

# Plant pathogens as biocontrol agents of *Cirsium arvense* – an overestimated approach?

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## Abstract

*Cirsium arvense* is one of the worst weeds in agriculture. As herbicides are not very effective and not accepted by organic farming and special habitats, possible biocontrol agents have been investigated since many decades. In particular plant pathogens of *C. arvense* have received considerable interest and have been promoted as “mycoherbicides” or “bioherbicides”. A total of 10 fungi and one bacterium have been proposed and tested as biocontrol agents against *C. arvense*. A variety of experiments analysed the noxious influence of spores or other parts of living fungi or bacteria on plants while others used fungal or bacterial products, usually toxins. Also combinations of spores with herbicides and combinations of several pathogens were tested. All approaches turned out to be inappropriate with regard to target plant specificity, effectiveness and application possibilities. As yet, none of the tested species or substances has achieved marketability, despite two patents on the use of *Septoria cirsii* and *Phomopsis cirsii*. We conclude that the potential of pathogens for biocontrol of *C. arvense* has largely been overestimated.

## Keywords

*Cirsium arvense*, bioherbicide, biological control, fungi, bacteria

## Introduction

*Cirsium arvense* (L.) Scop. (Canada thistle) is a perennial root-budding geophyte capable of sprouting from creeping roots that make it a vigorous pioneer in open, disturbed habitats especially on nutrient-rich deep soils (Tiley 2010). Likely to be native of Europe, Western Asia and North Africa (Kazinczi et al. 2001), it has spread worldwide

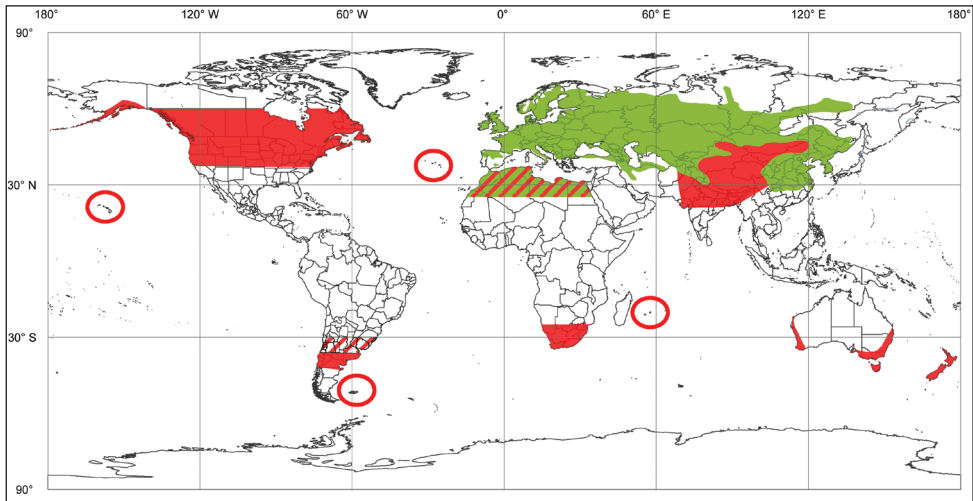
(Figure 1), to become one of the most noxious weeds on agricultural land (Skinner et al. 2000). The most severe problems are caused in cereal fields and pastures, especially in Europe (Guillerm and Maillet 1982, Franzini 1982, Dietl 1982, Niemeth 2001, Purgar and Hulona 2008, Macak et al. 2008, Privalov et al. 2008), North America (Alex 1966) and New Zealand (Rahman 1982). Canada thistle was introduced to North America probably in the 17<sup>th</sup> century from Eurasia (Moore 1975, Tiley 2010). There it has become an invasive weed that aggressively suppresses crops on cultivated land and native plants on fallow land (Moore 1975, Stachion and Zimdahl 1980).

*C. arvensis* reproduces sexually with seeds and vegetatively with an expanding system of root buds. While seeds aid long distance dispersal, the clonal propagation via the root system is considered to be most important for the effective colonization of a given location. New shoots develop out of root buds and build up dense patches of thistle shoots over the whole growth period. The formation of 106 shoots per square metre supported by a root system measuring 399 m in total length was observed by Stach (1996). With respect to the effect of Canada thistle on agriculture it is noteworthy that new shoots can develop out of very short root parts if the latter bear at least one root bud. At the end of the growing season only the above ground green parts of the plants die, while the root system overwinters.

The density of shoots and the long root system suppress the growth of most other plants. In the case of arable crops this causes a suppression of the cultivated plants. Yield losses of up to 60% have been reported depending on the kind of crop and on the weed density. In cereal crops for example, densities of 6 to 20 Canada thistle shoots per square metre cause up to 30% loss in grain yield. The overall global annual losses have been estimated at 320 million US\$ (Bailey et al. 2000).

In conventional farming, herbicides are commonly applied to control Canada thistle. However, herbicides can damage non-target plant species (Matarczyk et al. 2002, Rodwell and Sheffield 2005), other trophic levels (Bunemann et al. 2006) and adjacent ecosystems (Hayes et al. 2002, Relyea 2005, Perez et al. 2007). Additionally, in the case of *C. arvensis* herbicides mostly affect the aboveground plant parts and not the root system. Therefore, they need to be applied several times a year and every year anew, making this procedure ineffective and expensive. In New Zealand, for example, the annual costs for herbicides, mowing and vaccination of grazing animals wounded by the thistle's spines (Gourlay 2004) amount to NZ\$ 27 million just for the pastoral industry in two regions of New Zealand.

In organic farming, where herbicides are not accepted, several other methods for thistle growth control are used. Hoeing and mowing, for example, are used as mechanical control methods (Hurrell and Bourdot 1996, Bacher et al. 1997, Kluth et al. 2003, Graglia et al. 2006, Lukashyk et al. 2008). Both of them do not harm the thistle substantially as they do not destroy the root system. On the contrary, hoeing can even support the clonal spread of thistles, because they are able to form new shoots out of very short root cuttings. Mowing may even have a positive effect on the performance of the thistle as it can reduce the competitiveness of associated plant species (Edwards et al. 2000).



**Figure 1.** Distribution area of *Cirsium arvense*. The green area represents the native area, red indicates the invaded area. Circles indicate major invaded island groups. For northern Africa it seems not to be clear if this is part of the native area or already invaded. In South America the hatched area indicates that the invaded area could be larger but references seem to be scarce. According to Meusel and Jäger (1992), Weber (2003), Tiley (2010), ISSG (2011).

Another possibility to curtail weeds is biological control with the help of biocontrol agents, usually insects, fungi, bacteria or viruses (McFadyen 1998). In the case of Canada thistle, useful control agents have been sought especially among competing plant species, herbivorous insects and fungus species. Experiments were performed with different competing clover species (Lukashyk et al. 2008) and grass/clover mixtures (Graglia et al. 2006) which, together with mowing, resulted in a reduction of *C. arvense* shoot density of up to 90%. Additionally, a strongly decreased above ground biomass was achieved, presumably by suppressing the regrowth of thistle shoots after mowing. Ang et al. (1994) showed for arable crops that increased interspecific competition from non-crop plants can reduce the abundance of *C. arvense*. Though Edwards et al. (2000) found similar results in a permanent grassland community, this technique has been classified as too intense and costly to be accepted among organic growers (Graglia et al. 2006). This technique is also not applicable in ruderal sites or habitats of conservational value.

Additionally, numerous studies about herbivores as potential biocontrol agents of *C. arvense* were performed. In a recent review, Cripps et al. (2011) reviewed five insect species that have been released in North America and New Zealand, however without any indications of successful control. Neither the coleopterans *Altica carduorum* Guérin-Ménéville, *Lema cyanella* (L.) (Chrysomelidae), *Hadroplontus litura* (F.) (= *Ceutorhynchus litura*), and *Rhinocyllus conicus* (Frölich) (Curculionidae), nor the dipteran *Urophora cardui* (L.) (Tephritidae) could be established at all locations, where they were released. Additionally, none of the species had a significant influence on the Canada

thistle populations (Cripps et al. 2011, Julien and Griffith 1999). Some other herbivorous beetles, like the chrysomelid *Cassida rubiginosa* Müller (e.g. Ang et al. 1995, Bacher and Schwab 2000, Clough et al. 2007) or the curculionid *Larinus planus* (F.) were accidentally introduced to North America. They became established at several locations, but also had little or no impact on Canada thistle (Julien and Griffiths 1999). The curculionid *Cleonus piger* Scop. (Watson and Keogh 1980) showed a considerable impact on *C. arvensis* but has never been released as a biocontrol agent. A reason for this is certainly that the host range of *Cleonus piger* includes the artichoke and it could therefore not be considered as a suitable biocontrol agent (Cripps et al. 2011).

In their review Cripps et al. (2011) concluded that none of the herbivorous arthropod species had a significant influence on Canada thistles. Since plant pathogens are often cited as second major group of biocontrol agents (e.g., Charudattan and Dinoor 2000), we analysed the existing studies on plant pathogens, mainly fungi and bacteria, as biocontrol agents to close this review gap. There are plenty of studies on this topic adopting a range of taxonomically diverse organisms and approaches. With the present paper we aim at summarizing the results of these works and at presenting a comprehensive review on the application of fungi and bacteria for biocontrol of Canada thistle, *C. arvensis*.

Biological control is usually defined as the usage of living organisms to control other organisms. The current praxis, however, ranges from whole organism applications to the use of reproductive stages such as spores, parts of organisms and purified compounds. Such secondary metabolites may be included or excluded when defining biological control, see Ash (2010). The wide usage of the term “mycoherbicide” also plays with the obvious similarity between organisms, isolated compounds and synthetic herbicides, when applied as an aerial spray. Therefore, we decided to include also fungal and bacterial products into this review, especially since six out of eleven biocontrol agents as listed below served as compound source and since they were specifically targeted against *C. arvensis*.

## Fungi as biocontrol agents

A total of 10 fungal species have been tested as biocontrol agents of *C. arvensis* (Table 1). Some experiments tested the performance of the living fungi while others used fungal products, such as toxins.

### *Puccinia punctiformis*

Most work was done on the biotrophic rust fungus *Puccinia punctiformis* (syn. *P. obtegens* (Link) Tul. and C. Tul. and *P. suaveolens* (Pers.) Rostr.), which is considered to have the highest potential as a mycoherbicide (French and Lightfield 1990). The big advantage of *P. punctiformis* for a use as a biocontrol agent is its species specificity to

**Table 1.** Pathogens of *Cirsium arvense*, proposed for biocontrol. For further details, compare text. Effectivity is subdivided into high (ability to kill the plant) and limited (not able to kill the plant). Specificity is subdivided into very high (specific to one species), high (specific to a few species of one family), low (many species of one family), very low (many species of different families).

Systematics	Pathogen	Affected plant part	Effectivity	Specificity	Main references
Basidiomycota	<i>Puccinia punctiformis</i>	leaves, shoots	limited, local	very high	Frantzen (1994), French et al. (1988), French and Lightfield (1990), Kluth et al. (2003)
Ascomycota	<i>Phomopsis cirsii</i>	dead stems and leaves, roots	high	high	Leth and Andreasen (1999), Leth and Andreasen (2000), Leth et al. (2008)
	<i>Sclerotinia sclerotiorum</i>	dead and decaying stems and leaves	limited, local	very low	Brosten and Sands (1986), Bourdot et al. (1993, 1995)
	<i>Alternaria cirsinoxia</i>	leaves	limited	low	Berestetskii et al. (2010), Green and Bailey (2000 a, b), Green et al. (2001a)
	<i>Phoma destructiva</i>	dead and living plant material	high	unclear	Guske et al. (1996), Guske (2002), Kruess (2002)
	<i>Phoma exigua</i>	leaves	inconsistent	very low	Bithell and Steward (2001), Waipara (2003), Bilder and Berestetsky (2006), Scott et al. (1975)
	<i>Stagonospora cirsii</i>	leaves	high, with restrictions	low	Gasich and Berestetskiy (2006), Mitina et al. (2005), Yuzikhin et al. (2007)
	<i>Septoria cirsii</i>	leaves	high	very high	Leth (1985, 1990)
	<i>Phyllosticta cirsii</i>	unknown, only extracted phytotoxins tested	unknown	unknown	Berestetskiy et al. (2005), Evidente et al. (2007, 2008a)
	<i>Fusarium spec.</i>	seeds, seedlings, leaves, roots	inconsistent	low	Bailey BA et al. (1997 b, 2000), Bailey KL et al. (2000), Gronwald et al. (2004)
Bacteria	<i>Pseudomonas syringae</i> pv. <i>tagetis</i>	leaves, shoots	high	low	Bailey KL et al. (2000), Johnson and Wyse (1991), Johnson et al. (1996), Lukens and Durbin (1985), Rhodehamel and Durbin (1985), Tichich and Doll (2006)

*C. arvense*. However, single reports of *P. punctiformis* on other *Cirsium* species and Asteraceae genera (Tykhonenko and Minter 2002, Berner et al. 2002) require further investigation. Research on *P. punctiformis* in biocontrol started almost 100 years ago when Olive (1913) studied how *C. arvense* became infected by the rust fungus and pro-

duced systemically infected shoots. The importance of these observations for a possible control of *C. arvense* was recognized by Cockayne (1915) and Ferdinandsen (1923). Later studies were carried out attempting to stimulate spore germination (French et al. 1988, French 1990, French and Lightfield 1990, Frantzen 1994, French et al. 1994), to artificially spread spores in order to obtain higher infection rates (Thomas et al. 1994, Guske et al. 2003, Kluth et al. 2003, Demers et al. 2006, Wandeler and Bacher 2006, Müller et al. 2011) and studying interactions between *P. punctiformis* and insects (Friedli and Bacher 2001a, Kluth et al. 2001, Kluth et al. 2002, Cripps et al 2009).

*Puccinia punctiformis* causes two different kinds of infections, local and systemic infections. While local infections cause only small lesions on thistle leaves and influence the plant's performance only marginally (Kluth et al. 2005), systemic infections usually kill the infected shoots within a few months, mostly before flowering (French and Lightfield 1990). Most studies were unable to reach higher rates of systemic infection than 20 to 50% by artificial inoculation (e.g., Van den Ende et al. 1987, French et al. 1988, Frantzen 1994, Wandeler and Bacher 2006, Müller et al. 2011). This is considered inadequate for a successful suppression of *C. arvense* (Van den Ende et al. 1987, Van Leest and Scheepens 1994).

Wandeler and Bacher (2006) observed that the weevil *Ceratapion* (= *Apion*) *onopordi* Kirby (Coleoptera: Curculionidae) acts as a vector of *P. punctiformis* and that *C. arvense* becomes systemically infected after spore transmission. Only females were found to cause systemic infection (Friedli and Bacher 2001 a, b) suggesting that egg-laying, not feeding on the host plant is likely to be the underlying mechanism. Unfortunately, spore transmission by female *C. onopordi* did not result in an adequate infection and control level, either. The highest infection rate reached in this semi-field study was about 42%, whereas a rate of more than 80% or 90% would be necessary for effective control. Moreover, Cripps et al. (2009) found that rust infection rates were similar in areas with or without the weevil, indicating that its presence does not enhance systemic rust infection.

One can conclude that *P. punctiformis* as a potential biocontrol agent against *C. arvense* presently has the serious handicap that there are no suitable methods to cultivate this biotrophic rust fungus, to produce sufficient amounts of infectious spores, and to apply spores in an effective and economic manner to obtain the necessary infection rate. The most difficult step in this chain of argumentation is obviously the lack of understanding of the process by which a systemic infection is initiated.

### ***Sclerotinia sclerotiorum***

*Sclerotinia sclerotiorum* (Lib.) de By. is able to attack shoots and roots and can kill Canada thistles (Brosten and Sands 1986). Under natural conditions this fungus leads to localised patches of dead thistle shoots, ranging from one to several dead shoots. The extent of the destruction is possibly limited by *Sclerotinia*'s slow rate of expansion (Brosten and Sands 1986). All studies based on artificial infection showed mortality of vegetative shoots and a reduction in the root biomass (Bourdote et al. 1993, 1995, Bourdote and Harvey 1996).



Higher infection rates were achieved with plants that were experimentally wounded before the treatment (Bourdôt et al. 2004). The potential of *S. sclerotiorum* as control agent seems to be limited, as thistle shoots need to be re-infected in the next growing season because the fungus seems to be unable to hibernate in the root system of the thistle (Bourdôt et al. 2006). A further limitation is the high variability of the impact of the fungus on the host population, leading to a reduction ranging between 20 and 95%. This depends on site, fungal strains and resistance of the *C. arvense* clones. Additionally, *S. sclerotiorum* needs a minimum of free water like rain or dew for a successful infection. This also limits the use as a biocontrol agent to some climate regions where free water is available (Brosten and Sands 1986). The major objection against the use of *S. sclerotiorum*, however, is its lacking host specificity and occurrence on several hundreds of known host plants. Whereas its virulence on *C. vulgare*, *Carduus nutans* and many more wildflower species (Bourdôt and Harvey 1996) may not pose a problem, the virulence on canola and many vegetable species (Pennycook 1989) limits its use as a biocontrol agent. As *S. sclerotiorum* is not virulent on grasses and *Trifolium* ssp. Hurrell and Bourdôt (1993) proposed using this pathogen on pastures. Since *S. sclerotiorum* can survive for a long time in the ground (Bourdôt et al. 2000) and as its spores are spread easily, its use on pastures may cause hazards after changes of land use and for adjacent areas, even if a safety zone is allowed (De Jong et al. 2002).

### ***Alternaria cirsinoxia***

Another fungus widely discussed as a biocontrol agent is *Alternaria cirsinoxia* E.G. Simmons and K. Mort., firstly isolated from *C. arvense* in Canada in 1993 (Simmons and Mortensen 1997). Though it causes severe foliar necrosis (Green and Bailey 2000 a, b, Green et al. 2001a) its usefulness as a biocontrol agent is limited by a number of shortcomings. First, the fungus is not species-specific. Green et al. (2001a) tested several plant species from different families. With the exception of leafy spurge (*Euphorbia esula*, Euphorbiaceae) only Asteraceae were infected, but among these crops like sunflower (*Helianthus annuus*) and safflower (*Carthamus tinctorius*) could be found. Secondly, climatic conditions must be appropriate for the formation of appressoria and penetration of the leaf epidermis by the pathogen (Green et al. 2001a). Climatic conditions are also a limiting factor for the performance of the mycelium. The mycelium survives at temperatures around 0°C and can also overwinter; temperatures above 40°C kill it, thus it could only be used in temperate climates. The growth optimum is reached at 20 to 25 °C (Green and Bailey 2000 b, Green et al. 2001b). Also humidity conditions are limiting for a survival of the fungus, as high air humidity or even free water is necessary for the germination of the conidia (Green and Bailey 2000b). *Alternaria cirsinoxia* is primarily pathogenic on older, senescing leaves of *C. arvense* and infected plants can recover by developing new, healthy leaves (Green and Bailey 2000a, Gannibal and Berestetskii 2008) which additionally limits the fungus' potential as a bioherbicide (Green and Bailey 2000a). Berestetskii et al. (2010) identified zinniol as one of the

phytotoxic substances in *A. cirsinoxia*. However, the use of zinniol as natural herbicide is apparently limited by its non-specific phytotoxic activity and its cytotoxicity.

A combined treatment of *A. cirsinoxia* and the herbicide glyphosate on *C. arvense* was also tested. In a controlled environment, the combination of herbicide and the fungus caused more severe damage to Canada thistle than glyphosate alone, but did not reach a sufficient level of control. Moreover, the effects of *A. cirsinoxia* and glyphosate were not consistent in repeated field trials (Green and Bailey 2001). In conclusion, *A. cirsinoxia* is not suitable for the biological control of Canada thistle due to its low host specificity, unspecific toxicity and limited infection power.

### ***Phomopsis cirsii***

*Phomopsis cirsii* Grove, a necrotrophic fungus, was found on dead stems and leaves of *C. arvense* and *C. eriophorum* in Great Britain (Grove 1935) and later on those of *C. palustre* in Norway (Jørstad 1965) and Denmark (Leth 1985). In 2008, Leth et al. also found the fungus on seeds of *C. arvense*. Early season symptoms are black leaf veins and small limited necrotic lesions on stems, dying back of young shoots and wilting of shoots. Late season symptoms are black necrotised peduncles and bracts, black veins and black or brown necrotic lesions on the mature stems, often containing yellow patches with sporulating pycnidia (Leth et al. 2008). It can overwinter in dead stems and forms conidia that are spread by rain splash or invertebrates. The fungus can be cultivated on artificial substrates, and several experiments showed that it is possible to infect shoots of *C. arvense* by spreading the fungal mycelium (Leth and Andreasen 1999, Leth and Andreasen 2000, Leth et al. 2008). Precondition is that conidia and mycelial fragments are in contact with free water at least for 18 h to cause infection. This time period can be shortened to 6 h by the addition of alginate (Leth and Andreasen 2000). Spraying the mycelium on two-year old thistle shoots resulted in a 50% reduction of fresh weight of the shoots (Leth and Andreasen 1999). In other experiments, isolates killed 100% of the inoculated plants (Leth et al. 2008), indicating a different virulence of different fungal strains. Leth et al. (2008) suggested that it may be possible to increase the pathogen's virulence against a broad range of genotypes of *C. arvense* by optimising the cultivation practices. It remains to be investigated whether this fungus is really restricted to *Cirsium* species and whether it is able to kill whole thistle clones. If this turns out to be the case, the pathogen could become a promising candidate for the biocontrol of Canada thistle. Some applications of *Ph. cirsii* were covered by a patent (Leth 1985), for more details see below.

### ***Phoma* species**

*Phoma destructiva* Plowr. was first mentioned in 1915 by Jamieson as the cause of a fruit rot in tomatoes. Later it was also mentioned to cause leaf blight in tomato (Eben and Critchle 1972) but the host spectrum is uncertain as Guske et al. (1996) and



Guske (2002) claim specificity of the fungus for *C. arvense*. This contradiction may be accounted for by the presence of different varieties or special forms within *Ph. destructiva* (Aulakh et al. 1969). Guske et al. (1996) were the first to mention this fungus as a biocontrol option against *C. arvense*. Germinating conidia cause systemic infections which influence the C/N ratio negatively and therefore reduce the plant growth (Huber 1998), leading to chlorosis of the above-ground plant parts, a reduction in the number of flower heads and seeds and a reduced biomass (Kruess 2002). It is possible to inoculate thistle shoots (Kruess 2002) with this perthotrophic (Guske 2002) fungus. Perthotrophic means that the fungus lives on dead plant material, killed before by the fungus itself. This reduced plant quality was mentioned as a contraindication against a combination of the fungal pathogen with the herbivorous beetle *Cassida rubiginosa*. Infected plants were less attractive as hosts and larval performance and survival of the beetle were reduced, so that synergistic effects were excluded (Kruess 2002) or perhaps masked through decreased attractiveness of thistles to this beetle.

Better results were reached by a combination of *Ph. destructiva* with other plant pathogens. The application of a mixture of four pathogens, *Ph. destructiva*, *Ph. hedericola* (Durieu and Mont.) Boerema, *Ph. nebulosa* (Pers.) Mont. and a *Mycelium sterillum* significantly reduced the reproduction of the plants and also affected their roots, shown by a loss of dry root weight of 32% (Guske et al. 2004). A combination of *Ph. destructiva* with *P. punctiformis* reduced the shoot density (Kluth et al. 2005) but not all tested combinations of pathogens enhanced the control effect. A combination of *Ph. hedericola* and *P. punctiformis* was less effective than *Ph. hedericola* alone. The single application of *Ph. hedericola* or *Ph. nebulosa* was less harmful to thistles than the combination of both. Application of *Ph. nebulosa* alone caused death of all main shoots. This fungus is nevertheless inappropriate as a biocontrol agent, as more secondary shoots arose after the primary ones died (Guske et al. 2004).

Another *Phoma* species found on *C. arvense* is *Ph. exigua* Desm. The weak leaf spot pathogen (Waipara et al. 1997), preliminarily identified as *Ascochyta sonchi* (Mel'nik 2000) and later reclassified to *Ph. exigua* (van der Aa et al. 2000, Boerema et al. 2004), parasitizes more than 300 plant species and is discussed as a biocontrol agent against *Taraxacum officinale* (Stewart-Wade and Boand 2004) and *Gaultheria shallon* (Zhao and Shamoun 2006). The Canada thistle was originally not identified as a host of *Ph. exigua* (van der Aa 2000, Boerema et al. 2004) but could later be confirmed as such (Bithell and Stewart 2001, Waipara 2003, Bilder and Berestetsky 2006). Inoculation experiments showed that an artificial infection with the fungus is possible, but with inconsistent results between different isolates. The disease development was much faster on detached than on attached leaves, but the short-term experiment described by Bithell and Stewart (2001) does not allow further conclusions on the progress of this infection. Scott et al. (1975) identified several phytotoxins in *Ph. exigua* which they recommended for biocontrol. However, among these phytotoxins unspecific phyto- and cytotoxic cytochalasins are common and cytochalasin A and B even cause potato gangrene (Scott et al. 1975). Moreover, the main toxin ascosonchine is not virulent (Evidente et al. 2006), so that *Ph. exigua* cannot be recommended for biocontrol (Cimmino et al. 2008).

### ***Stagonospora cirsii***

*Stagonospora cirsii* Davis is a causal agent of brown foliar lesions on *C. arvense*. If sprayed on seedlings during a dew period, it can kill nearly 100 % of the treated plants. The fungus can also be dusted as mycelium powder onto the soil surface which led to the death of 60% of treated seedlings in one study. Older plants are also affected but not killed. The fungus is able to survive over long periods, at least in sterile soil and remains viable on organic substrate after a cold winter period, but an infection of the thistle roots seemed to be impossible (Gasich and Berestetskiy 2006), which restricts its potential as a mycoherbicide.

*S. cirsii* also produces phytotoxins, demonstrated by the phytotoxic activity of culture filtrates to leaves and roots of *C. arvense* (Mitina et al. 2005). Yuzikhin et al. (2007) isolated a new phytotoxin, a nonenolid named stagonolide, from the fungus. The phytotoxin was shown to be unspecific in general but more selective against Asteraceae including sunflower (*Helianthus annuus*). Other crops, such as pepper (*Capsicum annuum*), tomato (*Lycopersicon esculentum*), wheat (*Triticum aestivum*), pea (*Pisum sativum*) and radish (*Raphanus sativus*) were also affected and displayed leaf necrosis. Stagonolide was most harmful to leaves and acts as a strong inhibitor of root growth in seedlings of *C. arvense* (30% decreased root length) and other Asteraceae. Other isolated nonenolides, stagonolide B-F, showed no toxicity against *C. arvense* (Evidente et al. 2008 b). Later, another four nonenolides were isolated by Evidente and coworkers (Evidente et al. 2008 c). Three were new compounds, named stagonolides G, H, and I, the fourth was identified as modiolide A, known from the fungus *Paraphaeosphaeria* sp., living on the horse mussel *Modiolus auriculatus* (Tsuda et al. 2003). Stagonolide G showed no toxic activity, whereas stagonolide H was most toxic to *C. arvense* leaves, causing necrotic lesions. Also other plant species tested showed necrotic lesions after inoculation with stagonolide H, but were less sensitive. The authors concluded that this phytotoxin is highly phytotoxic and selective and recommend it as a potential natural herbicide. However, as the fungus is highly infectious on seedlings of various plants and its extracted toxins are not specific and also not that selective as mentioned by the authors, we question the potential of *S. cirsii* as a biocontrol agent of *C. arvense*.

### ***Septoria cirsii***

*Septoria cirsii* Niessl causes leaf spot on Canada thistle. Because of its host specificity and effective control of Canada thistle in the field, it had been proposed as a biocontrol agent (Leth 1985). Cultures of *S. cirsii* produce copious amounts of a phytotoxin which was identified as beta-nitropropionic acid. The toxin inhibits seed germination, root elongation and causes chlorosis and necrosis of the leaves of Canada thistle (Hershenthorn et al. 1993). *S. cirsii* is considered to be specific to the genus *Cirsium*, though infections were also found on artichoke (*Cynara scolymus*), another Asteraceae. According to susceptibility tests, no signs of infection were found in plants outside the tribe

Cardueae of Asteraceae (Leth 1985). Active components of the fungus were suggested as a mycoherbicide and their application seemed to be rather promising.

The application of *Septoria cirsii* and *Phomopsis cirsii* as mycoherbicide had been covered by the patent of Leth (1985, 1990). This patent looked interesting but so far never reached the market. At that time, Leth worked for Novo Industri A/S, Denmark. In the 1990's, Novo Industri sold its plant protection division to Abbott including most of the patent rights, but not the *Phomopsis* patent. However, around 1999 Novo Industri abandoned the case due to lack of interest and eventually, all patents on bioherbicides were abandoned. If no other party showed interest in the meantime, the patents would have expired in 2004-2005 (personal communication Bo Hammer Jensen).

### ***Phyllosticta cirsii***

The fungus *Phyllosticta cirsii* Desm. has been evaluated as another possible biocontrol agent of Canada thistle (Berestetskiy et al. 2005). Since the genus *Phyllosticta* is known to produce bioactive metabolites, studies concentrated on the isolation of different phytotoxins. Evidente et al. (2007) identified the four phyllostictines A to D, and later isolated phyllostoxin and phyllostin as further compounds (Evidente et al. 2008 a), with phyllostoxin being highly phytotoxic and phyllostin not being toxic. Phyllostoxin was proposed as a potential natural herbicide but its toxicity against other plant species was not tested and thus its specificity is unknown. Evidente et al. (2008 a) also investigated potential side-effects of this substance and concluded that antimicrobial or zootoxic activities were lacking. However, these results base on only limited tests with three bacteria species, one fungus species and one crustacean species and cannot be generalised. Until further data become available phyllostoxin or *P. cirsii* itself cannot be regarded as suitable biocontrol agents of Canada thistle.

### ***Fusarium* species**

The genus *Fusarium* includes many species that are pathogenic to *C. arvense*, for example *F. equiseti* (Corda) Sacc. (Gasich and Berestetskiy 2007). Species that occur on seeds can cause the death of the seedlings, e.g. *F. solani* (Mart.) Sacc. and *F. oxysporum* E.F. Sm. and Swingle (Fischl et al. 2004). Isolates of different *Fusarium* species reduced the emergence of new shoots by 45-70% and shortened root growth by 25-52% when applied as a suspension on the surface of root cuts (Bailey et al. 2000). Nep 1, an extracellular protein produced by *F. oxysporum* f. sp. *erythroxyli* (Bailey 1995, Bailey et al. 1997 a), can cause necrosis of leaves of dicotyledonous plants after foliar application (Bailey et al. 1997b, 2000a, 2000b, Jennings et al. 2000). Gronwald et al. (2004) showed rapid desiccation and necrosis of leaves. The greatest effect was observed in recent, fully expanded leaves, with 60 to 80% of the leaves being necrotic after a few hours of foliar application. Two weeks after application the dry

weight of the shoots was reduced by 30 to 41%. Similar results were obtained by a foliar application of Nep 1 in combination with the bacterium *Pseudomonas syringae* pv. *tagetis*. However, as neither the *Fusarium* spp. nor the extracted protein Nep1 are species specific, they cannot be regarded as biocontrol agents.

## Bacteria as biocontrol agents

The bacterium *Pseudomonas syringae* pv. *tagetis* (Pst), first found on *Tagetes erecta* (Hellmers 1955), is able to cause leaf spot and apical chlorosis on a number of Asteraceae, including *C. arvensis* (Johnson and Wyse 1991, Johnson et al. 1996, Rhodehamel and Durbin 1985, Styer and Durbin 1982). The apical chlorosis is due to the production of the unspecific compound tagetitoxin (Lukens and Durbin 1985, Durbin 1990). This toxin causes decreased vigour, inhibition of flowering and increased winter mortality (Johnson et al. 1996) and it led to study Pst as a potential biological weed control agent. Bacteria have many advantages compared to fungi: they grow very fast in liquid culture, can be stored frozen or dried and are suited for genetic manipulation and selection (Johnson et al. 1996). Nevertheless, they were ignored for a long time as possible biocontrol agents mainly because of their inability to penetrate intact plants (Templeton 1982). Field studies with a spray application of Pst and a surfactant resulted in 100% disease incidence and greater severity of disease symptoms than observed in natural infections. This led to a mortality of 57% of the plants meaning a significant reduction of the thistle population (Johnson et al. 1996). Another field study by Hoeft et al. (2001) showed similar results.

Application of Pst resulted in reduced survival of *C. arvensis*, less height growth and seed production. Less seed production leads to a reduced soil seed bank and less regrowth of the thistle. Gronwald et al. (2002) tested different application methods and effects of repeated applications. The authors found apical chlorosis in 67% of the plants, resulting in a 31% reduction of plant height; they counted 81% fewer flower heads and a survival rate reduced by 20% after two applications. Tichich and Doll (2006) also found repeated applications to be more effective than a single one, as a single application causes chlorosis but no loss of dry weight (Bailey 2000). In a growth chamber experiment with foliar application of Pst, Gronwald et al. (2002) showed a loss of dry weight of 52% and a loss of chlorophyll content of emerging leaves of 92%. Tagetitoxin inhibits plastidic RNA polymerase III, thus preventing chloroplast biogenesis, so that infected plants produce new cells without chloroplasts and incapable of photosynthesis (Lukens and Durbin 1985, Lukens et al. 1987, Mathews and Durbin 1990, Steinberg et al. 1990). To target the photosynthetic activity of above-ground plant parts appears to be a much better strategy than to try to deplete the roots' reserves, followed by mechanical methods such as mowing (Tichich and Doll 2006).

However, also the repeated foliar application of the sap from naturally infected thistles led only to a 50% incidence of disease, still not sufficient to effectively suppress thistle growth (Tichich and Doll 2006). Further possibilities to increase the effectiveness of Pst as a biocontrol agent include a strict selection for humid application periods to ameliorate the

initial conditions for the plant pathogen (Tichich and Doll 2006, Tichich et al. 2006), selecting strains that produce more toxin (Gronwald et al. 2002, Tichich and Doll 2006), or increase toxin production by optimal environmental and nutritional conditions (Bender et al. 1999, Li et al. 1998), especially a high nitrogen supply during cultivation (Styer 1982).

These studies succeeded due to the combined application of Pst with Silwet L-77 or a similar organosilicone surfactant that facilitated the entry of bacteria into leaves (Zidack et al. 1992, Zidack and Backman 1996) via the stomata and hydathodes, because of their property to lower surface tension (Neumann and Prinz 1974, Field and Bishop 1988, Stevens et al. 1991). A combination of Pst with a chemical herbicide such as glyphosate further increased disease symptoms and reduction of fresh and dry weight significantly (Bailey et al. 2000). This suggests synergistic effects between the bacterial agent and the herbicide (Christy et al. 1993).

Host specificity tests showed that tagetitoxin acts on a variety of Asteraceae (Johnson and Wyse 1991, Johnson et al. 1996, Rhodehamel and Durbin 1985, Styer and Durbin 1982). Durbin et al. (1989) described that wheat seedlings, after a first contact with tagetitoxin, completely lacked chlorophyll and Durbin (1990) designated tagetitoxin a “non-host selective” compound. Obviously this substance is suitable as a non-selective herbicide but not as a highly selective biocontrol agent.

## Conclusion

Mycoherbicides have been praised since decades to solve problems of weeds in a variety of habitats and as an upcoming strategy in organic farming but today results are still disappointing: only eleven products seem to have made it to the market worldwide (Charudattan and Dinoor 2000, Khetan 2001, Ash 2010). A recent search among patents yielded 71 citations (Ash 2010) but this does not necessarily indicate a huge product pipeline but rather underlines that most of them never will be realised. On a global level, the reasons for this situation are multiple and heterogeneous but may be similar to those outlined for *C. arvense* and its pathogens. The primary reason for the failure of most of the tested plant pathogens against *C. arvense* is the missing host specificity (among the here presented pathogens, this refers, e.g., to *Alternaria cirsinoxia*, *Sclerotinia sclerotiorum*, *Phoma exigua*, and *Pseudomonas syringae*). A useful and safe biocontrol agent has to be as specific as possible. Species-specificity would be ideal but is obviously very difficult to find. Genus specificity may be acceptable quite often but has to be tested very carefully. Less pronounced specificity, e.g. on family level, usually cannot be accepted. Also the varying and low virulence of the pathogens pose a problem (e.g., *Alternaria cirsinoxia*, *Sclerotinia sclerotiorum*, *Phomopsis cirsi*) as constant levels of virulence must be ensured for a successful inhibition of the growth of the target weed. None of the proposed fungi is able to kill a thistle clone, thus confirming the conclusion in Charudattan's (2005) review that weeds with a robust capacity for vegetative regeneration are more difficult to control with pathogens. Another restriction en-

countered is the obligate biotrophic nature of the rust *Puccinia punctiformis* which poses the problem that this fungus cannot be cultivated in the laboratory to produce the necessary amount of inoculum.

This review shows for *C. arvensis*, one of the single most important weeds of the world, that despite nearly 100 years of research it was so far not possible to use fungi and other pathogens as biocontrol agents. While it is generally undoubted that pathogens are important regulators of plant populations (e.g., Mitchell and Power 2003), the specific situation in a highly disturbed agricultural landscape is different since natural regulation mechanisms are not strongly developed against *C. arvensis*. At least for Canada thistles, one could conclude that the potential of fungi as biocontrol agents has been overestimated even if Charudattan (2005) would state that this approach is still underdeveloped. There is always a chance to find new and suitable biocontrol agents when increasing the search effort. Nevertheless, for us it is today very difficult, to advice on suitable and promising future research approaches for a biological control of Canada thistles.

The current regulatory situation where microbial products need to go through the same registration procedure as conventional pesticides certainly represents a huge barrier for potential applicants. This may explain the considerable number of dead patents. Size and diversity of a research consortium and the financial power of the industrial partners may be further decisive parameters (Ash 2010, Bailey et al. 2010). Another problem is target selectivity. Good biocontrol praxis demands an as high target specificity as possible. Economically speaking, however, such a small application basis is not interesting at all. Therefore one could propose to accept agents of only medium target selectivity since most applications would only occur in monocultures. While this even may be correct for *C. arvensis*, further candidate habitats would certainly include more diverse landscapes and even natural habitats of conservational value. Since Canada thistles are invasive in most parts of the world, related, endemic thistle species, though protected and non-targets, suddenly could be affected by such an agent of low specificity.

In the case of *C. arvensis* the research development of the last years, however, points into the direction of applying secondary plant compounds. Such substances quite often are structurally modified and can be produced synthetically. By this, unspecific but powerful herbicides may come up. Though sometimes the term “bioherbicide” is still used to indicate the biotic origin of such compounds they are as good or bad as chemical herbicides with the classic problems of effectivity, selectivity, degradability and potential side effects.

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# Ploidy levels and reproductive behaviour in invasive *Hieracium pilosella* in Patagonia

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## Abstract

Within a population of invasive *Hieracium pilosella* in Chilean Patagonia we found two ploidy levels, pentaploid and hexaploid. Each ploidy level was represented by one clone. Their reproductive system was apomictic (and thus replicating the maternal genome), with a low degree of residual sexuality. It is necessary to prevent the evolution of new biotypes via hybridisation with different clones of *H. pilosella* or other *Hieracium* species introduced into Patagonia.

## Keywords

*Hieracium pilosella*, Patagonia, ploidy levels, hybridisation

## Introduction

In 2010, a paper on a *Hieracium pilosella* invasion in Patagonia (Tierra del Fuego, Argentina) was published by Cipriotti et al. (2010). Earlier, similar invasive behaviour by this species was described in Chilean Patagonia in an unpublished thesis by Cárdenas Vergara (2005). Thus, *Hieracium pilosella* has evidently invaded throughout southernmost South America. One important aspect of invasion biology is connected with species reproduction, namely, how easily a species produces progeny and how variable these progeny are. This aspect of invasion biology was not addressed in either of the two papers mentioned.

*Hieracium pilosella* (syn. *Pilosella officinarum*) consists of several ploidy races (cytotypes), which combine with different reproductive modes (reviewed in Fehrer et al. 2007). Three ploidy levels (the basic chromosome number is  $x = 9$ ) are common in Europe, which is the native distribution area of *H. pilosella*: tetraploids (mostly sexual), pentaploids (mostly apomictic, rarely with sexual individuals), and hexaploids (both sexual and apomictic). Heptaploid individuals have only been found rarely, and always in mixed populations with other cytotypes; hence, it has been proposed that the heptaploids originate from cytotypes of lower ploidy via conjugation of unreduced gametes (Mráz et al. 2008). Both interspecific and intercytotype hybridisation is rather common in the whole subgenus *Pilosella* (Fehrer et al. 2007). *Hieracium pilosella* is an invasive species in other parts of the world (Tasmania, New Zealand, North America, South America; a summary is given in Fehrer et al. 2007). Its invasion has been especially studied in New Zealand; apomictic tetra-, penta-, and hexaploids occur in this secondary distribution area, but sexual plants have also been observed only occasionally (Chapman and Bicknell 2000; Houliston and Chapman 2001).

Many species of the *Hieracium* subgen. *Pilosella* are facultatively apomictic, producing predominantly progeny, which is genetically identical with their maternal parent. Nevertheless, a (usually minor) proportion of their progeny are formed by sexual process. The degree of this residual sexuality varies among species and is generally unknown, having not been studied thoroughly (for the quantification of residual sexuality in three species, see Bicknell et al. 2003; Krahulcová et al. 2004). Residual sexuality may be extremely important for the formation of new genotypes, which could serve as a substrate for natural selection.

In 2005, we acquired seeds from *Hieracium pilosella* plants (and other subgen. *Pilosella* species) collected in Chilean Patagonia. Based on the collector's information on the highly extensive populations of *H. pilosella* in this area, we presumed that this species was reproducing apomictically. Therefore, we cultivated mature plants from seeds sampled in the field, and we determined their ploidy level and reproductive mode. Our data, which are complementary to those in a recently published paper (Cipriotti et al. 2010), are important for understanding the future of the *H. pilosella* invasion in Patagonia.

## Materials and methods

In the summer of 2004/2005, Ladislava Filipová collected herbarium specimens of *H. pilosella* with seeds from the following localities:

- Loc. 1. Patagonia, Kampenaike, Punta Arenas, Cerro Caballo; 52°43'02"S, 70°57'46"W, alt. 30 m (4 plants).
- Loc. 2. Patagonia, Kampenaike, Punta Arenas, Gali 2; 52°42'27"S, 70°59'48"W, alt. 29 m (3 plants).
- Loc. 3. Patagonia, Kampenaike, N margin of Punta Arenas, Domaie, 53°7'24"S, 70°52'14"W, alt. 4 m (2 plants).



The seeds were extracted from pressed fruiting plants and were sown in 2005 into pots with sterilised garden soil. Later, the seedlings were replanted, and the mature plants were kept in outdoor beds in an experimental garden at the Institute of Botany at Průhonice, the Czech Republic. Specimens of the plants sampled in the field and of the plants cultivated from their seeds are deposited in the herbarium of the Institute of Botany, Průhonice, the Czech Republic (PRA).

Ploidy level and reproductive mode were determined using standard methods and following the procedures described by Krahulcová et al. (2004). Flow cytometry of DAPI (4',6-diamidino-2-phenylindole)-stained nuclei was used to determine DNA ploidy level (Suda et al. 2006), and the relative seed-set of emasculated versus open-pollinated capitula was used to determine the reproductive system (sexual versus apomictic). Potential residual sexuality (i.e., the capability of the production of sexually derived progeny) was assessed with apomictic maternal plants that were pollinated in the greenhouse by an appropriate cytotype of the same species, allowing for the origins of the progeny to be detected (Krahulcová et al. 2004). Specifically, the two detected *H. pilosella* cytotypes, pentaploid and hexaploid, were crossed with tetraploid *H. pilosella*. The viability of the pollen from the tetraploid parent was sufficient for fertilisation (pollen stainability 75% – 89%), as pollen from this parent has been used successfully in previous intercytotype crosses (Krahulcová et al. 2004). Seeds obtained from pollinated apomictic plants were analysed using the Flow Cytometric Seed Screen (FCSS) method, either in its conventional version processing seed doublets (Matzk et al. 2000), or in its modified version processing pooled samples of ten seeds (Krahulcová and Suda 2006). The origins of the progeny were inferred either from the ploidy level of the embryos as compared to the maternal ploidy level (using modified FCSS for the progeny originating from hexaploid × tetraploid crosses), or from the ploidy of the embryo and of its endosperm (using conventional FCSS for the progeny originating from pentaploid × tetraploid crosses).

The clonal (or genotypic) identity of the material from different localities was determined by comparing the isozyme phenotypes of the respective cultivated plants; a combination of four enzymes (AAT, EST, LAP, PGM) was used because this system has sufficient resolution efficiency in *Hieracium* subgen. *Pilosella* (Krahulec et al. 2004). In addition, variations in chloroplast DNA (cp-DNA) were examined in selected clones and compared with that recorded in *H. pilosella* in Europe (Fehrer et al. 2005; Krahulec et al., unpublished data). The procedure used for cp-DNA analysis (Southern blotting and minisatellite fingerprinting) and the characteristic cp-DNA haplotypes distinguished in the subgenus *Pilosella* follow Fehrer et al. (2005).

## Results

A total of 57 plants were cultivated from seeds that were sampled from nine maternal plants at three localities in Patagonia. All 25 cultivated progeny plants originating from the four maternal plants at locality 1 (Materials and Methods) were pentaploid.

The other progeny plants, originating from both locality 2 (three maternal plants/11 cultivated progeny plants) and locality 3 (two maternal plants/21 cultivated progeny plants), were hexaploid. All of the progeny plants were apomictic, and their morphology was highly uniform within each cytotype: this fact implied an apomictic reproductive mode in the maternal plants collected in the field. For this reason, to detect the clonal structure among their presumably apomictic maternal parents, we chose only one progeny plant from each maternal array for isozyme analysis. Analysis showed that each cytotype was composed of only a single clone. Thus, the pentaploids and hexaploids were found to be clonally uniform. These two clones differed also in their cp-DNA haplotypes. The hexaploid clone had the main group II haplotype (namely subtype II/7), which predominates in *H. pilosella* in Europe (Fehrer et al. 2005). The haplotype detected in the pentaploid clone belonged to a main group I haplotype, namely subtype I/1.

The level of residual sexuality was low in both the pentaploid and hexaploid apomictic clones. A total of 30 progeny seeds produced by the pentaploid clone pollinated by tetraploid *H. pilosella* were analysed using the FCSS method (two seeds were analysed per sample). All of these seeds (100%) had pentaploid embryos and decaploid endosperm, corresponding to autonomous apomixis giving rise to pentaploid apomictic progeny. FCSS analysis of 190 progeny seeds showed that crossing the hexaploid clone with tetraploid *H. pilosella* also generated predominantly apomictic progeny. In the respective flow cytometric histograms (10 seeds were analysed per sample), 189 hexaploid embryos were recorded in total; a clearly detectable small peak of apomictic dodecaploid endosperm was present in all of the histograms, which again corresponds to autonomous apomixis. Only one octoploid embryo (out of 190 embryos analysed) originated from the hexaploid  $\times$  tetraploid cross, likely originating from an unreduced female gamete of the hexaploid maternal plant being fertilised by a diploid male gamete of the tetraploid pollen parent. Consequently, the frequency of apomixis in hexaploid apomictic *H. pilosella* was estimated to be 99.5%.

## Discussion

At all three ploidy levels that are most common in *H. pilosella*, both apomictic and sexual plants are known. Nevertheless, most of the data on chromosome number and reproductive system are based on plants from its native distribution area in Europe. The plants invading New Zealand are mostly pentaploid and apomictic, although tetraploids and hexaploids have been found there rarely (Houlston and Chapman 2001; Jenkins and Jong 1996). Apomictic reproduction is evidently advantageous especially for the colonisation of new areas. However, apomixis in *Hieracium* subgen. *Pilosella* is facultative because some degree of sexuality is still present in otherwise apomictic plants (Fehrer et al. 2007). This characteristic allows the production of some sexual progeny, provided that either another clone or another related cross-compatible species occurs together with an apomictic maternal parent. In New Zealand, sexual

plants have already been found, which supposedly originated from crosses between facultative apomicts (Chapman and Bicknell 2000; Houlston and Chapman 2001). In light of this finding, the low genetic variation and low degree of residual sexuality detected in *H. pilosella* in Tierra del Fuego decrease the chances for an analogous process in this part of its secondary distribution area. Importantly, the introduction of another *Hieracium pilosella* clone into Tierra del Fuego would be dangerous because occasional hybridisation between the different clones could result in the production of new genotypes. Also worrisome is the fact that several other *Hieracium* species with the potential to hybridise with *H. pilosella* have been introduced into this area: *H. aurantiacum*, *H. piloselloides* (syn. *H. praealtum*), and *H. flagellare* (a hybridogenous species originated from *H. caespitosum* and *H. pilosella*) – for references see Fehrer et al. (2007). In addition, among the herbarium specimens we received from Tierra del Fuego, *H. floribundum* (a hybridogenous species originated from *H. caespitosum* and *H. lactucella*) was also present. All of these species are known to hybridise with *H. pilosella* in Europe (Sell and West 1976), and at least some of them do so in New Zealand (Morgan Richards et al. 2004).

Species of *Hieracium* subgen. *Pilosella* are known as easily hybridising, forming both stabilised hybrids (hybridogenous species) and hybrid swarms, even between different ploidy levels (e.g., Fehrer et al. 2007; Sell and West 1976). Efficient hybridisation results in the formation of new forms (either sexual or facultatively apomictic) and increases the evolutionary potential of these species (e.g., Houlston and Chapman 2001; Morgan-Richards et al. 2004).

The following measures are recommended to prevent the rapid evolution of new biotypes of *Hieracium pilosella* (and its hybrids) in Patagonia: (i) prevent the introduction of both new clones and new cytotypes of *H. pilosella*, as well as of new species of the *Pilosella* subgenus (ii) look for possible hybrids among introduced *Hieracium* species and (iii) eradicate these hybrids from sites where they currently occur.

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# Modelling the distribution of the invasive Roesel's bush-cricket (*Metrioptera roeselii*) in a fragmented landscape

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## Abstract

The development of conservation strategies to mitigate the impact of invasive species requires knowledge of the species ecology and distribution. This is, however, often lacking as collecting biological data may be both time-consuming and resource intensive. Species distribution models can offer a solution to this dilemma by analysing the species-environment relationship with help of Geographic information systems (GIS). In this study, we model the distribution of the non-native bush-cricket *Metrioptera roeselii* in the agricultural landscape in mid-Sweden where the species has been rapidly expanding in its range since the 1990s. We extract ecologically relevant landscape variables from Swedish CORINE land-cover maps and use species presence-absence data from large-scale surveys to construct a species distribution model (SDM). The aim of the study is to increase the knowledge of the species range expansion pattern by examining how its distribution is affected by landscape composition and structure, and to evaluate SDM performance at two different spatial scales. We found that models including data on a scale of 1 × 1 km were able to explain more of the variation in species distribution than those on the local scale (10 m buffer on each side of surveyed road). The amount of grassland in the landscape, estimated from the area of arable land, pasture and rural settlements, was a good predictor of the presence of the species on both scales. The measurements of landscape structure – linear elements and fragmentation – gave ambivalent results which differed from previous small scaled studies on species dispersal behaviour and occupancy patterns. The models had good predictive ability and showed that areas dominated by agricultural fields and their associated grassland edges have a high probability being colonised by the species. Our study identified important landscape variables that explain the distribution of *M. roeselii* in Mid-Sweden that may also be important to other range expanding orthopteran species. This work will serve as a foundation for future analyses of species spread and ecological processes during range expansion.

## Keywords

Orthoptera, presence-absence data, spatial scale, landscape structure, land use

## Introduction

The development of effective strategies to manage the spread of invasive organisms requires data on species habitat preferences and knowledge of how landscape characteristics influence species dispersal and establishment (Cote and Reynolds 2002; Rosin et al. 2011). However, the collection of fine-detailed distribution data over large scales is time consuming and logistically challenging, hence data is missing for many species (Jimenez-Valverde et al. 2008). Management decisions have often to be taken swiftly (Morueta-Holme et al. 2010) and species distribution modelling becomes a handy tool when dealing with limited observation data and large spatial and temporal extents (Guisan and Thuiller 2005). By modelling species distribution as a function of ecologically relevant data on climate conditions and/ or landscape characteristics, it is possible to describe occupancy patterns and predict species range expansions (Hein et al. 2007; Early et al. 2008; De Groot et al. 2009; Bonter et al. 2010). Estimates of current and future species distributions rely on: (1) the strength of the relationship between environmental variables and the organism in question (Cote and Reynolds 2002), and (2) the availability of ecological relevant environmental data that can be applied at a range of geographic scales (Scott et al. 2002). It is also important to consider the impact of scale on the performance of the models (Scott et al. 2002), i.e. we need to know which environmental predictors give the best estimates for species presence at a given spatial scale.

Some species of orthopterans (grasshoppers and bush-crickets) have recently shown a rapid response to changed environmental conditions and are invading new areas outside their common range (Sword et al. 2008; Bazazi et al. 2011). Orthopterans are well suited for studying distribution patterns across a range of spatial and temporal scales, because they are relatively easy to survey and their ecology is well studied (Ingrisch and Köhler 1998; Gwynne 2001; Hein et al. 2003; Holzhauer et al. 2006). *Metrioptera roeselii* is an example of a range expanding species in northern Europe (Simmons and Thomas 2004; Gardiner 2009; Hochkirch and Damerau 2009; Species Gateway 2010). Detailed studies on the species' ecology (e.g. Ingrisch 1984; Berggren et al. 2001; Poniatowski and Fartmann 2005; Holzhauer et al. 2006) and movement behaviour (Berggren et al. 2002; Berggren 2004, 2005) have increased the understanding of how *M. roeselii* responds to local biotic and abiotic factors. However it is currently unknown which of the factors are shaping the regional occupancy pattern of *M. roeselii*, and to what extent readily-available landscape data can be used to predict the regional distribution of the species.

The aim of this study is to model the distribution of *M. roeselii* at a large scale (>2000 km<sup>2</sup>) using species presence-absence data from field surveys and digital landscape data available from the national cartographic agency. Since the predictive ability of occupancy models is known to be scale sensitive (Scott et al. 2002) we model the distribution of *M. roeselii* at two different spatial scales ('landscape' and 'local' scale) and compare model performance. At the 'landscape' scale, we measure the landscape composition and structure, factors that affect colonisation and establishment of populations (Werling and Gratton 2008). At the local scale we use land cover type as a

predictor for species occurrence as it is thought to reflect closely species habitat requirements (Hirzel and Le Lay 2008).

The questions we sought to answer in this study were: (1) is there any difference in predictive ability of models which use landscape composition and structure versus those that only include local land cover type to explain the distribution pattern of *M. roeselii* and (2) which landscape variables explain best the occurrence of *M. roeselii* and are these variables consistent between the landscape and local scale?

## Material and methods

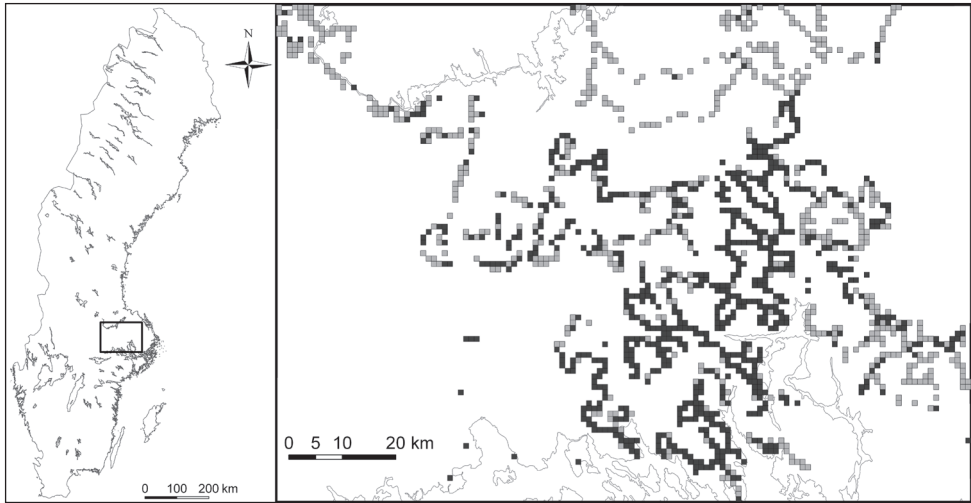
### Study species

*Metrioptera roeselii* (Orthoptera: Tettigoniidae) (Hagenbach 1822) is a small (12–18 mm) predominantly short-winged and flightless bush-cricket commonly found in grasslands of central and northern Europe (Bellmann 2006). In Sweden *M. roeselii* occurs mainly in the Lake Mälaren region and the position of the population core area suggests that the species has been introduced via sea cargo (de Jong and Kindvall 1991). There are indications that the expansion of *M. roeselii* may cause the displacement of a native orthopteran species (Berggren and Low 2004), but its impact on the insect community as whole is largely unknown. *Metrioptera roeselii* is an omnivorous generalist that prefers tall grassland habitats. In the agricultural landscape the species is found in extensively grazed pastures, leys, grassy field margins, ditches, and road verges (Marshall and Haes 1988; Berggren et al. 2001). Forests, arable crop fields and intensively grazed pastures are considered to be unsuitable habitat for the species and urban areas are usually avoided (Ingrisch and Köhler 1998; de Jong and Kindvall 1991; Wissmann et al. 2009).

The reproductive season of *M. roeselii* in Scandinavia is between July and September. Males stridulate to attract females and the species-specific call makes the species easy to census (Marshall and Haes 1988). *Metrioptera roeselii* is a wing polymorphic species; extremely favourable weather conditions (mild springs and hot summers) and high population densities trigger the development of long winged morphs (macropters) (Poinatowski and Fartmann 2010). However, in normal years and at range margins the proportion of macropters in *M. roeselii* populations rarely exceeds two percent and the vast majority of individuals disperse by walking and jumping (Vickery 1965; Wissmann et al. 2009; pers. obs.).

### Data collection

During 2008 and 2009 we surveyed an area of 2554 km<sup>2</sup> in the Lake Mälaren region (mid-point 59°44'N, 16°52'E) for the presence of *M. roeselii* (Fig. 1). The landscape in this region consists of a mosaic of agricultural land (46%), forest (43%), scattered settle-



**Figure 1.** Survey area (mid-point 59°44'N, 16°52'E) in south-central Sweden covering 2554 km<sup>2</sup>. The 1 × 1 km grid squares (n = 874) show presence (black) and absence (grey) of *Metrioptera roeselii*.

ments and small towns (5%), lakes and waterways (3%) and a small proportion of other land use types (3%). In our surveys we sampled the land cover types proportionally to their occurrence in the landscape. We used known locations of *M. roeselii* (de Jong and Kindvall 1991; Berggren et al. 2001; Species Gateway 2010) as starting points for our surveys and surveyed the wider surroundings to map the current distribution of the species. We conducted auditory surveys by car (de Jong and Kindvall 1991; Berggren et al. 2001) on sunny days, between 10 am – 5 pm, from mid July until the end of August. Since the species' call is strong and can be heard over distances of approximately 10 m (Fischer et al. 1997; Bellman 2006), it is possible to listen for stridulating males from the car window while driving slowly (~30 km/ h) along countryside roads (Berggren et al. 2001). We recorded our survey routes and observations of *M. roeselii* using a GPS (Garmin 60XL).

### Variable selection

We used ArcGIS 9.2 (ESRI 2006) to plot and analyse the survey and landscape data. Information on landscape structure and landscape composition was extracted from a topographic map (Geographic Sweden Data (GSD) 1:50 000) and a Swedish CO-RINE (Coordination of Information on the Environment) land cover map (resolution 30 × 30 m) both available from the Swedish mapping, cadastral and land registration authority. We analysed the effect of landscape variables on the species' distribution at two spatial scales: the landscape and the local scale. We placed a 1 × 1 km grid across the study area to create presence-absence squares from the species survey data and to design units in which we measured the predictor variables for the landscape scale analysis (Fig.1). For the analysis at the local scale we use the same 1 × 1 km grid for the spe-

cies data but extracted the land use data from a 10 m wide buffer strip running parallel to each side of the surveyed roads (i.e. the search area). We compared the models from the search area with the models at the landscape scale to test if we find similar effects of land use on species occurrence at a larger spatial scale.

The distribution of *M. roeselii* was treated as presence-absence data within the  $1 \times 1$  km squares for both spatial scales of the analysis ( $n_{total} = 874$  with 318 absence and 556 presence squares). Squares where *M. roeselii* was absent were only included in the analysis if they were adjacent to a presence square. Based on our knowledge of the species dispersal behaviour (Berggren et al. 2001, 2002) we excluded distant and isolated absence squares from the analysis because we considered those squares to lie outside the species immediate colonisable area. We chose this conservative approach in order to minimise the number of false absences in the data which otherwise inflates the omission error, lowering the accuracy of the models (Guisan and Thuiller 2005). Because we were primarily interested in modelling the distribution of populations rather than dispersing in individuals, we only included squares in the analysis that contained at least two observations of male *M. roeselii*. Previous studies have shown that the species has a good colonising ability and propagules consisting of two males and two females can found sustainable populations (Berggren 2001). Because survey length affects detection probability of the species, we used survey length as a covariate in all models, and only included squares in the analyses in which more than 100 m of road was surveyed.

We used GIS to extract landscape variables that are of ecological relevance for *M. roeselii* (Berggren et al. 2001; Berggren et al. 2002; Berggren 2004) and which represent predefined categories in the maps that we used. The land cover categories were generic and consisted of sub-categories of land-use types that resembled each other in terms of vegetation- and management type: (1) arable land (under crop rotation; includes cultivation of cereals, fodder - and root crops, fallow land), (2) forest (includes broadleaved, coniferous and mixed forest, clear-cuts and young plantations), (3) pasture (includes dense herbaceous vegetation dominated by grasses under different grazing regimes), (4) urban areas (includes land with buildings and other man-made structures, small towns and villages), (5) rural settlements (includes solitary houses and farm buildings surrounded by grasslands and gardens), (6) linear elements (combined lengths of streams and roads), and (7) number of fragments of arable land (see Table 1).

We used Pearson's product-moment correlations to test for the relationships between landscape variables using JMP version 8.0.1 (SAS Institute Inc. 2009). Arable land and forest were highly negatively correlated ( $r = -0.86$ ,  $p < 0.0001$ ), suggesting they are mutually exclusive in the landscape. Thus, we choose to exclude forest and include arable land in the analyses as previous studies have shown that *M. roeselii* does not occur in forest areas and arable land under intensive cultivation but occurs and spreads along grassy field margins (Ingrisch and Köhler 1998; Berggren et al. 2001). Linear elements were positively correlated with urban areas ( $r = 0.56$ ,  $p < 0.0001$ ) as road length increases with urban development. We excluded urban areas from the analyses since we know from personal observations and records in the national species base

**Table 1.** Descriptive statistics for the major landscape features and predictor variables used in the regression analyses to explain the distribution of *Metrioptera roeselii* in south-central Sweden.

Variable	Presence squares (1 × 1 km)				Absence squares (1 × 1 km)			
	Min	Mean	Max	SE	Min	Mean	Max	SE
Survey length [km]	0.06	1.02	2.62	0.02	0.01	0.69	2.06	0.02
<i>eLandscape scal</i>								
Arable land [ha]	0.00	53.09	100.00	1.10	0.00	39.96	100.00	1.68
Forest [ha]	0.00	36.91	99.12	1.10	0.00	45.94	100.00	1.75
Pasture [ha]	0.00	4.37	45.12	0.23	0.00	5.19	34.94	0.37
Urban [ha]	0.00	1.67	62.38	0.20	0.00	2.93	59.82	0.45
Rural settlements [ha]	0.00	1.65	17.06	0.10	0.00	0.58	8.88	0.08
Fragments† [count]	0.00	1.43	5.00	0.03	0.00	1.42	6.00	0.05
Linear Elements‡ [km]	0.31	3.45	13.59	0.06	0.20	3.37	10.21	0.09
Stream length [km]	0.00	0.95	3.94	0.04	0.00	0.98	3.36	0.05
Road Length [km]	0.11	2.50	11.91	0.05	0.14	2.39	10.21	0.08
<i>eLocal scal</i>								
Arable land [ha]	0.00	1.21	4.11	0.03	0.00	0.56	3.89	0.04
Forest [ha]	0.00	0.46	3.46	0.03	0.00	0.54	3.76	0.04
Pasture [ha]	0.00	0.14	2.28	0.01	0.00	0.10	1.26	0.01
Rural settlements [m²]	0.00	0.20	3.15	0.02	0.00	0.12	2.20	0.02

Min = Minimum, Max = Maximum, SE is the standard error of the mean.  
† = Number of fragments of arable land, ‡ = the sum of the length of streams and roads.

(Species Gateway 2010) that *M. roeselii* is rarely found in urban areas due to the lack of suitable habitat. All other landscape variables showed low to moderate r-values ( $r \leq 0.3$ ) and were included in analyses. Moran’s I values indicated that the response variable was spatially structured which would cause our estimates of variable significance in the models to be exaggerated (Legendre 1993). However, our primary aim was not to elicit precise species-habitat relationships but rather to produce a general applicable model to predict the species distribution over a large spatial extent. We therefore chose a non-spatial modeling approach over explicitly accounting for spatial dependency in the species distribution model.

Statistical analyses

We used logistic regression models to investigate the relationship between the landscape variables and *M. roeselii* occurrence at two scales: the landscape scale (1 × 1 km units) and the local scale (10 m area either side of surveyed roads). For both analyses a balanced set of candidate models were considered (i.e. all possible combinations of the variables of interest) and these were ranked according to the relative strength of support for each model using Akaike’s information criterion (AIC). We used AIC weights ( $\omega_i$ ) to generate weighted model-averaged parameter estimates when there was no clear best model by including all models within 5 AIC ( $\sum \omega_i = 0.95$ ) from the highest-ranked model (Burnham



and Anderson 2002). We also estimated the relative importance of the predictor variables by summing the AIC weights over all the models in which the variable was contained (Burnham and Anderson 2002). Parameter estimates and AIC for all models were calculated using the 'glm' function in the R 2.8.1 software (R Core Development Team 2008).

We used *v*-fold cross-validation (Witten and Frank 2000), to evaluate the prediction accuracy of the highest-ranked models from our analyses (i.e. survey scale and landscape scale). Of the 874 survey squares, 80% were randomly sub-sampled as the training set and used to parameterise the model. The coefficients of this model were then used to derive probabilities of occurrence for the remaining 20% of the survey squares. Among the number of data partitioning methods in model evaluation (Fielding and Bell 1997) this ratio of 80% training and 20% test data has been previously found useful (Dormann et al. 2008). The square-specific probabilities were used to calculate a random draw from a Bernoulli probability distribution for each square to produce a prediction (0 or 1) and these were compared to the observed data in the validation set (0 or 1) for each square. Differences in observation versus prediction were then recorded as a proportion of mismatches for the training data set. This was repeated 1000 times, with the proportion of mismatches being modeled as a distribution of errors; i.e. the proportional deviation of the predicted versus the observed – similar to a probability density curve. The median and 95% confidence intervals of these errors were then calculated using the cumulative distribution function (ecdf) in R 2.13.1 (R Development Core Team 2009).

## Results

Models at the landscape scale had lower AIC values when compared to equivalent models at the local scale (Table 2), suggesting that variables measured at the landscape scale were better predictors of *M. roeselii* presence than those measured in the immediate survey area (local scale). There was strong support for arable land as an important positive predictor for this species, as it was the only variable present in all models with AIC support (Table 2). By comparing different scales in the analyses (landscape versus local) we show that the habitat variables were differently associated with the species presence depending on the spatial scale at which they were measured (Tables 2 and 3).

At the landscape scale, *M. roeselii* presence was best explained by the full model, containing arable land, rural settlements, pasture, number of arable land fragments and linear elements (Table 2). The second- and third-ranked models differed in either number of fragments or linear elements, suggesting that structural landscape variables had weaker support in explaining *M. roeselii* occurrence. Contrary to expectation, occurrence of *M. roeselii* was negatively correlated with the amount of pasture and linear elements, and positively correlated with the number of fragments of arable land (Table 3). The three land-use variables (arable land, rural settlements and pasture) had the highest relative-importance weights (1.0), followed by linear elements (0.927) and number of fragments (0.778).

**Table 2.** Model selection results for the effect of landscape variables on the occurrence of *Metrioptera roeselii*. The model selection statistics are number of parameters (K), Akaike’s information criterion (AIC), difference between model and minimum AIC values ( $\Delta$ AIC), and AIC weights ( $\omega_i$ ). Only models with  $\Delta$ AIC < 10 are shown.

Rank	Model			K	AIC	$\Delta$ AIC	$\omega_i$
<i>eLandscape scal</i>							
1	Sur + Ara + Rural + Pas + Lin + Frag			7	969.08	0	0.662
2	Sur + Ara + Rural + Pas + Lin			6	971.39	2.31	0.209
3	Sur + Ara + Rural + Pas + Frag			6	973.62	4.54	0.068
4	Sur + Ara + Rural + Pas			5	974.65	5.57	0.041
5	Sur + Ara + Rural + Lin + Frag			6	977.51	8.43	0.010
6	Sur + Ara + Rural + Lin			5	977.86	8.78	0.008
<i>eLocal scal</i>							
1	Sur + Ara + Rural			4	983.48	0	0.581
2	Sur + Ara + Rural + Pas			5	984.65	1.17	0.324
3	Sur + Ara			3	987.82	4.34	0.066
4	Sur + Ara + Pas			4	989.49	6.01	0.029

Abbreviations used for the explanatory variables in the models: Sur = Survey length, Ara = Arable land, Rural = Rural settlements, Pas = Pasture, Lin = Linear Elements, Frag = Number of fragments of arable land.

**Table 3.** AIC-weighted model-averaged parameter estimates generated from the top three models ( $\Sigma \omega_i = 0.95$ ) presented in Table 2.

Averaged Model	Variable	Coeff	SE
<i>eLandscape Scal</i>			
(1 × 1 km)	(Intercept)	-1.478	0.282
	Survey length	1.791	0.199
	Arable land	0.014	0.003
	Rural settlements	0.259	0.051
	Pasture	-0.042	0.013
	Linear Elements‡	-0.124	0.050
	Fragments†	0.159	0.078
<i>eLocal Scal</i>			
(10 m buffer)	(Intercept)	-0.996	0.169
	Survey length	0.643	0.228
	Arable land	1.071	0.142
	Rural settlements	0.559	0.228
	Pasture	0.100	0.111

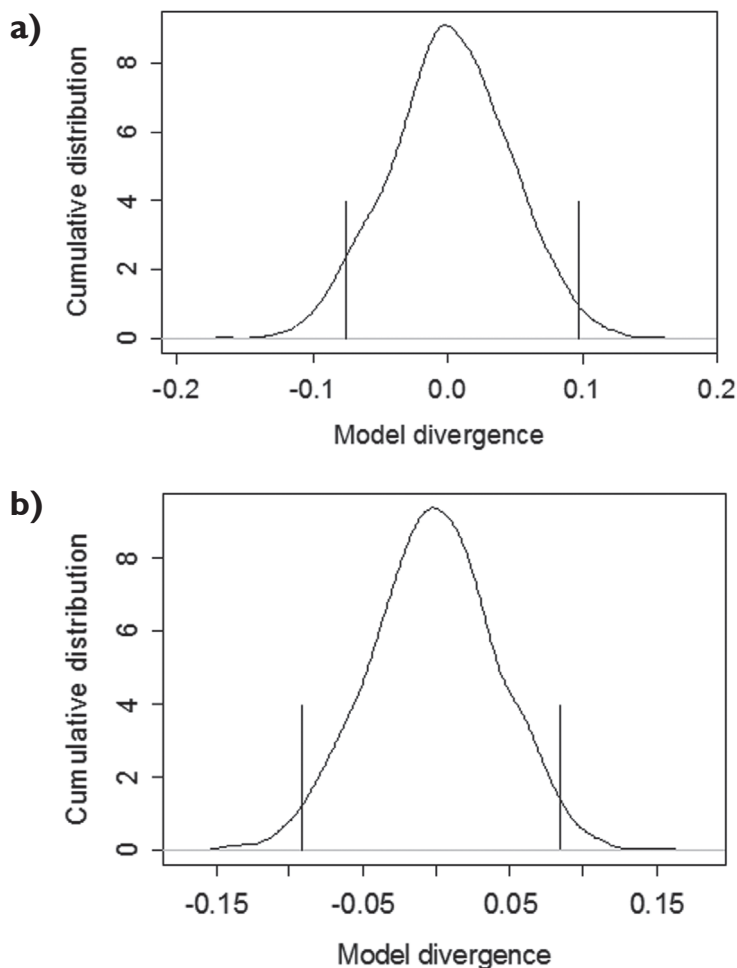
† = Number of fragments of arable land, ‡ = the sum of the length of streams and roads.

Coeff = coefficient; SE = Standard error of coefficient.

At the local scale, the two highest-ranked models contained arable land and rural settlements. This, in combination with their relative-importance weights (1.0 and 0.905 respectively), demonstrates the strong support for them as positive predictors (Tables 2 & 3). Although pasture was included in the second-highest-ranked model,

an examination of Table 2 shows that its inclusion in models generally results in a lower ranking than models without it – suggesting very weak support for it as a predictor of *M. roeselii* presence (relative-importance of pasture = 0.353).

Cross-validation showed that the models were generally accurate in their predictions of species occurrence across the spatial scales for the environmental gradients examined in the study. The landscape-level model prediction for the probability of *M. roeselii* being detected in a square had an error which ranged from -0.091 to +0.080 (95% CI; Fig. 2a). At the survey scale, model prediction error for the probability of detection ranged between -0.075 to +0.097 (95% CI; Fig. 2b).



**Figure 2.** Cross-validation accuracy of 1000 models using randomly selected training and validation sets (80% and 20% respectively). The curves show the relative deviation of prediction accuracy when comparing estimated to observed occurrence of *Metrioptera roeselii* being detected in a square at **a** the survey scale (vertical bars show the 95% CI for model prediction error: -0.075 to +0.097), and **b** the landscape scale (-0.091 to +0.080).

## Discussion

In our study, the distribution of *M. roeselii* was best explained by models at the landscape scale. This indicates that measuring the landscape characteristics within  $1 \times 1$  km units captures both the availability of habitat for the species and incorporates ecological functions of the landscape features (Crawford and Hoagland 2010). The weaker relationship between land use and species occurrence at the local scale could be attributable to the coarse grain size of the land-cover data failing to capture local aspects of habitat quality, i.e. vegetation heterogeneity, microclimate (Gardiner and Dover 2008) and its temporal variability (Gardiner et al. 2008; Poniatowski and Fartmann 2008) as well as important biotic interactions (Huston 2002) that are influencing the distribution of the species. Our study shows that landscape data extracted from digital map sources can be used to explain the regional distribution pattern of this expanding species. Determining biologically important variables and the optimal spatial scale is a prerequisite to predict the likelihood of occurrence of a species in non-surveyed sites with a resolution of  $1 \text{ km}^2$  and form the base for monitoring species spread, serving conservation planning and future research on spatial processes shaping species distributions. The models can also be further developed and used for region-wide predictions in areas similar to the study area, assisting in devising management actions and possible control of undesired species expansion (Hutto and Young 2002; Scott et al. 2002). However, extrapolation of model results should be treated with caution. Abiotic factors such as land cover can generally be applied only within a limited spatial extent and time frame because the same variables can differ in habitat suitability moreover the same species may respond to different sets of variables in different parts of its distributional range (Guisan and Zimmerman 2000).

When modelling species distributions in fragmented landscapes it is important to incorporate the landscape structure into the analyses (Umetsu et al. 2008). The number of fragments of arable land was a positive predictor for the occurrence of *M. roeselii*, indicating that the field margins offer important edge habitat and serve as dispersal paths in the agricultural landscape (Berggren et al. 2001). Similar dispersal behaviour has been observed in the wood cricket *Nemobius sylvestris* that moves along habitat edges (Brouwers et al. 2011). Contrary to expectations, linear landscape elements (roads and streams) had a negative effect on species occurrence at the landscape scale. One possible explanation is that although linear elements have been associated with increased dispersal opportunities in small-scaled studies, at larger scales linear landscape features such as major roads and streams act as a barrier for the species dispersal if they separate suitable habitat areas (de Jong and Kindvall 1991). Due to the large spatial extent of our study it was not possible for us to explicitly incorporate spatial configuration and orientation of linear landscape features in the model.

At both spatial scales that we analysed, arable land and rural settlements turned out to be strong predictors for the presence of *M. roeselii* suggesting that these land use types can be used as surrogate measure for grassland habitat in the region. The positive effect of arable land on the occurrence of *M. roeselii* might be surprising at first since it is known that *M. roeselii* avoids crop fields because of the lack of shelter, food and egg laying places

(Ingrisch and Köhler 1998). However, arable land is a generic land use description and vegetation cover varies with the type of crop cultivated. In Sweden, crop rotation is commonly practised (Söderberg 2006) and arable land becomes temporally a suitable habitat for orthopterans and other grassland living insects when crop fields are shifted into fallows or leys (Duelli et al. 1999). The ability to track resources is particularly important for species in dynamic landscapes. In areas with intensive agricultural production the grassy field margins and hedgerows often have high species richness and function as dispersal corridors and source habitats for colonisers of crop fields (Marshall and Moonen 2002; Meek et al. 2002). The present findings support our assumption that grassland insects like *M. roeselii* benefit from habitat heterogeneity in arable landscapes. Bräschler et al. (2009) found that cricket (Ensifera) density was higher in fragmented plots, as uncut patches of grassy vegetation play an important role in maintaining insect diversity in the agricultural landscape by offering shelter from predators and serving as mating and egg laying sites. A previous study by Bieringer and Zulka (2003) showed that orthopteran species richness increases with distance to forest edge. We believe that the positive effect of arable land in our study was not simply because the bush-crickets avoided forest, but rather that agricultural areas contain a larger amount of suitable grassland vegetation than forests.

In cultivated landscapes, generalist species that are able to occupy a broad range of habitat types are less sensitive to local habitat loss (Marini et al. 2008, 2009a). *Metrioptera roeselii* is an example of a grassland generalist (Ingrisch and Köhler 1998) colonising a range of grassland types (Gardiner et al. 2008; Poniowski and Fartmann 2005). Like its close relative *M. bicolor* (Kindvall 1996) it is able to sustain populations in small patches of habitat. Rural settlements, despite covering only a small area of the landscape, have been shown to provide important habitat for a range of species (Belfrage et al. 2005; Rosin et al. 2011) and may function as source patches for *M. roeselii* enabling the species to colonise surrounding areas. Extensive farming practices and small field sizes are positively correlated with habitat heterogeneity, which in turn has a positive effect on the local diversity of species with limited movement ability like pollinators and grassland living insects (Benton et al. 2003; Marini et al. 2009b; Steck et al. 2007).

We expected that the amount of pasture and the presence of *M. roeselii* would be positively correlated on both scales since *M. roeselii* has been found to colonise extensively grazed pastures (Poniowski and Fartmann 2005). The negative correlation of pasture on *M. roeselii* occurrence at the landscape scale is difficult to interpret. A possible explanation could be that the overall proportion of pastures in the landscape is small and its distribution scattered which makes it more difficult for the species to colonize.

Species ecology, range size and rarity have an influence on model performance (Franklin et al. 2009; Syphard and Franklin 2009). Results from other studies (Heikkinen et al. 2006; Segurado and Araújo 2004) have shown that specialist species and species with a limited range are generally more accurately modeled than generalist species and species with a wide geographic range, *M. roeselii* is an example of the latter. The natural dynamics of the study species makes it more difficult to model its distribution because the assumption of the species being in equilibrium with the environment is violated and dispersal contributes to spatial autocorrelation in the data (Franklin et

al. 2009). With these limitations in mind we thoroughly surveyed the range of environmental conditions present in the distribution area from the core of the study area to the margin aiming to obtain a large sample size as possible. Despite our surveys were conducted by car, we sampled all important habitat types (arable land, forests, pastures and human settlements) proportionally to their occurrence in the landscape. Aware of the trade-off between model generality, reality and precision (Guisan and Zimmerman 2000), we prioritized the former as our primary aim in this study was to develop predictive model for *M. roeselii* within the study region. The model can be further developed and applied to other grassland insects with similar traits.

## Conclusions

Type of land use and structural landscape elements describing the amount of available habitat are important predictors for species occurrences (Hein et al. 2007; Kemp et al. 1990; Crawford and Hoagland 2010). The possibility to model *M. roeselii* distribution using survey data and available land-cover data on a scale that is easy to extract and utilise for managers is promising in that it will enable us to predict the direction and possible extent of future range expansion of the species. As many Orthopterans disperse and interact with the environment in a similar way (Hjermann and Ims 1996; Diekötter et al. 2007; Brouwers et al. 2011), the results from this study may also be valid for other related species that are now expanding their distribution areas. This is very useful, as many studies on grassland living insects face a similar dilemma: a limited availability of distribution data for species that are living in highly dynamic landscapes (Marini et al. 2009b). The possibility to utilise available distribution data in combination with land-cover data enables us to improve our understanding of the species ecology, to highlight areas of conservation concern and to predict species occurrences in a time of environmental change (Bonter et al. 2010).

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# Impact of the introduced small Indian mongoose (*Herpestes auropunctatus*) on abundance and activity time of the introduced ship rat (*Rattus rattus*) and the small mammal community on Adriatic islands, Croatia

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## Abstract

The small Indian mongoose (*Herpestes auropunctatus*) is one of the world's 100 worst invasive species (IUCN 2000). It has negative impacts on several small mammals on islands where it was introduced. We assess the abundance of small mammal populations and the activity time of introduced ship rats (*Rattus rattus*) on three mongoose-infested and three mongoose-free islands in the Adriatic Sea, Croatia. We set up three transects on each island with a trapping system consisting of 30 small live traps to capture small mammals under 30 grams and 30 larger traps to capture ship rats and mongooses, on each transect. Our results support an already large but mostly speculative literature that suggests inability of the small Indian mongoose to reduce high abundances of introduced *R. rattus*. Further, we suggest that the low abundance of native small mammals is probably not solely caused by the mongoose but also by high *R. rattus* populations on all six islands. In addition, we provide evidence that *R. rattus* has changed its activity time to become more nocturnal on mongoose-infested islands, possibly to avoid predation by the mongoose. As *R. rattus* became more nocturnal, the diurnal mongoose may have become the main predator on amphibians, reptiles, and poultry.

## Keywords

introduced predator, *Apodemus*, *Crocodyra*

## Introduction

The small Indian mongoose (*Herpestes auropunctatus*) has been listed by the IUCN (2000) as one of the world's 100 worst invasive species. Native to southern Asia, it was introduced to many islands in the Pacific, the Indian Ocean and the Caribbean Sea (Simberloff et al. 2000, Thulin et al. 2006).

Most mongoose introductions were in the late 19<sup>th</sup> and early 20<sup>th</sup> century to control introduced rats in sugar cane fields, but evidence of its success as a ratter is conflicting and mostly negative (Espeut 1882, Urich 1914, Pemberton 1925, Barnum 1930, Doty 1945, Seaman 1952, Hinton and Dunn 1967, Stone et al. 1994, Hays and Conant 2007). Statements on this matter are mostly anecdotal, and there are no controlled studies looking at the mongoose's ability to control rats.

No comprehensive study has been devoted to the impact of the mongoose on the abundance of native small mammal populations, although several studies have proposed the mongoose as a major cause for the decline of species. For example, Woods and Ottenwalder (1992) suggested that introduction of the mongoose has contributed to extinction of four species of Haitian island shrews (*Nesophontes* spp.). Borroto-Paéz (2011) believed that the mongoose has been largely responsible for the endangered status of the Cuban solenodon (*Solenodon cubanus*) and is suspected in the likely extinction of the dwarf hutia (*Mesocapromys nanus*). Yamada and Sugimura (2004) linked the decline in the abundance of the threatened native rabbit (*Pentalagus furnessi*) on the Japanese island of Amami-Oshima to the spread of the mongoose across the island.

On Adriatic Islands, the mongoose was introduced in 1910 to Mljet Island to control a poisonous viper (*Vipera ammodytes*) and subsequently to several other islands (Korčula in 1921, Hvar (early 1950's), Čiovo (ca. 1950's), Škrda (ca. 1950's), Kobrava (unknown) (Tvrković and Kryštufek 1990, Barun et al. 2008). It was introduced to the Pelješac Peninsula repeatedly from 1921 to 1927, and it is spreading along the southernmost part of the Dalmatian coast and has reached the Neretva River in the north (Barun et al. 2008) and Albania in the south (Ćirović et al. 2011). Nearly all Croatian large islands host a native carnivore, the stone marten (*Martes foina*), plus feral domestic cats (*Felis sylvestris*) and the ship rat (*Rattus rattus*). The latter was introduced to the western Mediterranean region over 2000 years ago (Audouin-Rouzeau and Vigne 1994, 1997, Martin et al. 2000). The impact of the mongoose on rat and native small mammal abundance is unknown, but assessing the impact of one particular species among a predator community is not easy. Fortunately, the mongoose has been introduced to some but not all islands of Dalmatia. Although we do not have censuses of small mammals before and after the introduction, we attempted to compensate for this shortcoming by comparing mongoose-infested and mongoose-free islands to try to determine the impact of the mongoose on the abundance of rats and native small mammals.

If introduced predators are capable of changing the abundance of their prey, conversely, prey may be able to assess predation risk and may behave accordingly, shifting their feeding, social, or escape behavior (Lima and Dill 1990, Kronfeld and Dayan

2003). For example, *R. rattus*, generally nocturnal, will be active and forage during the day if benefits outweigh risks. Berdoy and Macdonald (1991) have shown that socially subordinate individuals were forced to be diurnal to escape competition from dominants, and Fenn and Macdonald (1995) have shown that nocturnal visits by predators made it more dangerous for rats to be active by night than by day, forcing rats to be diurnal. Nellis and Everard (1983) found that rats on a Caribbean island became primarily nocturnal and arboreal after the introduction of the mongoose. In sum, rats can become more active diurnally, but cases of such a shift are scarce and possible mechanisms untested.

The goals of this study are: i) to assess the abundance of introduced rats and native small mammals on mongoose-infested and mongoose-free islands; ii) to compare rat activity times on mongoose-infested and mongoose-free islands, to test the hypotheses that activity times will be primarily diurnal where only the nocturnal marten is present (all the mongoose-free islands), but shifted towards night time when the diurnal mongoose is also present.

## Methods

### Study area and field methods

We conducted this study in 2008 on six islands in the southern part of Adriatic Sea: Lastovo (5,300 ha), Brač (39,400 ha), Dugi Otok (11,400 ha), Mljet (10,000 ha), Korčula (27,000 ha) and Hvar (29,900 ha). The first three are mongoose-free and the others are mongoose-infested. These islands are relatively similar in elevation, karst geology, Mediterranean climate and vegetation, but vary in surface area. They have a similar history of agricultural practices, human occupation, and timing of introduction of most exotic species. Their landscape is a fine-grained mosaic of small agricultural fields, scrublands (garrigue), thickets (maquis, mattoral), and forests. Agricultural production is mainly for local consumption and consists of olive groves and vineyards, with a few small vegetable fields with rich soil. A full description of these habitats is provided by Barun et al. (2010).

To determine small mammal abundance on every island, we set up three transects of 30 trapping spots distributed at 30 meter intervals in 900m long transects along narrow dirt roads, each running through all four vegetation types described previously in a proportion that may vary among transects. On each transect, trapping spots were placed alternatively on one side of the road and its opposite, and each trapping spot received two live traps: one INRA trap (stainless steel, horizontal bar-sprung trap similar to Sherman traps) to capture mammals weighing less than 30 g and one ratière trap (collapsible, wire and hanging bait-sprung trap, Guédon et al. 1990) to trap heavier mammals, particularly ship rats and mongooses. All traps were baited with a mixture of oat-flakes, peanut butter, and sardine oil, and bait was changed once during the three-day trapping period or just after rain. We ran the trapping system for three days

and three nights in April and repeated the procedure in May at the same locations. We did not trap during rainy nights. We checked each trap early in the morning to collect nocturnal specimens and before sunset to collect the diurnal ones. Trapped animals were either euthanized and preserved for museum deposition or released at least one kilometer away from the transects.

### **Local habitat structure and analysis**

To describe vegetation structure, four sample locations were evenly spaced along each transect, and the following data were collected within a 50-meter radius: % cover of bare ground, dead wood, rock, detritus, grasses in three layers (0–0.25 m, 0.25–0.5 m, 0.5–1 m); % cover of vegetation layers (0–0.25 m, 0.25–0.5 m, 0.5–1 m, 1–2 m, 2–4 m, 4–8 m, 8–16 m, 16–32 m, >32 m), maximum height of vegetation, canopy height, and % cover of each woody plant species. Within each vegetation layer, the relative cover was defined as the projection of the foliage volume of the layer on a horizontal plane. This was estimated by comparison with a reference percent cover chart (Prodon and Lebreton 1981). At each point we also recorded percent cover of each woody plant species present and its average height.

We used PRIMER (Plymouth Marine Laboratory, UK) to conduct an analysis of similarity (ANOSIM) followed by pairwise comparisons to examine if two habitat variables (habitat characteristics and percent cover of each woody plant species) differed between islands with and without the mongoose. In the analysis, we nested six islands into two main grouping factors: mongoose present and mongoose absent. For each habitat variable, habitat characteristic, and percent cover of each woody plant species, we constructed a nonmetric multidimensional scaling (NMDS) plot, a nonparametric approach, using Bray–Curtis similarity coefficients from a triangular matrix (Bray and Curtis 1957) of Euclidean distances of islands with the mongoose versus islands without it. The NMDS plot can also illustrate similarity and/or dissimilarity in habitat characteristics between the two island groups.

### **Abundance analysis**

To compare abundances of single species between islands with and without the mongoose, we calculated a Minimum Number Alive index (MNA) (Krebs 1966, Hilborn et al. 1976). This index is a ratio of the number of trapped animals belonging to one species to the number of trap-nights. However, several traps may be inoperative for one or all target species during parts of trapping sessions. Traps were inoperative for all species when they were found closed and empty (NTO). Traps were inoperative for a species when they contained an individual of any other species (Sum AllSpp). The number of trap-nights used to compute the MNA index was the number of functional

trap-nights for each target species (Pascal et al. 2009). The species one (*Sp1*) MNA index was computed as follows:

$$Sp1MNA = Sp1C / (NT - NTO - \text{Sum AllSpp})$$

*Sp1C* is the number of captures for species one, NT is the total number of trap-nights, and NTO is the number of trap-nights the trap was inoperative for all species, whereas SumAllSpp is the total number of individuals of all other species captured.

To compare *R. rattus* and wood mouse (*Apodemus sylvaticus*) abundances between islands with and without mongooses, we calculated mean MNA indexes for each species for the three transects for each island and compared those values for the three islands with mongooses vs. the three mongoose-free islands with a t-test. To compare *R. rattus* activity times on mongoose-infested and mongoose-free islands, we performed Fisher's exact test on the total number of captured rats for all three transects for each island, but we kept daytime captures separate from night captures. We performed all analyses in JMP, Version 8. (SAS Institute Inc., Cary, NC).

## Results

ANOSIM indicated that composition of habitat characteristics did not differ between islands with the mongoose and islands without it (global  $R = 0.359$ ,  $P = 0.136$ ), nor did the percent cover of woody plant species differ (global  $R = -0.457$ ,  $P = 0.115$ ).

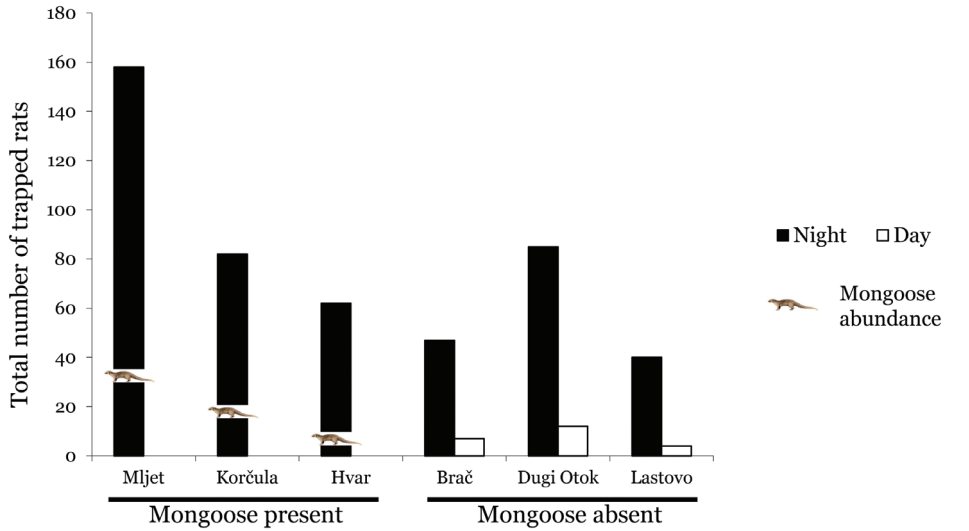
In Table 1 we list the mammal species found on each island according to Kryštufek and Kletečki (2007) and the number of specimens trapped during our field operations. Apart from 23 reptiles (*Pseudopus apodus* and *Dalmatolacerta oxycephala*) and one amphibian (one *Bufo viridis*), the 699 other captures belonged to eight mammal species among the 14 species recorded as present on the studied islands. The largest samples came from three species, two aliens, *R. rattus* (499) and *H. auropunctatus* (57), and one native, *A. sylvaticus* (122). Specimen numbers of these three species together constitute 97 % of all mammalian captures.

Mongooses were most abundant on Mljet and Korčula and much scarcer on Hvar (Fig. 1), where local hunters have conducted intensive, island-wide predator-control operations for several years (Barun et al. 2010). Edible dormice (*Myoxus glis*) were not caught, likely because of the largely arboreal habits of this species and its long hibernation time during trapping months. MNA of rats did not differ between islands with the mongoose and those without it ( $F = 0.291$ ,  $df = 5$ ,  $p = 0.619$ ). Similarly, MNA of *A. sylvaticus* did not differ between mongoose-infested and mongoose-free islands ( $F = 3.523$ ,  $df = 5$ ,  $p = 0.134$ ).

The frequency of rats trapped during the day on mongoose-free islands exceeded that on mongoose-infested islands, ( $P < 0.001$ , Fisher's exact test, Fig. 1); in fact no rats were trapped on mongoose-infested islands during the day.

**Table 1.** Mammalian species distributions on the islands under study, after Kryštufek and Kletečki (2007). X : present; - : absent; numbers are numbers of trapped individuals during our study.

	Mongoose PRESENT			Mongoose ABSENT		
	Mljet	Korčula	Hvar	Brač	Lastovo	Dugi Otok
<i>Herpestes auropunctatus</i>	31	21	5	-	-	-
<i>Martes foina</i>	X	X	X	X	X	X
<i>Canis aureus</i>	-	X	-	-	-	-
<i>Felis sylvestris</i> (feral)	X	X	X	X	1	X
<i>Rattus rattus</i>	158	83	62	55	44	97
<i>Mus musculus</i>	1	X	X	X	X	X
<i>Apodemus sylvaticus</i>	-	22	4	54	29	13
<i>Apodemus epimelas</i>	1	X	-	-	-	-
<i>Suncus etruscus</i>	-	-	X	-	-	-
<i>Crocidura suaveolens</i>	2	1	1	6	1	4
<i>Eliomys quercinus</i>	-	3	X	X	X	-
<i>Myoxusglis</i>	X	X	X	X	-	-
<i>Erinaceus concolor</i>	X	X	X	X	X	-
<i>Lepus europaeus</i>	X	X	X	X	X	X



**Figure 1.** Total number (April and May) of trapped rats during the night and day on three islands with the mongoose and three islands without the mongoose. Mongoose abundance is illustrated with the picture of a mongoose for each island.



## Discussion

Our data are too scant to allow a precise sense of the impact of the mongoose on small mammals on these islands. However, combined with previous work on the mongoose diet on these islands (Barun et al. 2010), our results are suggestive. We have previously reported the following results from stomachs of 57 trapped mongooses: 19 were empty, 39 contained vegetation and/or animal remains, and only five produced hairs, one identified to *A. sylvaticus* (Barun et al. 2010). The dietary results accord with those of several studies devoted to mongoose diet in insular ecosystems, which concluded that the spectrum of items is very large and encompasses many plants and animals (i.e., Nellis and Everard 1983). It is likely that few of the small mammals we targeted were potential prey for the mongoose. Among the 14 mammalian species recorded on these islands, three are large and carnivorous, and two are semiarboreal Myoxidae, all out of reach of the mongoose, which cannot confront the carnivorous species and is a poor climber. Among the nine remaining species, the hedgehog (*Erinaceus concolor*) and the hare (*Lepus europaeus*) both have natural defenses against mongoose predation (spines for the hedgehog and speed for the hare). Among the remaining species that may constitute prey for the mongoose are two shrews, *Suncus etruscus* and *Crocidura suaveolens*, and four rodents, of which two (*Apodemus epimelas* and *A. sylvaticus*) are cryptogenic (Carlton 1996) but probably native, and two are alien and invasive (*Mus musculus* and *R. rattus*).

Although the INRA traps and the bait we used are effective for capturing *C. suaveolens* (Pascal et al. 2009), and despite a significant trapping effort, the number of trapped *C. suaveolens* was small (n=15). Nevertheless, even though the species has been captured on the six islands under study, and even though the total number of captures on mongoose-free islands is higher (11) than on islands with mongooses (4), the sample sizes are insufficient to allow strong conclusions. Moreover, several *R. norvegicus* eradication attempts on islands of the English Channel and French Atlantic coast have shown a strong detrimental effect of that rat on two shrew species, *C. suaveolens* and *C. russula* (Pascal et al. 2005). One cannot yet exclude a similar effect of *R. rattus* on *C. suaveolens* for Croatian populations, and perhaps also on *S. etruscus*, recorded previously only on Hvar, where we did not record it.

As stated previously, the small Indian mongoose has frequently been cited as a species that could send already low island populations to the brink of extinction. In addition to the examples cited above, on Amami-Oshima Island, the shrew *Crocidura orii* is considered endangered because of the mongoose introduction (Yamada and Sugimura 2004). On Adriatic islands, the lesser white-toothed shrew *C. suaveolens* is already considered rare (Dulić 1969), but whether an introduced predator is to blame cannot be determined.

As with *C. suaveolens*, INRA traps and the bait used are efficient for capturing house mice on islands (Pascal et al. 2009). Despite this efficiency and the trapping effort, we captured only one mouse, the species having been recorded previously on

these six islands. This result suggests that this mostly synanthropic species is scarce in natural habitats. However, several rodent eradication attempts have shown that mouse outbreaks occur when rats are successfully eradicated (references in Caut et al. 2007), suggesting mouse suppression by rats. Thus, our result does not by itself strongly implicate an impact by the mongoose. Moreover, interaction among several Muridae species in insular ecosystems has been suspected elsewhere. For example, an inventory of the micro-mammalian fauna of the insular system located at the Atlantic mouth of the English Channel and composed of the large island of Ushant (1560 ha) and the 16 islands of the Molène Archipelago (all less than 100 ha) was performed between 1992 and 2000. Four murid species were recorded, three introduced (*R. rattus*, *R. norvegicus* and *M. musculus*) and one native (*A. sylvaticus*). These four species are present on Ushant, but only one or none of the four on each island in the Molène Archipelago (Pascal 2002). Preliminary results of archaeological research suggest that *A. sylvaticus* had been present on all these islands before invasion by the three other murids. These results suggest that strong interactions occur between these species, leading to replacement if island area is small.

Experimental conditions and our protocol do not allow us to address rigorously the question of the specific consequences of the introduction of the two major alien species, *H. auropunctatus* and *R. rattus*, on the native mammals. Nevertheless, the number of individuals captured of native species was more than three times greater on islands without the mongoose (107) than on islands with the mongoose (33); the number of *R. rattus* captures was one-third higher in the first situation (303) than in the second (196). This general trend suggests that at least one of the alien species has a detrimental effect on the native mammalian fauna, and probably both do.

In either case, our analyses show no statistical difference in *R. rattus* abundance on islands with and without the mongoose, and this result is in accordance with an already large but mostly speculative literature suggesting that, in spite of its reputation as a good rat, the small Indian mongoose does not substantially control introduced *R. rattus*.

Our analyses show that the number of rats trapped during the day on mongoose-free islands exceeded those on mongoose-infested islands. This result accords with the proposed mechanism explaining the poor performance of the mongoose in reducing rat populations (Nellis and Everard 1983) and the shift of rat activity under predation pressure (Fenn and Macdonald 1995). Additionally, as rats become less vulnerable to mongoose predation through modification of their activity time, the mongoose may increase predation pressure on amphibians, reptiles, and poultry (Barun et al. 2010). Our results expand on previous work and show that the mongoose may not only have detrimental effects on native species of conservation concern but may also affect behavior of another introduced species, *R. rattus*, that is a major target species of insular eradication attempts (Howald et al. 2007). Consequences of such interspecific interactions must be taken into consideration in planning eradication operations (Courchamp et al. 2003).

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