSW), Hannover, Germany.

Bicchi, C., D'Amato, A. & Cappelletti, E. (1985) Determination of pyrrolizidine alkaloids in Senecio inaequidens D.C. by capillary gas chromatography. *Journal of Chromatography* **439** (1): 23-29.

Böhmer, H.J., Heger, T. & Trepl, L. (2001) *Fallstudien zu gebietsfremden Arten in Deutschland [Case Studies on Alien Species in Germany].* Umweltbundesamt, Germany, Berlin.

Bonser, S.P. & Reader, R.J. (1995) Plant competition and herbivory in relation to vegetation biomass. *Ecology* **76** (7): 2176-2183.

Brandes, D. (1999) *Senecio inaequidens* am Monte Baldo. *Abhandlungen des Naturwissenschaftlichen Vereins zu Bremen* **44** (2-3): 245-256.

Bromilow, C. (1995) *Problem plants of South Africa.* Briza Publications cc for Bayer AG, Arcadia, South Africa.

Cameron, E. (1935) A study of the natural control of ragwort (*Senecio jacobaea* L.). *Journal of Ecology* **23** (2): 265-322.

Case, C.M. & Crawley, M.J. (2000) Effect of interspecific competition and herbivory on the recruitment of an invasive alien plant: *Conyza sumatrensis*. *Biological Invasions* **2**: 103-110.

Clapham, A.R., Tutin, T.G. & Moore, D.M. (1987) *Flora of the British Isles, 3rd ed.* Cambridge University Press, Cambridge.

Colling, G. & Reichling, L. (1996) Floristic notes 1994-1995. *Bulletin de la Société des Naturalistes Luxembourgeois*: 25-38.

Crawley, M.J. (1983) *Herbivory*. Blackwell Scientific Publications, Oxford.

Crawley, M.J. (1989) The Relative Importance of Vertebrate and Invertebrate Herbivores in Plant Population Dynamics. In: *Insect-Plant Interactions* (Ed. E.A. Bernays), Vol. 1, pp. 45-71. CRC Press, Inc., Boca Raton, Florida.

Crawley, M.J. (1997a) Biodiversity. In: *Plant Ecology* (Ed. M.J. Crawley). Blackwell Science, 2nd ed., Oxford.

Crawley, M.J. (1997b) Plant-Herbivore Dynamics. In: *Plant Ecology, 2nd edition* (Ed. M.J. Crawley). Blackwell Science Ltd., Oxford.

Crawley, M.J. (2002) *Statistical Computing. An Introduction to Data Analysis using S-Plus*. John Wiley & Sons, Ltd., Chichester, West Sussex, UK.

D'Antonio, C.M. & Vitousek, P.M. (1992) Biological invasions by exotic grasses, the grass/fire cycle, and global change. *Annual Review of Ecology and Systematics* **23**: 63-87.

de Candolle, A.P. (1837) *Prodromus systematis naturalis regni vegetabilis*. Treuttel & Würtz, Paris.

De Langhe, J.E., Delvosalle, L., Duvigneaud, J., Lambinon, J. & van den Bergen, C. (1973) *Nouvelle Flore de la Belgique, du Grand-Duché de Luxembourg, du Nord de la France et des Régions voisines*, Bruxelles.

Deinzer, M.L., Thomson, P.A., Burgett, D.M. & Isaacson, D.L. (1977) Pyrrolizidine Alkaloids: Their Occurence in Honey from Tansy Ragwort (*Senecio jacobaea* L.). *Science* **195**: 497-499.

Dukes, J.S. & Mooney, H.A. (1999) Does global change increase the success of biological invaders? *Trends in Ecology & Evolution* **14** (4): 135-139.

Edwards, G.R., Bourdot, G.W. & Crawley, M.J. (2000) Influence of herbivory, competition and soil fertility on the abundance of *Cirsium arvense* in acid grassland. *Journal of Applied Ecology* **37**: 321-334.

Edwards, G.R. & Crawley, M.J. (1999) Herbivores, seed banks and seedling recruitment in mesic grassland. *Journal of Ecology* **87**: 423-435.

Elton, C.S. (1958) *The ecology of invasions by animals and plants*. Methuen & Co. Ltd., reprint 1966, London.

Ernst, W.H.O. (1998) Invasion, dispersal and ecology of the South African neophyte *Senecio inaequidens* in The Netherlands: from wool alien to railway and road alien. *Acta Botanica Neerlandica* **47** (1): 131-151.

Frick, K.A. (1970) *Longitarsus jacobaeae* (Coleoptera: Chrysomelidae), A Flea Beetle for the Biological Control of Tansy Ragwort. 1. Host Plant Specifcity Studies. *Annals of the Entomological Society of America* **63** (1): 284-296.

Gaida, R. & Schneider-Gaida, M. (1999) Die Dynamik der Entwicklung des Neophyten *Senecio inaequidens* DC. (Schmalblättriges Greiskraut) am Sandberg in der Hildener Heide (Hilden und Haan, Rheinland) in den Jahren 1993 bis 1997. *Jahresbericht der naturwissenschaftlichen Vereinigung Wuppertal* **52**: 206-220.

Griese, D. (1998) Die viatische Migration einiger neophytischer Pflanzensippen am Beispiel norddeutscher Autobahnen. In: *Vegetationsökologie von Habitatinseln und linearen Strukturen. Tagungsbericht des Braunschweiger Kolloquiums vom 22.-24. November 1996 / Braunschweiger Geobotanische Arbeiten, Band 5* (ed. D. Brandes), pp. 263-270. Universitätsbibliothek der TU Braunschweig, Braunschweig.

Grime, J.P. (1997) Climate Change and Vegetation. In: *Plant Ecology (2nd edition)* (Ed. M.J. Crawley). Blackwell Scientific Publishing, Oxford.

Hartmann, T. (1999) Chemical ecology of pyrrolizidine alkaloids. *Planta* **207**: 483-495.

Hartmann, T. & Dierich, B. (1998) Chemical diversity and variation of pyrrolizidine alkaloids of the senecionine type: biological need or coincidence? *Planta* **206**: 443-451.

Hartmann, T., Theuring, C., Schmidt, J., Rahier, M. & Pasteels, J.M. (1999) Biochemical strategy of sequestration of pyrrolizidine alkaloids by adults and larvae of chrysomelid leaf beetles. *Journal of Insect Physiology* **45**: 1085-1095.

Henker, H. (1996) Erstnachweise und Einbürgerungen bemerkenswerter Pflanzenarten in Mecklenburg-Vorpommern. *Botanischer Rundbrief Mecklenburg-Vorpommern* **29**: 135-140.

Herrera, J.M.A. (2000) Identificacion y seleccion de agentes potencialmente biocontroladores de especies malezas de clima frio, Resumen ejecutivo, http://www.pronatta.gov.co/info_proyectos2/resumenes%20ejecutivos/961252175-r.doc. Corporacion Colombiana de Investigacion Agropecuaria (CORPOICA).

Herrera, J.M.A. (2002) Estudios para el control de malezas compuestas en pastos dedicados a la ganaderia de leche (con enfasis en *Senecio inaequidens* DC). In: *Programma Nacional de Manejo Integrado de Plagas - MIP.CORPOICA, C.I. Tibaiata [unpublished; author's e-mail address: jarrieta@corpoica.org.co]*, Colombia.

Heywood, V.H. (1989) Patterns, Extents and Modes of Invasions by Terrestrial Plants. In: *Biological Invasions: a Global Perspective (SCOPE report)* (Ed. J.A. Drake, et al.), pp. 31-60. John Wiley & Sons Ltd.

Hilliard, O.M. (1977) *Compositae in Natal.* University of Natal Press, Pietermaritzburg.

Hiscock, S.J. (2000) Genetic control of self-incompatibility in *Senecio squalidus* L. (Asteraceae): a successful colonizing species. *Heredity* **85**: 10-19.

Hutchings, M.J. (1986) The Structure of Plant Populations. In: *Plant Ecology* (Ed. M.J. Crawley). Blackwell Science - 1st edition, Oxford.

James, R.R., Mc Evoy, P.B. & Cox, C.S. (1992) Combining the cinnabar moth (*Tyria jacobaeae*) and the ragwort flea beetle (*Longitarsus jacobaeae*) for control of ragwort (*Senecio jacobaea*): an experimental analysis. *Journal of Applied Ecology* **29**: 589-596.

Jermy, T., Hanson, F.E. & Dethier, V.G. (1968) Induction of specific food preference in Lepidopterous larvae. *Entomologia Experimentalis et Applicata* **11**: 211-230.

Keane, R.M. & Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* **117** (4): 164-170.

Kennedy, T.A., Naeem, S., Howe, K.M., Knops, J.M.H., Tilman, D. & Reich, P. (2002) Biodiversity as a barrier to ecological invasion. *Nature* **417**: 636-638.

Kowarik, I. (1990) Ecological Consequences of the Introduction and Dissemination of New Plant Species: An Analogy with the Release of Genetically Engineered Organisms. In: *European Workshop on Law and Genetic Engineering* (eds. D. Leskien & J. Spangenberg), pp. 67-71. BBU Verlag, Bonn.

Kowarik, I. (1995a) On the role of alien species in urban flora and vegetation. In: *Plant Invasions - General Aspects and Special Problems* (Eds. P. Pysek, K. Prach, M. Rejmanek *et al.*), pp. 85-103. SPB Academic Publishing, Amsterdam, The Netherlands.

Kowarik, I. (1995b) Time lags in biological invasions with regard to the success and failure of alien species. In: *Plant Invasions - General Aspects and Special Problems* (Eds. P. Pysek, K. Prach, M. Rejmanek *et al.*), pp. 15-38. SPB Academic Publishing, Amsterdam, The Netherlands.

Kowarik, I. (1999) Neophytes in Germany: Quantitative Overview, Introduction and Dispersal Pathways, Ecological Consequences, and Open Questions. In: *Alien Organisms in Germany* (Ed. U. Doyle). Federal Environmental Agency (Umweltbundesamt), Berlin.

Kuhbier, H. (1977) *Senecio inaequidens* DC.- ein Neubürger der nordwestdeutschen Flora. *Abhandlungen des Naturwissenschaftlichen Vereins zu Bremen* **38** (21): 383-396.

Lagey, K., Duinslaeger, L. & Vanderkelen, A. (1995) Burns induced by plants. *Burns* **21** (7): 542-543.

Litterski, B. & Berg, C. (2000) Naturräumliche Bindung und Einbürgerung von Neophyten in Mecklenburg-Vorpommern. *Gleditschia* **28** (1-2): 47-63.

Lonsdale, W.M. (1999) Global patterns of plant invasions and the concept of invasibility. *Ecology* **80** (5): 1522-1536.

Lövei, G.L. (1997) Global change through invasion. *Nature* **388**: 627-628.

Lutz, W., Sanderson, W. & Scherbov, S. (2001) The end of world population growth. *Nature* **412**: 543-545.

Marohasy, J. (1989) A survey of Fireweed (*Senecio madagascariensis* Poir.) and its natural enemies in Madagascar with a view to biological control in Australia. *Plant Protection Quarterly* **4** (4): 139-140.

Marohasy, J. (1991). A survey of the insect fauna of the *Senecio madagascariensis* complex (Compositae) in South Africa (unpublished project report). Plant Protection Institute, Pretoria (South Africa).

Marquardt, H. & Schaefer, S.G.e. (1997) *Lehrbuch der Toxikologie*. Spektrum Akademischer Verlag, Heidelberg.

Mazel, R. & Garrigue, J. (2000) La conquête du Séneçon du Cap par quelques Insectes phytophages (Lepidoptera; Diptera; Homoptera; ...). *R.A.R.E.* **9** (3): 72-78.

McEvoy, P.B. & Cox, C. (1991) Successful biological control of ragwort, *Senecio jacobaea*, by introduced insects in Oregon. *Ecological Applications* **1** (4): 430-442.

McEvoy, P.B. & Rudd, N.T. (1993) Effects of vegetation disturbances on insect biological control of Tansy Ragwort (*Senecio jacobaea*). *Ecological Applications* **3** (4): 682-698.

McEvoy, P.B., Rudd, N.T., Cox, C.S. & Huso, M. (1993) Disturbance, competition, and herbivory effects on ragwort *Senecio jacobaea* populations. *Ecological Monographs* **63** (1): 55-75.

McLaren, D.A., Ireson, J.E. & Kwong, R.M. (2000) Biological Control of Ragwort (*Senecio jacobaea* L.) in Australia. In: *Proceedings of the X International Symposium on Biological Control of Weeds, 4-14 July 1999* (ed. N.R. Spencer), pp. 67-79, Montana State University, Bozeman, Montana, USA.

McPhee, C.S. & Aarssen, L.W. (2001) The separation of above- and below-ground competition in plants. A review and critique of methodology. *Plant Ecology* **152**: 119-136.

Mooney, H.A. (1999) Species without frontiers (in book reviews). *Nature* **397**: 665-666.

Naumann, C., Hartmann, T. & Ober, D. (2002) Evolutionary recruitment of a flavin-dependent monooxygenase for the detoxification of host-plant acquired pyrrolizidine alkaloids in the alkaloid-defended arctiid moth Tyria jacobaeae. *PNAS* **99** (9): 6085-6090.

Noble, J.W., Crossley, J., Hill, B.D., Pierce, R.J., McKenzie, R.A., Debritz, M. & Morley, A.A. (1994) Pyrrolizidine alkaloidosis of cattle associated with Senecio lautus. *Australian Veterinary Journal* **71**: 196-200.

Oberdorfer, E. (1994) *Pflanzensoziologische Exkursionsflora, 7th ed.* Ulmer Verlag, Stuttgart (Germany).

Often, A. (1997) Skrotemark i Oslo med nye korgplanter: Senecio inaequidens DC. og Solidago rugosa Mill. *Blyttia* **55**: 141-144.

Pelser, P.B., Gravendeel, B. & van der Meiden, R. (2002) Tackling speciose genera: Species composition and phylogenetic position of *Senecio* sect. *Jacobaea* (Asteraceae) based on plastid and nrDNA sequences. *American Journal of Botany* **89** (6): 929-939.

Perrings, C., Williamson, M. & Dalmazzone, S. Eds.) (2000) *The economics of biological invasions*. Edward Elgar Publishing Ltd.

Peterson, C.H. & Renaud, P.E. (1989) Analysis of feeding preference experiments. *Oecologia* **80**: 82-86.

Pierson, M.L., Cheeke, P.R. & Dickinson, E.O. (1977) Resistance of the rabbit to dietary pyrrolizidine (*Senecio*) alkaloid. *Res Commun Chem Pathol Pharmacol* **16** (3): 561-564.

Pignatti, S. (1982) *Flora d'Italia, Vol. 3*. Edagricole, Bologna.

Polatschek, A. (1984) *Senecio inaequidens* DC. neu für Österreich und Spanien. *Verhandlungen der Zoologisch-Botanischen Gesellschaft Österreich* **12**: 93-95.

Prakash, A.S., Pereira, T.N., Reilly, P.E.B. & Seawright, A.A. (1999) Pyrrolizidine alkaloids in human diet. *Mutation Research* **443**: 53-67.

Radford, I.J. & Cousens, R.D. (2000) Invasiveness and comparative life-history traits of exotic and indigenous *Senecio* species in Australia. *Oecologia* **125**: 531-542.

Radford, I.J., Muller, P., Fiffer, S. & Michael, P.W. (2000) Genetic Relationships between Australian Fireweed and South African and Madagascan Populations of *Senecio madagascariensis* Poir. and Closely Related *Senecio* species. *Australian Systematic Botany* **13**: 409-423.

Radkowitsch, A. (1997) *Senecio inaequidens* DC. - ein Beitrag zur Verbreitung in Deutschland unter besonderer Berücksichtigung von Bayern. *Hoppea, Denkschriften der Regensburger Botanischen Gesellschaft* **58**: 389-404.

Rejmanek, M. (2000) Invasive plants: approaches and predictions. *Austral Ecolgy* **25**: 497-506.

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

Robinson, G.S., Ackery, P.R., Kitching, I.J., Beccaloni, G.W. & Hernandez, L.M. (2002) HOSTS - a database of the hostplants of the world's Lepidoptera - http://www.nhm.ac.uk/entomology/hostplants/, Vol. 2002. Department of Entomology, The Natural History Museum, Cromwell Road, LONDON SW7 5BD.

Rubiolo, P., Pieters, L., Calomme, M., Bicchi, C., Vlietinck, A. & Vanden Berghe, D. (1992) Mutagenicity of pyrrolizidine alkaloids in the *Salmonella typhimurium* / mammalian microsome system. *Mutation Research* **281**: 143-147.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughmann, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C. *et al.* (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics* **32**: 305-332.

Sax, D.F. & Brown, J.H. (2000) The paradox of invasion. *Global Ecology and Biogeography* **9**: 363-371.

Schaefer, M. (2000) *Brohmer, Fauna von Deutschland.* Quelle und Meyer, 20th ed., Wiebelsheim (Germany).

Schafer, A. & Victor, D.G. (1999) Global passenger travel: implications for carbon dioxide emissions. *Energy* **24**: 657-679.

Schmitz, G. & Werner, D.J. (2000) The importance of the alien plant *Senecio inaequidens* DC. (Asteraceae) for phytophagous insects. *Zeitschrift für Ökologie und Naturschutz* **9**: 153-160.

Schubert, R. & Vent, W. Eds.) (1990) *Exkursionsflora von Deutschland, Band 4 - Kritischer Band, 8.Auflage.* Volk und Wissen Verlag, Berlin.

Scott, L.J., Congdon, B.C. & Playford, J. (1998) Molecular evidence that fireweed (Senecio madagascariensis, Asteraceae) is of South African origin. *Plant Systematics and Evolution* **213** (3-4): 251-257.

Silvertown, J. & Charlesworth, D. (2001) *Introduction to Plant Population Biology.* Blackwell Science, 4th edition, Oxford.

Sindel, B.M., Radford, I.J., Holtkamp, R.H. & Michael, P.W. (1998) The Biology of Australian Weeds. 33. *Senecio madagascariensis* Poir. *Plant Protection Quarterly* **13** (1): 2-15.

Smil, V. (1999) How many billions to go? The peaking of the population growth rate deserves wider recognition. *Nature* **401**: 429.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry. The principles and practice of statistics in biological research.* W.H.Freeman and Company, New York.

Stace, C. (1997) *New Flora of the British Isles, 2nd ed.* Cambridge University Press, Cambridge.

Stengl, P. (1982) Zur Isolierung, Strukturaufklärung und Analytik der Pyrrolizidinalkaloide aus *Senecio ovirensis* ssp. *gaudinii* GREMLI, *Senecio inaequidens* DC. und *Symphytum officinale* L. Inaugural-Dissertation, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn.

Stewart, M.J. & Steenkamp, V. (2001) Pyrrolizidine Poisoning: A Neglected Area in Human Toxicology. *Therapeutic Drug Monitoring* **23**: 698-708.

Strauss, S.Y. & Zangerl, A.R. (2002) Plant-insect interactions in terrestrial ecosystems. In: *Plant-Animal Interactions* (Eds. C.M. Herrera & O. Pellmyr). Blackwell Science Ltd, Oxford.

Syrett, P. (1985) Host specificity of the ragwort flea beetle Longitarsus jacobaeae (Waterhouse) (Coleoptera: Chrysomelidae). *New Zealand Journal of Zoology* **12**: 335-340.

Thompson, K. & Grime, J.P. (1983) A comparative study of germination responses to diurnally-fluctuating temperatures. *Journal of Applied Ecology* **20**: 148 (cited in: ECOFLORA database).

Tilman, D. & Lehman, C. (2001) Human-caused environmental change: Impacts on plant diversity and evolution. *PNAS* **98** (10): 5433-5440.

Tinney, G.W., Hatcher, P.E., Ayres, P.G., Paul, N.D. & Whittaker, J.B. (1998) Inter- and intra-species differences in plants as hosts to *Tyria jacobaeae*. *Entomologia Experimentalis et Applicata* **88**: 137-145.

Troussel, R. (1998) Caractérisation de la résistance des communautés végétales a l'invasion par *Senecio inaequidens*. Diplôme d'etudes approfondies (DEA), Université Montpellier II Sciences et Techniques du Languedoc - École Nationale Supérieure Agronomique Montpellier, Montpellier, France.

UN (2001). World Population Monitoring 2001. Population, environment and development. United Nations, Department of Economic and Social Affairs, Population Division, New York.

Vicens, J. (1996) GEA, Flora et Fauna - Notes breus (Flora): *Cistus* x *ledon* Lam., *Aster lynosiris* (L.)Bernh. and *Senecio inaequidens* DC., three interesting plants from Catalan Flora. *Butletti de la Institutio Catalana d'Historia Natural, Barcelona* **64**: 73-80.

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

Vitousek, P.M., Mooney, H.A., Lubchenco, J. & Melillo, J.M. (1997) Human Domination of Earth's Ecosystems. *Science* **277**: 494-499.

Watkinson, A.R. (1997) Plant Population Dynamics. In: *Plant Ecology* (Ed. M.J. Crawley). Blackwell Science Ltd., Oxford.

Way, M.L. & Ahmad, S. (1983) Host Location in the Colorado Potato Beetle: Searching Mechanisms in Relation to Oligophagy. In: *Herbivorous Insects. Host-Seeking Behavior and Mechanisms* (Ed. S. Ahmad). Academic Press, Inc., New York.

Werner, D.J. (2000) Neue Aspekte und Daten zu Herkunft, Ausbreitung, Ökologie und Vergesellschaftung von *Senecio inaequidens* DC. *Flora Colonia* **8**: 21-48.

Werner, D.J., Rockenbach, T. & Hoelscher, M.-L. (1991) Herkunft, Ausbreitung, Vergesellschaftung und Ökologie von *Senecio inaequidens* DC. unter besonderer Berücksichtigung des Köln-Aachener Raumes. *Tuexenia* **11**: 73-107.

Whittaker, J.B. (1978) Invertebrate grazing, competition and plant dynamics. In: *Population Dynamics. The 20th Symposium of the British Ecological Society* (eds. R.M. Anderson, B.D. Turner & L.R. Taylor). Blackwell Science, London.

Wieners, A. (1994) Transpiration unter verschiedenen Standortbedingungen des Neophyten *Senecio inaequidens* und die Trockenresistenz dieser Ruderalpflanze im Vergleich mit der von *Senecio jacobaea* (Diploma Thesis), Heinrich-Heine-Universität, Düsseldorf.

Windig, J.J. (1993) Intensity of *Longitarsus jacobaeae* herbivory and mortality of *Senecio jacobaea*. *Journal of Applied Ecology* **30**: 179-186.

Wink, M. & Legal, L. (2001) Evidence for two genetically and chemically defined host races of *Tyria jacobaeae* (Arctiidae, Lepidoptera). *Chemoecology* **11**: 199-207.

Wisskirchen, R. & Haeupler, H. (1998) *Standardliste der Farn- und Blütenpflanzen Deutschlands*. Ulmer Verlag, Stuttgart.

Zangerl, A.R., Hamilton, J.G., Miller, T.J., Crofts, A.R., Oxborough, K., Berenbaum, M.R. & de Lucia, E.H. (2002) Impact of folivory on photosynthesis is greater than the sum of its holes. *PNAS* **99** (2): 1088-1091.

Colour Plates

Plate 1 a) *Senecio inaequidens*, pollinated by a hoverfly (Diptera: Syrphidae); October 2001, Rostock, Germany; **b)** *Longitarsus jacobaeae* feeding on *Senecio inaequidens;* August 2002, field plot at Silwood Park .

Plate 2 a) *Tyria jacobaeae*, 4th and 5th instar larvae collected for a feeding trial; **b)** *Tyria jacobaeae* adult female laying egg batches on *Senecio jacobaea* (June 2002, Silwood Park)

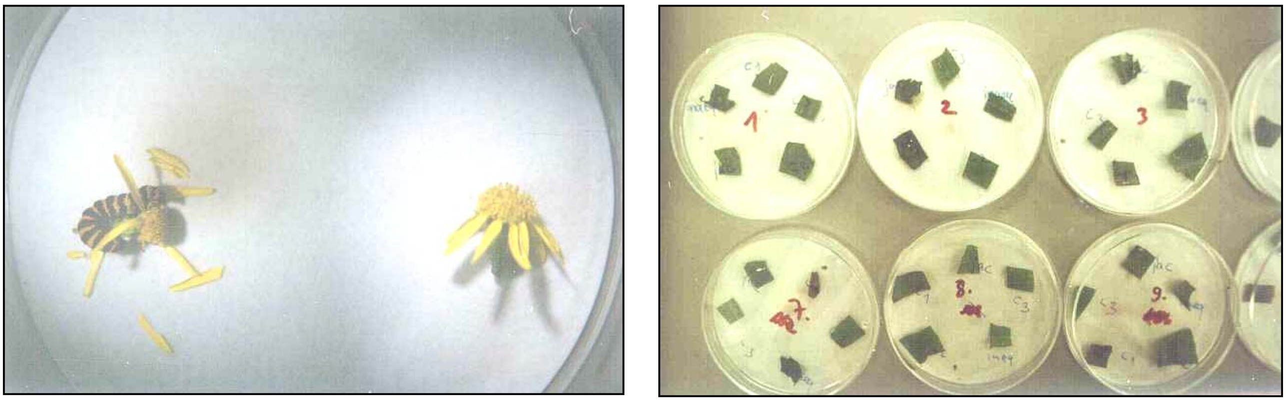

Plate 3 a) Two-way choice feeding trial with *Tyria jacobaeae*; clearly, the larva prefers feeding on the capitulum of *Senecio jacobaea* (left); **b)** *Longitarsus jacobaeae* preliminary feeding trial with rectangular leaf pieces.

Plate 4 a) Field plot 1 in June 2002; the rabbit fence and the cultivation strips are clearly recognisable; **b)** *Tyria* enclosure at field plot 2, note the difference between the cultivated and the uncultivated part.

Plate 5 a) arrangement of container pots in the greenhouses; **b)** pot with monoculture of *Senecio inaequidens* (four plants; left) and *Senecio inaequidens* growing in competition with *Festuca rubra* in the greenhouses.

Plate 6 a) Difference between monoculture pots of *Senecio inaequidens* (tall plants) and plants growing under competition with *Festuca rubra* (pot in the middle); note the acetate cages constructed to serve as enclosures for *Tyria*; **b)** the "JIF" greenhouses where the experiments were conducted; chambers 10/9 and 10/8 are located at the right front corner and behind it, respectively.

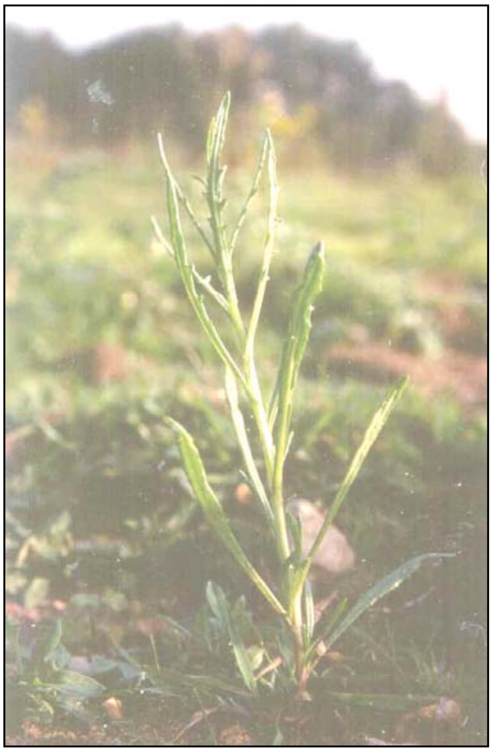

Plate 7 a) *Tyria jacobaeae* feeding on *Senecio inaequidens* in a no-choice trial in the greenhouse; note that preferably rayflorets are eaten; **b)** transplanted *Senecio inaequidens* seedling on a cultivated "no competition" plot in the field (July 2002)

Plate 8 a) *Senecio inaequidens* clipped by rabbits (field experiment, July 2002); note that the first regrowth shoots are still eaten; **b)** plant that has recovered from clipping (regrowth shoots) and has started to produce flowerheads (September 2002).

Plate 9 a) the characteristic "shotholes" caused by *Longitarsus* herbivory; **b)** heavy mollusc damage to a *Senecio inaequidens* leaf. Both photographs show young leaves from a freshly transplanted *Senecio inaequidens* seedlings in the field, (July 2002).

Plate 10 Senecio inaequidens growing under high competition, two weeks after transplantation; **a)** in the field; **b)** in the greenhouse.