

Dynamics of a mutualism in a multi-species context

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Despite recent findings that mutualistic interactions between two species may be greatly affected by species external to the mutualism, the implications of such multi-species interactions for the population dynamics of the mutualists are virtually unexplored. In this paper, we ask how the mutualism between the shoot-base boring weevil *Apion onopordi* and the rust fungus *Puccinia punctiformis* is influenced by the dynamics of their shared host plant *Cirsium arvense*, and vice versa. In particular, we hypothesized that the distribution of the weevil's egg load between healthy and rust-infected thistles may regulate the abundance of the mutualists and their host plant. In contrast to our expectations we found that the dynamics of the mutualists are largely determined by the dynamics of their host. This is, to our knowledge, the first demonstration that the dynamics of a mutualism are driven by a third, non-mutualistic species.

Keywords: benefit trade-offs; frequency dependence; herbivory; multitrophic; plant pathogen; regulation

1. INTRODUCTION

The importance of a multi-species perspective for understanding the population dynamics of trophic or competitive systems has often been demonstrated in the past two decades. By contrast, our understanding of the dynamics of mutualism is poor and still largely limited to two-species systems (e.g. Holland & DeAngelis 2001). This is surprising because mutualistic species obviously also interact with species other than their mutualistic partners, often in a way that third species are even essential to the mutualism itself. For example, in the widespread class of protection mutualisms the benefits conferred to the protected partner largely depend on the presence and abundance of their natural enemies (e.g. Agrawal & Fordyce 2000; Morales 2000; Fischer *et al.* 2001). Moreover, herbivores in such mutualisms obligatorily depend on the presence of their host plants. The fact that two-species mutualistic systems may be greatly affected by species external to the mutualism has received more attention only recently (summarized in Bronstein & Barbosa 2002). However, the importance of multi-species interactions for the abundance and population dynamics of the mutualists is virtually unexplored.

In this paper, we develop a framework of interactions between the oligophagous shoot-base boring weevil, *Apion onopordi* Kirby, and the monospecific rust fungus *Puccinia punctiformis* (Str.) Röhl., both parasites of the agricultural weed creeping thistle, *Cirsium arvense* (L.) Scop. *Apion onopordi* larvae develop and pupate in the stem of thistles and adults emerge in late summer. The weevil and the fungus engage in a mutualistic relationship: while female *A. onopordi* spread the plant pathogen, leading to an increase of systemically rust-infected thistle shoots in the year following weevil infestation, weevils benefit from higher adult body weight, fecundity and survival during winter diapause when developing in rust-infected shoots (Friedli & Bacher 2001a; Bacher *et al.* 2002). This

interaction can be classified as a by-product—purloined mutualism (Connor 1995); the weevil purloins benefits by feeding on rust-infected plant tissue and the rust receives by-product benefits from the weevil promoting systemic infections in healthy thistles in the year following weevil attack. Creeping thistle is a clonal plant; shoots die in winter and are regrown from the root system in the spring. While the weevil alone has a limited impact on thistle performance (Friedli & Bacher 2001b), the rust is detrimental to thistle shoots, killing them before flowering and reducing the vegetative growth of the root system (Watson & Keogh 1980; Thomas *et al.* 1994). Because the rust seems to depend to a large extent on insect vectors (Friedli & Bacher 2001a), the mutualistic interaction between the weevil and the rust may greatly affect the dynamics of the shared host plant, *C. arvense*. Vice versa, because both mutualists depend on thistles as hosts, the plant's dynamics will also affect the dynamics of the mutualists.

The distribution of the weevil's egg load over healthy and rust-infected shoots may have a critical impact on the dynamics of this tripartite system, because it determines the number of systemically rust-infected thistle shoots in the following generation, and thus the population size of the shared host plant. The weevil prefers to oviposit into systemically rust-infected over healthy thistle shoots, although both are accepted in the field (Friedli & Bacher 2001a). During the oviposition period females lay approximately one egg per day, independent of the availability of superior and inferior host plants (Friedli & Bacher 2001b). Because it should be easier to locate the preferred host in a population when it is abundant, we hypothesize that the proportion of weevil eggs laid in rust-infected thistle shoots depends on the number and/or relative frequency of rust-infected shoots in the thistle population. There is a trade-off in the benefits of this mutualism for the weevil: ovipositing in systemically infected shoots has a direct advantage for the weevil's offspring (the F_1 generation), but does not spread the rust, thereby leaving the F_2 generation with suboptimal food (Friedli & Bacher 2001a; Bacher *et al.* 2002). However, ovipositing in healthy *C. arvense* shoots increases the num-

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ber of systemically infected shoots in the following season, thereby improving food quality for their offspring's offspring (the F_2 generation), but the female's direct offspring (the F_1 generation) develops in a suboptimal host and suffers fitness reductions. Foraging theory predicts that weevils should deposit their eggs in the optimal (i.e. rusted) host, but that the likelihood of ovipositing in a suboptimal (i.e. healthy) host increases with the number of encounters of healthy thistles before a rust-infested thistle is found (e.g. Courtney *et al.* 1989). Therefore, our second hypothesis is that *A. onopordi*, at the population level, employs a mixed strategy, depositing some eggs in healthy and some in rust-infested shoots, which will ensure persistence of the rust.

In the first part of this paper, we validate and quantify our hypotheses about the weevil's oviposition decisions with field data. In the second part, we develop a simple deterministic model for the growth of creeping thistle clones, incorporating the interaction between the weevil and the rust fungus. With the aid of the model, we investigate if the mutualism is regulated by the interaction between the weevil and the rust alone, and how the dynamics of the plant as a resource for both mutualists modulate the system. The biology of the three species is detailed in Friedli & Bacher (2001a).

2. MATERIAL AND METHODS

Field data on infestation levels of *A. onopordi* and *P. punctiformis* were collected in spring 1998 and 1999 at 42 *C. arvensis* sites in Switzerland and the French Alsace. A site was defined as the total area covered by creeping thistle. Plant population size (defined as the number of shoots) and the proportion of thistle shoots infested with *P. punctiformis* were estimated from five 1 m × 1 m plots at each site. From each site, between 15 and 38 healthy and rust-infested shoots (including the below-ground shoot parts) were arbitrarily collected and dissected for eggs and larvae of *A. onopordi*. The relationship between *A. onopordi* infestation and frequency of rust-infested shoots, thistle population size and shoot density (shoots m⁻²) was established by multiple regression analysis (SYSTAT 1999). Linear multiple regression with backward elimination was performed when investigating the influence of the proportion of rust-infested shoots, thistle population size and density on the number of weevil individuals per thistle shoot (including all shoots of the population) and the proportion of thistle shoots infested by *A. onopordi*. However, there is a theoretical upper limit of 100% in the case of the proportion of thistle shoots infested by *A. onopordi* (i) as the dependent variable. Therefore, in order not to overestimate i at large values of either the proportion of rust-infested shoots or the thistle population size, we carried out a nonlinear least squares regression of the form $i = s(1 - (ae^{-bx}))$ on the variable combinations that proved significant in linear multiple regression analysis. In this function, the dependent variable i asymptotically approaches an upper threshold value s , a and b are positive parameters determining how steep this approach happens, and x is the independent variable (either the proportion of rust-infested shoots or the thistle population size). The parameters s , a and b were estimated with the nonlinear curve fitting function in SYSTAT v. 9.0 (SYSTAT 1999).

3. RESULTS AND DISCUSSION

(a) Frequency dependence

We found the weevil and the rust to be highly associated in the field: in 13 of 42 *C. arvensis* populations examined, both *A. onopordi* and *P. punctiformis* were found; in the other 29 populations neither the weevil nor the rust were observed ($\chi^2_{\text{Yates corrected}} = 37.451$; $p < 0.001$). In linear multiple regression analysis both dependent variables were always significantly dependent on one of the independent variables only. The proportion of thistle shoots infested with *P. punctiformis* had a significant influence on the average number of weevils per shoot in rust-infested ($y = 0.35 + 0.14x$; $r^2 = 0.57$; $p = 0.002$), but not in healthy shoots (figure 1a). By contrast, the average number of weevils per healthy ($y = 0.15 + 0.0002x$; $r^2 = 0.48$; $p = 0.008$), but not per rust-infested thistle shoot was affected by the thistle population size (figure 1b). The proportion of thistle shoots infested by *A. onopordi* increased with the proportion of shoots infested by *P. punctiformis* in both rust-infested (linear regression: $p = 0.003$; nonlinear regression: $a = 0.92$, $b = 19.35$; $s = 1$; $r^2 = 0.78$) and healthy thistle shoots (linear regression: $p = 0.006$; nonlinear regression: $a = 0.76$, $b = 6.84$; $s = 0.4$; $r^2 = 0.45$; figure 1c). The effect of thistle population size on the infestation rate of *A. onopordi* was not significant for either rust-infested or healthy shoots (figure 1d). Shoot density was never significant. Combining the data from figure 1a,c, we calculated the proportion of eggs laid into healthy and rust-infested shoots in dependence of rust frequency (figure 2). At low rust frequencies the majority of eggs were deposited into healthy shoots. The proportion of eggs laid into rust-infested shoots increased as rusted shoots became more abundant. At frequencies of more than ca. 15% of rust-infested shoots in the thistle population, more offspring of *A. onopordi* developed in rust-infested than in healthy shoots. Both our hypotheses stated in § 1 were supported by field data: at the population level, *A. onopordi* indeed employed a mixed strategy at all field sites, depositing some eggs in healthy and some in rust-infested shoots, and the proportion of eggs laid into rust-infested thistle shoots depended on the relative frequency of rust-infested shoots, but not on thistle population size or density. Thus, there seem to be decision rules for the weevil on how to divide its egg load between healthy and rust-infested thistles depending on the relative frequency of rusted shoots.

(b) Regulation of benefits

For both partners, the benefits of the mutualistic association of the weevil and the rust fungus depended on the frequency of the rust in the thistle population. At higher rust frequencies, a larger proportion of *A. onopordi* larvae developed in rusted shoots (figure 2), i.e. the per capita benefit for the weevil increased with increasing rust frequencies. This is supposedly due to the increased ability of weevils to locate their preferred host due to its higher abundance. However, ovipositing in rust-infested shoots does not promote systemically infected shoots in the following season. The benefits for the fungus, however, depend on the number of healthy thistle shoots infested by the weevil. At low frequencies of rust infection, most of the weevil's eggs were deposited into healthy thistle shoots (figure 2). This leads to an increase in the number of rust-

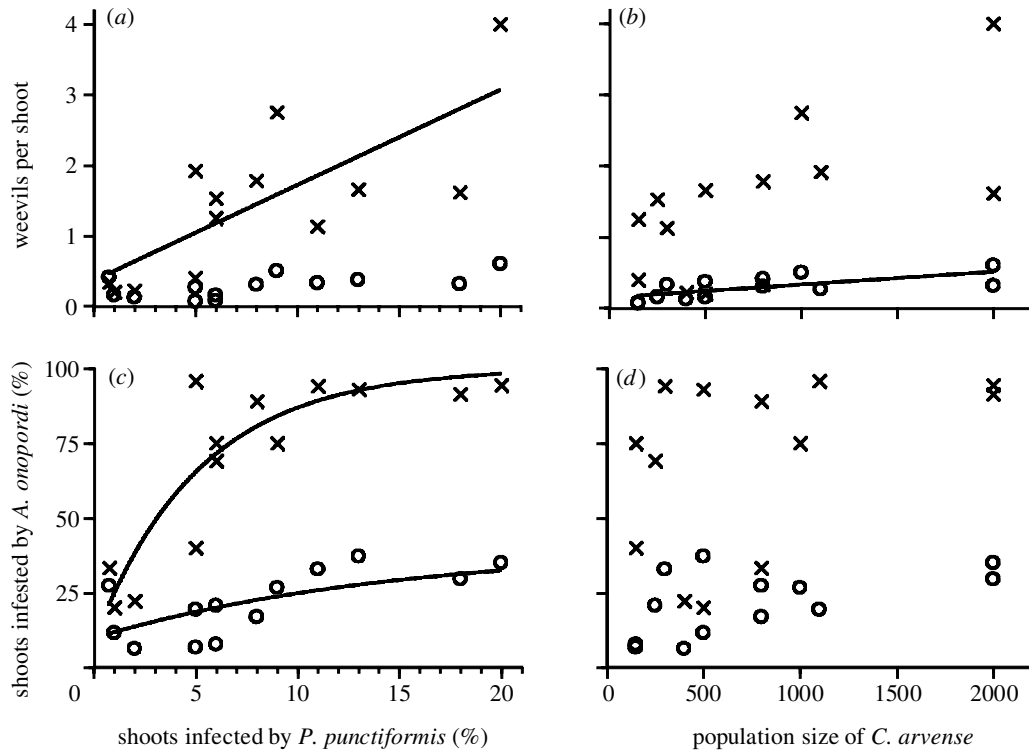


Figure 1. (a–d) Effect of the frequency of thistle (*Cirsium arvense*) shoots systemically infected by rust fungus (*Puccinia punctiformis*) and thistle population size on the average number of weevils (*Apion onopordi*) per shoot and proportion of thistle shoots infested by *A. onopordi* for healthy (circles) and systemically rust-infected (crosses) thistle shoots. Only significant regression curves are plotted (multiple regression analysis; $p < 0.05$).

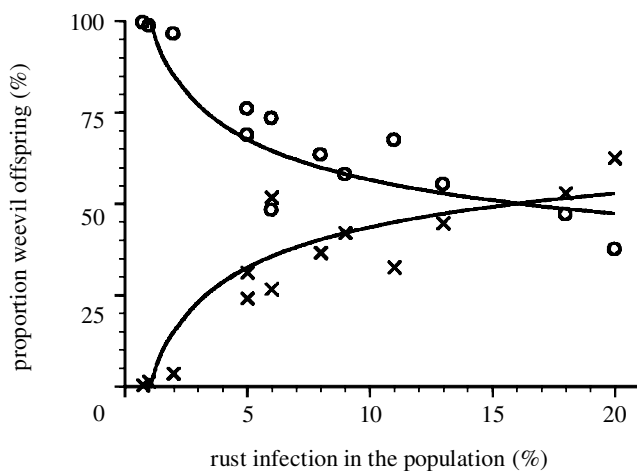


Figure 2. Proportion of weevil eggs laid into healthy (circles) and rust-infected (crosses) shoots in dependence of rust-infected shoots in the thistle population.

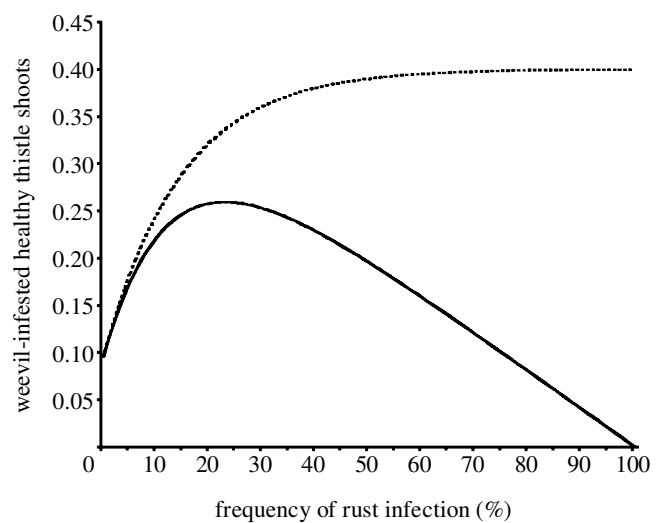


Figure 3. Proportion (i ; dotted line) (see figure 1c) and absolute number ($i^*(1-x)$; solid line) of weevil-infested healthy thistle shoots in dependence of rust frequency. The absolute number of weevil-infested thistle shoots was calculated by multiplying the proportion of weevil-infested shoots at each rust frequency x by the proportion of healthy thistle shoots at that frequency ($1-x$). The absolute number of healthy thistle shoots is expressed as fraction of the total thistle shoot population.

infested thistles in the following year. Although at high rust frequencies a larger proportion of healthy thistle shoots was infested by the weevil (figure 1c), this does not necessarily translate into a higher absolute number of healthy thistles infested, because with increasing rust frequencies the absolute number of healthy shoots decreases (assuming constant thistle abundance). At rust frequencies above 23% of the total thistle population size, the absolute number of healthy shoots infested by the weevil, and therefore the per capita benefit for the rust,

decreased with increasing rust frequency (figure 3). Thus, at high rust frequencies the number of systemically rust-infested thistle shoots in the following year will decrease. Considering only the interaction between the mutualists it

appears that the partition of the weevil's egg load between healthy and rust-infected thistle shoots and the associated benefit trade-offs are capable of regulating the system. However, including the growth pattern of creeping thistle may change this conclusion.

(c) Population model

In order to explore the effect of the weevil's oviposition decisions on the dynamics of the host plant *C. arvensis* mediated by the dynamics of systemic rust infections, we built a deterministic population dynamics model of a creeping thistle clone which incorporates weevil-induced rust infections. Strictly speaking, the word 'population' does not apply to the growth of a clone; however, the principle remains the same as in true populations composed of individuals. Individuals in our model refer to the modular parts of the clone, the ramets (a ramet is a shoot and its connected root part). In a first model, we assume that, in the absence of the rust, a clone of creeping thistle grows vegetatively (we ignore sexual reproduction because it hardly has a role in the growth of established clones) at an exponential rate until it reaches the carrying capacity K of the site which is determined by the dimensions of the site, the resource availability, herbivore pressure and the competing vegetation. Because the above-ground thistle parts die in the autumn and are regrown in spring from the root system, we assume discrete thistle population growth in which the number of thistle ramets N in generation $t + 1$ is determined by the number of ramets in generation t according to the difference equation

$$N_{t+1} = \left(rN_t \left(1 - \frac{N_t}{K} \right) + N_t \right), \quad (3.1)$$

where r is the maximum growth rate of the ramets in that particular environment. If we introduce the thistle rust *P. punctiformis* to the plant population, the number of thistle ramets alive in a generation is diminished by the number of systemically rust-infected ramets M in the same generation.

$$N_{t+1} = \left(rN_t \left(1 - \frac{N_t}{K} \right) + N_t \right) - M_{t+1}. \quad (3.2)$$

Müller (1976) and Watson & Keogh (1980) estimated that the impact of a rust-infected ramet is larger than only the loss of this one ramet to the clone, due to the attempt of the clone to compensate for the loss by reallocation of nutrients from healthy to rust-infected ramets, thereby reducing the vegetative growth of healthy ramets. It is also known from other plant pathogens that infected plant parts constitute a sink of resources that impedes the growth of the entire plant (Hatcher 1995). Thus, a rust-infected thistle ramet can be considered as a sink of nutrients. We introduced a factor f to quantify the impact of an infected ramet on the number of reproductive ramets in a thistle clone.

$$N_{t+1} = \left(rN_t \left(1 - \frac{N_t}{K} \right) + N_t \right) - M_{t+1}f. \quad (3.3)$$

If $f = 1$, then the impact of a systemically infected ramet on the clone is equal to its loss; if $f > 1$, then a rust-infected ramet impairs the growth of other ramets. For example, if $f = 2$ then a systemically rust-infected ramet

not only dies but prevents the growth of another ramet that would have been grown without rust infection.

We further assume that the rust cannot induce systemic infections on its own, and that all systemically infected ramets are caused by weevil-mining in the previous year (Friedli & Bacher 2001a,b). Under this assumption, we can calculate the number of systemically infected ramets in the thistle population as

$$M_{t+1} = N_t i_t c, \quad (3.4)$$

where i_t is the proportion of healthy thistle ramets infested by *A. onopordi* in the previous year and c the proportion of weevil-infested ramets that are converted into systemically rust-infected ramets in the next generation. i_t can be estimated from field data and depends on the frequency of systemically infected thistle ramets in the *C. arvensis* population (figure 1c, lower graph), but is independent of the plant population size (figure 1d):

$$i_t = s \left(1 - \left(0.76e^{-6.84 \left(\frac{M_t}{M_t + N_t} \right)} \right) \right), \quad (3.5)$$

with s representing the maximum proportion of healthy thistle ramets infested by *A. onopordi* ($0 \leq s \leq 1$). The proportion of weevil-infested ramets that are converted into systemically rust-infected ramets in the following generation c may vary between zero and any positive value. For example, $c = 1$ would indicate that for every weevil-infested shoot a systemically rust-infected shoot is produced in the following season. Larger values of c would signify that a weevil-infested shoot produces c rust-infected shoots in the following growth season. In summary, the total number of systemically rust-infected thistle shoots is therefore given by

$$M_{t+1} = N_t s \left(1 - \left(0.76e^{-6.84 \left(\frac{M_t}{M_t + N_t} \right)} \right) \right) c. \quad (3.6)$$

The total number of thistle ramets T in the population at time t is

$$T_t = N_t + M_t. \quad (3.7)$$

Although the model explicitly considers only the dynamics of the host plant and the rust, the population dynamics of the weevil is implicitly incorporated and can be calculated by multiplying the number of healthy and rusted thistles in each generation by their corresponding weevil infestation rates (figure 1). The model assumes that weevil and rust populations persist as long as healthy thistles are present. We ran the model varying the parameters c , f , r , and s over a wide but biologically meaningful range (c : 0, 0.5, 1, 1.5, 2; f : 1, 1.5, 2; r : 0.5, 1, 1.5, 2; s : 0, 0.1, 0.2, ..., 1). With each parameter combination the model was run until either an equilibrium thistle population size $T^* = \text{constant} > 0$ was reached or the thistle population became extinct. If an equilibrium was reached we calculated the proportion of the carrying capacity of the thistle population realized (% K) under the conditions given by the parameter combination at the equilibrium population size as fraction of the carrying capacity without rust (% $K = T^*/K$). We modelled two different starting conditions: introducing the pathogen (i) to a thistle population that had reached the carrying capacity of the site, and (ii) to an expanding population (starting thistle popu-

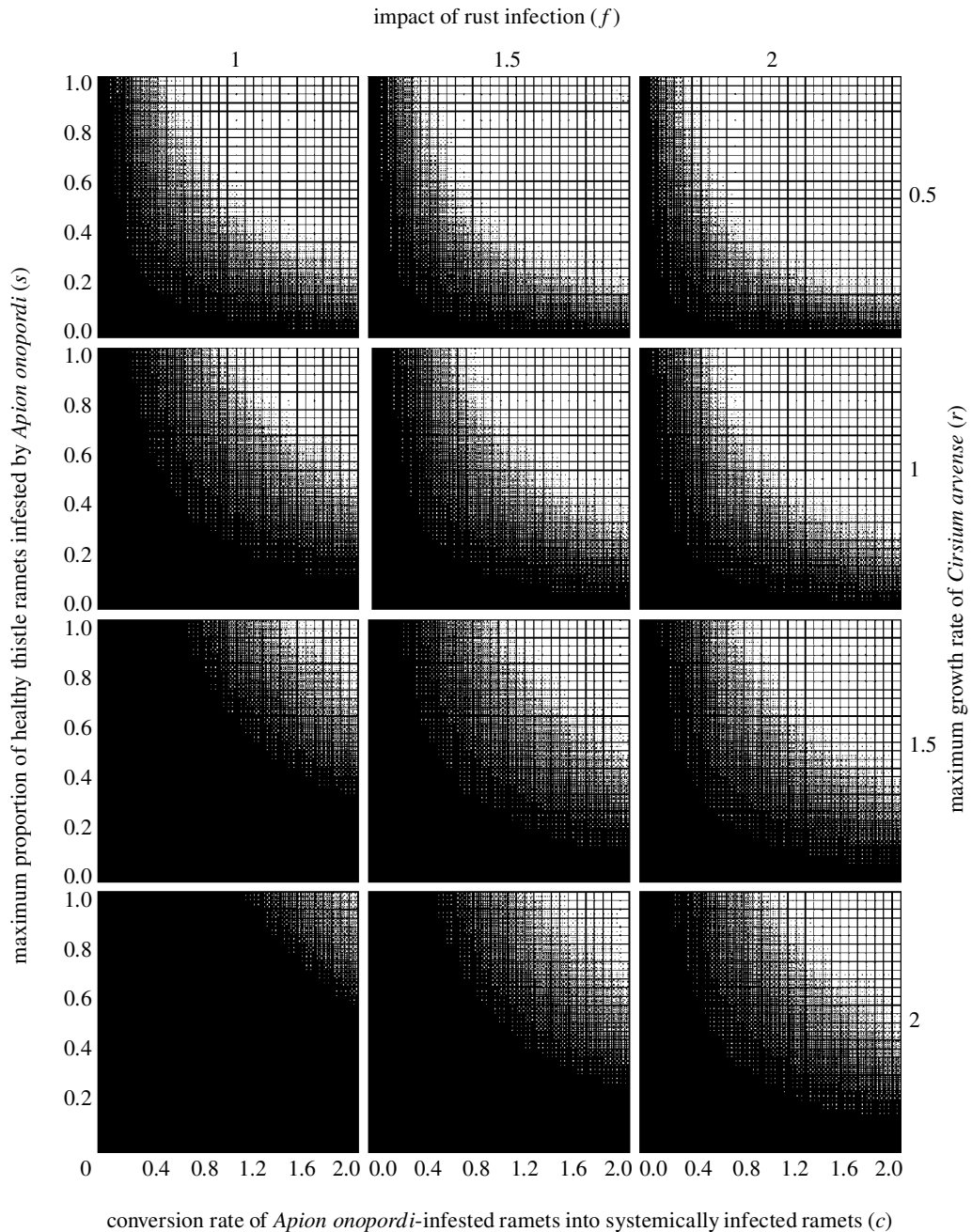


Figure 4. Outcome of thistle growth running the population model varying the parameters c , s , f and r expressed as proportion of the carrying capacity (% K). The model produces the whole range of outcomes from extinction (white, % K = 0) over stable equilibria (grey shades, % K = constant > 0) up to % K \leq K , the carrying capacity for the thistle population (black).

lation size = 0.1 K). In a second model, we allowed unlimited thistle population growth in order to evaluate the influence of the form of the host plant dynamics on the dynamics of the tripartite system:

$$N_{t+1} = (rN_t + N_t) - M_{t+1}f. \tag{3.8}$$

In the second model we only recorded whether or not an equilibrium was reached.

(d) Dynamics of the three-species interaction

In the first model, neither the starting thistle population size nor the carrying capacity had an influence on the proportion of the carrying capacity of the thistle population realized at the equilibrium (% K). This indicates that the system is well balanced and tolerates even severe disturb-

ances. With the above-mentioned parameter combinations the model produced the whole range of outcomes from extinction of the thistle clone (and the associated extinction of the mutualists) via stable equilibria to no effect of rust infection on realized population size (figure 4). At high growth rates of *C. arvense* ($r = 2$) and a limited impact of systemically infected ramets on the thistle clone ($f = 1$), rust infection can even lead to a stable equilibrium of up to 12% higher than the carrying capacity K . In general, the larger f (the impact of an infected ramet on the number of reproductive ramets in a thistle clone), c (the probability that a weevil-infested thistle ramet is converted into a systemically rust-infected ramet in the next generation) and s (the maximum infestation rate of healthy thistle ramets by *A. onopordi*), the lower was the proportion of the carry-

Table 1. Estimates for the model parameters.

parameter	estimate	source	reliability
<i>r</i>	ca. 2	Frantzen (1994)	uncertain, only one estimate from one field site
<i>s</i>	0.4	figure 1c	based on estimates from 13 natural populations
<i>c</i>	0.12–0.80	Friedli & Bacher (2001a,b)	variable, seems to depend on surrounding vegetation
<i>f</i>	> 1	Müller (1976)	not quantified

ing capacity of the thistle population realized at the equilibrium (%*K*). By contrast, the larger *r* (the intrinsic rate of increase of a thistle clone in a particular environment) the higher was %*K* (figure 4). Using field estimates for the model parameters (table 1), the model predicts that thistle populations should not be at all reduced by the weevil spreading the rust (see coordinates in figure 4). Although thistle population dynamics in the field cannot be accurately predicted by the model, it indicates that the efficiency of the weevil spreading the rust may be low and that the plant's growth rate compensates for losses inflicted by the rust. This may help to explain the fact that the rust rarely reaches outbreak densities in the field that drive its host, creeping thistle, towards extinction (e.g. Frantzen 1994) and thus so far has remained unsuccessful as a biocontrol agent (Julien 1998). The importance of the plant's growth pattern in driving the dynamics of the system is emphasized by the second population model. If we allowed unlimited thistle population growth, all three species either grew indefinitely or became extinct, depending on parameter values. An equilibrium population size was never reached. This indicates that the mutualistic interaction between the weevil and the rust alone cannot regulate the system. By contrast, the dynamics of the resource largely determined the dynamics of the mutualists in both models. This is, to our knowledge, the first demonstration that the dynamics of a mutualism are driven by a third, non-mutualistic species. Our results indicate that major determinants of the dynamics of mutualism may not be the mutualists themselves and that the dynamics of this and other mutualisms can only be understood in a multi-species context.

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