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SHORT COMMUNICATION

Establishing systemic rust infections in *Cirsium arvense* in the field

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An existing method to induce systemic rust infections in creeping thistles (*Cirsium arvense*) using an insect vector, the weevil *Ceratapion onopordi*, was successfully applied in the field. Urediniospores of *Puccinia punctiformis* were experimentally transmitted to naturally grown thistles, to our knowledge for the first time resulting in systemically infected shoots in an established thistle population.

Keywords: biological weed control; *Ceratapion* (= *Apion*) *onopordi; Cirsium arvense;* field experiment; *Puccinia punctiformis;* rust fungus; systemic infection

Creeping thistle *Cirsium arvense* (L.) Scop. is a noxious weed that is difficult to control due to its deep-growing and perennial root system (Donald 1994), particularly in areas that are set aside for ecological compensation. Such ecological compensation areas are part of integrated and organic farming systems in Europe, designed to increase the species diversity in agricultural areas. Since the use of chemical and mechanical control measures is restricted in ecological compensation areas, biological control may provide a promising alternative. The biotrophic rust fungus *Puccinia punctiformis* (Str.) Röhl is specific to creeping thistle, having a recognised potential for biocontrol of its host (Watson and Keogh 1980; French and Lightfield 1990; Frantzen 1994b; Thomas, Tworkoski, French and Leather 1994; Völker and Boyle 1994). *Puccinia punctiformis* infects the thistle either locally or systemically (Buller 1950). Local infections only have a marginal influence on the plant's fitness (Kluth, Kruess and Tscharntke 2003), but systemically infected shoots show large growth disturbances (Bailiss and Wilson 1967) and usually die before flowering (Watson and Keogh 1980).

In recent years, systemic infections of the weed *C. arvense* by the rust fungus *P. punctiformis* have been induced in a few experiments. However, no successful attempts to induce systemic infections in the field in naturally grown thistle plants have been reported (Frantzen and Scheepens 1993; Van Leest and Scheepens 1994). In laboratory studies, root pieces of *C. arvense* plants were treated with teliospores, resulting in systemically infected shoots (Van den Ende, Frantzen and Timmers 1987; French and Lightfield 1990; Frantzen and Scheepens 1993; Frantzen and Van der Zwerde 1994). Afterwards, it was shown in several pot experiments and under a variety of conditions that the weevil *Ceratapion* (= *Apion*) *onopordi* Kirby

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(Coleoptera: Apionidae) induces systemic infections in the year following weevil infestation by transmitting spores of the rust fungus to *C. arvense* plants (Friedli and Bacher 2001a,b; Wandeler and Bacher 2006). Furthermore, studies on *P. punctiformis* revealed that neither rust spores nor weevils alone seem to be able to induce systemic rust infections in established *C. arvense* clones (Friedli and Bacher 2001b; Kluth et al. 2003; Wandeler and Bacher 2006). However, until there is a means to increase the systemic rust infection rate in the field, biological control of creeping thistle will not be a realistic option.

When systemic, *P punctiformis* is able to infect new shoots within a clone by internal spread within the horizontal roots. Little is known about the spatial spread of systemic *P. punctiformis* infections in the root system of *C. arvense* (but see Frantzen 1994a,b). However, such knowledge is crucial to estimate the fraction of healthy *C. arvense* shoots that needs to be treated for successful biological control.

In this study, we applied a method that had been successfully tested several times in experiments on potted plants (Friedli and Bacher 2001a,b; Wandeler and Bacher 2006) to infect naturally growing thistles. The aim was to test the basic value of this method for inducing systemic rust infections in the field. In addition, the spatial growth pattern of systemically infected shoots was used to estimate the spread of the rust fungus in the root system.

Study organisms

Ceratapion onopordi is a small oligophagous weevil that lives on several genera in the sub-tribe Carduinae (Zwölfer 1965; Freese 1995). Females lay their eggs in the shoot-base of the plants; the larvae feed inside the shoots above and below ground and pupate in the root collar (Dieckmann 1977). Friedli and Bacher (2001a,b) hypothesised that females of the weevil transmit spores of the rust to healthy plants during oviposition. Once in the stem, the mycelium is thought to enter the root system where it overwinters and infects root buds. In the following spring, systemically infected shoots emerge from such a root system.

Infection experiment

The experiment was conducted in an ecological compensation area of 1.5 ha, 3 km north of Bern (Switzerland). The site was installed 5 years before by sowing a mixture of wild flower seeds (standard mixture, see Pfiffner and Schaffner 2000) and it was dominated by *Dipsacus fullonum*, *Centaurea jacea*, *Leucanthemum vulgare*, grasses and clover species. No rust incidence was found when searching the entire site before the experiment. However, shortly after the start of the experiment in spring 2004, three thistle shoots systemically infected by *P. punctiformis* emerged in the wildflower strip. At all three places, rust-infected shoots grew again in 2005, but these were excluded from the analysis. The mean natural *C. arvense* density was 1.15 shoots per m^2 with a range between 0 and 9 shoots per m^2. We did not find any *C. onopordi* on this site prior to the experiment. Field-collected weevils were kept singly on healthy *C. arvense* leaves for at least 7 days in the laboratory. Only egg-laying females were used for the experiment. Urediniospores of *P. punctiformis* were collected from systemically infected thistle shoots from a site in the Swiss Valais and deep-frozen in airtight plastic vials (volume 1 cm^3) at $-20°C$ for up to 2 weeks until

they were used for the experiment. After urediniospores had been deep-frozen for 2 weeks, still about 30% of them germinated within 24 h at room temperature in a drop of tap water on an object slide in the laboratory (see also Wandeler and Bacher 2006). Spore germination rates directly after harvest are between 50 and 80% (personal observation).

In late April 2004, when the thistle shoots in the field had a maximum height of 14 cm, 60 *C. arvense* shoots with a minimum distance of 1 m from each other were chosen in the wildflower strip. The 60 shoots were randomly assigned to one of two experimental treatments ($N = 30$ shoots per treatment): (1) single females of *C. onopordi* were powdered with urediniospores using a fine brush (in the order of magnitude of about 1000 spores per weevil; see Wandeler and Bacher 2006) and immediately confined to the *C. arvense* shoot by means of a transparent plastic cylinder (10.5 cm diameter, 14 cm height) sealed on top with gauze mesh; and (2) as a control, only a transparent plastic cylinder was placed over the thistle shoot. After 72 h, weevils and cylinders were removed. We did not include a urediniospore-only treatment, because previous experiments showed that urediniospore application alone does not lead to systemic infections in established plants (Friedli and Bacher 2001b). Nor did we include a non-dusted weevil control, because (1) it is difficult to ensure that a weevil is effectively not contaminated with rust spores (we have an outside garden rearing), and (2) without spores no rust infection can result.

From April 2005 to July 2005, the spatial position of all systemically infected plants in the wildflower strip was recorded with the help of a map, an aerial photograph and measuring tapes (50 m) to an accuracy of 5 cm. Each infected shoot was assigned to the nearest experimental shoot. Four different spatial scales were used for determining which infected shoots were included (radii around the treated shoots were 0.3, 1, 2 and 3 m, respectively). Rusted shoots often grow together in patches where many shoots originate from the same thistle shoot (Moravie, Borer and Bacher 2006), and probably originate from the same rust infection. The number of rusted shoots therefore may overestimate successful rust transfer. Instead, we used in our analysis the more conservative measurement of rust presence or absence (rust incidence). We analysed rust incidence (yes/no) around an experimental shoot in dependence of the treatment with an exact logistic regression (LogXact 6, Cytel Software Corporation, Cambridge, MA).

In 2005, we found a total of 51 systemically rust-infected thistle shoots within a 3-m radius around the places where the 60 experimental shoots were growing the year before. Of these 51 shoots, 35 rust-infected shoots were growing within a radius of 1 m around experimental shoots; 34 around weevil-treated shoots and one around control shoots. Within a radius of 1 m, rust incidence was higher around weevil-treated shoots than around control shoots (Table 1): 26.7% of weevil-treated shoots had associated systemically rust-infected shoots compared to 3.3% of the control shoots. The number of rust-infected shoots around treated thistles ranged between 1 and 18. No significant treatment effect was found for the smallest radius of 0.3 m, as only each one rust shoot was growing that close to the experimental shoot in the treatment and control groups, respectively (Table 1 and Figure 1). Above a 1 m radius, the *P* values were also not significant (Table 1), indicating that most rust infections were induced at distances between 30 cm and 1 m around weevil-infested thistles (Figure 1).

Table 1. Effect of treating experimental shoots with weevils and urediniospores on the occurrence of systemically infected shoots within the different radii of 0.3, 1, 2 and 3 m.

Radius		Summary statistics (df = 2)		Parameter estimates	
		Value	P value		P value
0.3 m	Likelihood Ratio	65.64	<0.0001	Constant	0.139
				Treatment	**1.0**
1 m	Likelihood Ratio	39.61	<0.0001	Constant	0.006
				Treatment	**0.026**
2 m	Likelihood Ratio	33.69	<0.0001	Constant	0.005
				Treatment	**0.079**
3 m	Likelihood Ratio	27.02	<0.0001	Constant	0.006
				Treatment	**0.104**

Data were analysed by exact binary logistic regression.

This study demonstrates, to our knowledge for the first time, that systemic rust infections of *C. arvense* can successfully be induced in the field using the weevil *C. onopordi* as vector. A major difference between pot experiments and field studies is the space available for root growth. Friedli and Bacher (2001a) found evidence that already in the first year the growth of the root system of *C. arvense* was limited by the soil volume. Furthermore, the rust-infected shoots in pots were forced to grow in direct vicinity to the treated ones. In the field study reported here, the growth pattern of the systemically infected shoots showed a different pattern: only in two cases infected shoots were growing directly next to treated ones and the majority of rusted shoots appeared at distances between 30 cm and 1 m from treated shoots. However, the mycelium rarely seems to grow distances exceeding 1 m in the horizontal root system (Figure 1). Therefore, the spread of the disease within thistle clones via horizontal root connections appears to be incomplete, as *C. arvense* roots can grow more than 5 m per year (Donald 1994). The restricted fungal growth in combination with the attraction of weevils to thistles growing in the vicinity of rusted shoots
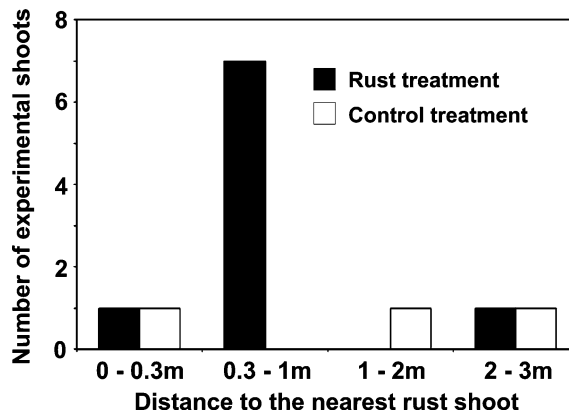

Figure 1. The number of experimental *C. arvense* shoots with the nearest systemically rust-infected shoot growing at different distances. Most of the nearest infected shoots were growing between 0.3 and 1 m away from the experimental one.

(Moravie et al. 2006) creates patches of rusted shoots at places where the rust is already present.

There is still discussion about how systemic infections develop naturally in the field. Our results strongly support the hypothesis that insect-transmitted rust spores are the major source of systemic infections (Friedli and Bacher 2001b; Wandeler and Bacher 2006). By contrast, several authors argue that teliospores infect root buds of *C. arvense* after a passage through the soil (French and Lightfield 1990; Frantzen 1994a). However, infections of root buds by teliospores from local infections would not produce the pattern we found. Systemically infected shoots resulting from external root bud infection by teliospores would have been concentrated close to the treated plants, as deposition of teliospores around rust-infected shoots strongly decreases with increasing distance (Frantzen 1994b). In this study, only two of 51 infected shoots were found at the smallest spatial scale (0.3 m).

The question posed by Frantzen (1994a), how large the fraction of *C. arvense* shoots should be systemically infected to sustainably damage the thistle clone, is still unanswered. Results from a population dynamics model suggest that, at the observed conversion rates of weevil-infested shoots into systemically rust-infected shoots, a larger number of healthy shoots have to be infested by weevils in order to substantially reduce thistle population size (Bacher and Friedli 2002). However, it should be noted that the conversion rate depends on many factors, among others on the weevil's origin and timing of the weevil infestation (Wandeler and Bacher 2006). Likewise, the choice of an aggressive/virulent rust line might increase the conversion rates (Wyss and Müller-Schärer 1999). More studies are needed before more general statements can be made about the level of infection that can be obtained in the field. Nevertheless, this study shows that the induction of systemic rust infection in the field is possible by means of an insect vector. This is an encouraging step towards the development of successful biological control techniques against creeping thistle.

Acknowledgements

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