

Population biology of plants infected by systemic pathogens

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Population biology of plants infected by systemic pathogens

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracoval samostatně a že jsem uvedl všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Abstract

Three separate studies of the effect of plant pathogen on population biology of its host are presented in the thesis. Two are using field data about a widespread system of plant *Falcaria vulgaris* and its systemic rust fungus *Puccinia sii-falcariae*.

The first study shows, that the disease prevalences in 40 populations of the plant were correlated with the moisture, the soil reaction and the cover of the herb layer at the localities. This was probably a result of the interaction of the life history of the plant and different effect the disease has at various localities.

Similar pattern was found in the second study that aimed to determine long-term effect of the disease at the population level at four chosen localities over 4-5 years. Population growth rates were only rarely predicted to be higher for the healthy part of the population when compared with the whole population. Other analyses have however found big differences among years and localities. The locality type (slope vs. field populations) was important factor influencing population stage composition and importance of life cycle transitions for the growth of the population.

Finally, the last study explores the possibility that systemic infection in clonal plants might be able to select against clonality. The result of the modelling showed that more integrated plants cope better with the spread of disease than the less integrated plants. The study also shows that the potential for selection against clonality is further restricted by limited range of suitable infection rates.

The thesis as a whole demonstrates that both population biology methods and modelling can answer some of the important questions of ecology of plant pathogens and their hosts.

Abstrakt

Tato disertační práce prezentuje tři práce o vlivu systemických chorob na populační biologii rostlin. První dvě se zabývají analýzou terénních dat o široce rozšířeného systému srpku obecného (*Falcaria vulgaris*) a jeho systemické rzi *Puccinia sii-falcariae*. První studie se zabývá vlivem prostředí na prevalenci choroby na krajinné úrovni. Pomocí dat ze 40ti populací ve třech regionech bylo zjištěno, že prevalence choroby koreluje s vyšší vlhkostí a půdní reakcí (měřeno pomocí Ellenbergových indikačních hodnot) a dále vzrůstá se zvyšující se pokryvností bylin. Tento výsledek je pravděpodobně způsoben vyšším přežíváním infikovaných rostlin na stepních lokalitách, kde je půda tvořena vlhkými vápnitými jíly a slíny.

Druhá práce zkoumá do hloubky životní cyklus *Falcaria vulgaris* za pomoci dat ze čtyř lokalit sbíraných po 4-5 let. Použité maticové modely sloužily ke srovnání základních populačních charakteristik nakažených populací s teoretickými populacemi, které by rostly na stejném místě a byly složeny pouze ze zdravých rostlin. Další analýzy však ukázaly, že existují významné rozdíly mezi lety a populacemi. Typ populace (na slínovcových svazích vs. mezi poli) se ukázal být zásadním faktorem určujícím jak velikostní složení populace, tak důležitost různých životních fází pro přežívání populace.

Konečně třetí práce měla za cíl zjistit, zda systemické infekce mohou nebo mohly vést k selekci proti klonalitě u rostlin. Výsledky získané pomocí prostorově explicitního modelu klonálního růstu ukazují, že oproti očekávání více integrované klonální rostliny se se systemickou infekcí vyrovnávají obecně lépe, než rostliny s nepropojenými oddenkovými systémy. Model dále ukazuje, že prostor pro výraznou selekci proti klonalitě je pouze v úzkém rozmezí hodnot míry nakažlivosti.

Disertační práce jako celek ukazuje, že tradiční metody terénního výzkumu a modelování populační biologie rostlin lze použít k zodpovězení zajímavých otázek týkajících se vztahu rostlin a jejich patogenů.

Introduction

Plant pathogens play important role in the dynamics and evolution of natural plant populations. They are able to cause mortality, change competitive ability, decrease fitness and change spatial structure of their hosts' populations. At the same time, they can affect their hosts' genetic structure and evolutionary processes through selection for resistance or tolerance of the disease. The outcome of the interaction of a host plant population and a pathogen largely depends on the chosen spatial and temporal scale, on life histories of both the host plant and the pathogen and on external factors such as environmental conditions and their variability. This thesis concerns three of these effects in three separate papers. The first two deal with one particular plant-pathogen system, its interaction with environmental factors and quantification of the population level effect of the disease. The third paper explores possible effects of diseases on evolution in clonal plants.

Plant pathogens and their effects on host plants

There are many causal agents of plant diseases – the most notable are viruses, bacteria, phytoplasmas, oomycetes, nematodes and fungi (Agrios 1997). The environmental factors themselves can also cause plant damage but in natural systems they mostly act only as a predisposition influencing the relationship between the host plant and the pathogen. The viruses and their importance for plant populations were mentioned already by Harper (1977) in his basic population biology textbook but they were mostly studied in crops until recently so our knowledge of viruses in wild plants is limited (Wren et al 2006). Recent works suggest that the viruses are certainly not only simple agent causing agricultural losses because there is considerable diversity of asymptomatic or even mutualistic viruses waiting to be uncovered in natural ecosystems (Roossinck et al 2010; Roossinck 2011).

From the rest of the causal agents the fungi are the most widespread and studied and they possess great scale of life-strategies (Burdon 1987; Alexander 1992). These range from aggressive necrotrophic, soil-borne or canker and wilt diseases on one side over biotrophic foliar pathogens till sexually transmitted floral pathogens and various systemic pathogens on the other side (Jarosz and Davelos 1995). The most studied and species abundant groups are the rusts, anther smuts and smuts (three separate groups within phylum *Basidiomycota*). The rusts typically produce various kinds of spores that are employed in specific parts of the disease life cycle (Petersen 1974). The complexity of the life cycle is determined by the

number of hosts (one – autoecic or two – heteroecic) and by number of spore types that were given up in evolution. As the thesis uses rust fungus as the pathogen of interest, overview of the cycle is given here with generally used symbols. The spermatia (0) are sexual stage that fertilizes unrelated receptive hyphae in spring within the first host. Aecia (I) are formed consequently producing aeciospores that infect the other host species. Within the second host plant, uredospores (II) are formed infecting new individuals of the same species. At the end of the season, teliospores (III) are produced serving as overwintering stage, which in the spring germinates into basidia. The basidiospores (IV) then infect the first host species individuals thus completing the cycle. The macrocyclic (complete) rust cycle can be shortened by omitting uredospores (demicyclic rusts) or by omitting all stages but teliospores and basidiospores (microcyclic rusts). Other variants are possible but not very common. The autoecic rusts complete all stages within one species.

Finally, there is an important group of fungi that are in facultatively or obligately mutualistic relationship with their hosts (Clay 1988) often recruited from group of *Clavicipitaceae* endophytes but with representatives among rust fungi as well (Wennstrom and Ericson 1991).

Major distinction among different pathogens is whether they only exploit the host locally (non-systemic pathogens) or whether they can grow through the plant tissues and are able to occupy the whole host plant individual (systemic pathogens). This distinction to large extent correlates with aggressiveness as the non-systemic pathogens rely on resource limited in time and space leading to strategy of fast exploitation and reproduction. The systemic pathogens can spread their reproduction in time, which may lead to lower aggressiveness to avoid death of the host (Jarosz and Davelos 1995; Gilbert 2002). Within the group of systemic pathogens, the result of the relationship depends on other factors as well, notably on growth pattern of the host. The species with extensive clonal growth tend to suffer from higher disease incidence rate and severity than species with no or weak lateral growth (Wennstrom 1994). This is probably caused by the ability of the clonal plants to outgrow the infection and fragment into independent ramets. Such defence reaction might favour aggressive strains of the pathogen because if they cause mortality of the affected ramet, the genet still persists and multiplies the susceptible genotype, which are perfect substrate for the growth of the specific strain.

The thesis focuses on systemic pathogens because in the long-term there is greater capacity for coevolution of the pathogen and the host plant and emergence of interesting patterns of behaviour. All three papers deal with systemic pathogen; the first two a specific

example of autoecic rust *Puccinia sii-falcariae* on *Falcaria vulgaris* while the third, modelling paper, uses simulated systemic disease with attributes resembling viral pathogen (fast growth and simple resource-reducing effects).

Plant pathogens and environmental conditions

As already mentioned, the environmental factors affect the host plant, the pathogen and their interaction. This is commonly referred to as the disease triangle (Agrios 1997), which represents the fact that for successful infection of the host there must be both the pathogen and the favourable environmental conditions present. For most of the fungi, humidity and temperature are the important factors influencing growth of the hyphae and sporulation (Colhoun 1973). In biotrophic fungi such as in the rusts, the successful infection of the host is dependent on presence of humidity on the leaves (Mendgen and Hahn 2002) with the exception of powdery mildews that germinate better in dry conditions (Bushnell 2002).

Besides of the direct effect on the pathogen, environmental conditions affect the host plant as well leading to well known results e.g. drought, waterlogging, frost, high temperature damage (Harper 1977). Yet even if the effect of the environment is not detrimental by itself, exposure to some factors can make the host more vulnerable for the pathogen. The effect of predisposition of the host to the disease is known to be caused by e.g. moisture (Warren and Mordecai 2010), temperature, light and nutrient availability (Colhoun 1973). All the listed effects can finally interact with life histories of both the host and the pathogen (Wennstrom and Ericson 1992; Wennstrom and Hagner 1999; Barrett et al 2008). In the first and second paper of thesis, one can see that environmental conditions such as moisture availability and soil type can affect life-history rates (mortality, reproduction, etc.) of the host leading to changes in population structure and prevalences of the disease in the populations.

Evolutionary consequences of plant diseases

The plant-pathogen relationships in wild plants are more thoroughly studied since circa 1980 with the basic textbook written by Burdon in 1987. Since then several important topics have been predominantly studied. The most important is the race-specific resistance variation within and among populations and its implications for disease epidemics (e.g. Thrall and Burdon 2000; Ericson and Burdon 2009). The studies most often show that for pathogens with epidemic kind of dynamics the resistance structure of the populations is important factor in determining disease incidence. The resistance is commonly divided into race non-specific resistance manifested in form of traits leading to lowering risk of infection and resistance race

specific (Burdon et al 1996). The latter is defined as ability of particular genotype of plant hosting a resistance gene to recognize the complementary avirulence gene of the pathogen and react with hypersensitive reaction ('gene-for-gene concept' – Flor 1955, 1971). Such reaction effectively suppresses any fungal growth of the pathogen within the host plant. However, this kind of resistance is typically studied in epidemic non-systemic pathogens. Burdon (1996) indeed proposes that race-specific resistance should be more important in populations (and metapopulations) of plants that cause substantial reduction in fitness of their host plants and that show epidemic rather than endemic dynamics.

Roy and Kirchner (2000) have proposed a general concept of resistance as a strategy of plant to limit infection as opposed to tolerance, which is a strategy of the host to lower the fitness effects of the disease after successful infection occurs. Roy and Kirchner show in their study that both tolerance and resistance are present within set of populations infected by various rusts as compiled from literature. In paper II. of the thesis, model system of *Falcaria vulgaris* and its often highly prevalent systemic rust fungus are studied. The results generally indicate that this particular disease is only rarely important factor in population dynamics suggesting that the system might have evolved towards the low-aggressiveness of the disease and/or tolerance strategy of the host.

In clonal plants, the systemic fungal infection is able to grow through the plant along the vascular vessels (Mendgen and Hahn 2002) and viral particles can spread in the vascular system (Cheng et al 2000). Both cases can eventually lead to infection of the whole interconnected genet. This poses a significant threat to the particular plant genotype as the uniform vegetative progeny can be easily accessed by the pathogen. It has been proposed a few times (Frantzen 1994; Stuefer et al 2004) that the systemic diseases might have lead to evolution of early genet fragmentation (as a sort of resistance sensu Roy and Kirchner 2000). This possibility is however hard to prove as one would need to compare clonal and non-clonal populations of the same species and their fitness under pressure of the pathogen. Because there is no such system known to us, the hypothesis can be only tested by modelling approach. This is thoroughly addressed in the Paper III.

Presentation of the papers

Paper I.

Are environmental factors influencing the prevalence of rust in pathosystem *Falcaria vulgaris–Pucinia sii-falcariae*?

This study aimed to test the hypothesis that environmental factors influence presence of the disease in populations of *Falcaria vulgaris* infected by its systemic rust *Puccinia sii-falcariae*. Data were collected for 40 populations in three regions in central Bohemia; the populations largely differed in prevalence. For each locality, vegetation data were collected and used for computing the Ellenberg indicator values (EIVs) for light, temperature, continentality, moisture, soil reaction and nutrients; further data characterizing the locality were collected as well. The regression analysis has shown that disease prevalence positively correlates with EIVs for soil reaction and moisture and with herb cover. The prevalences were further influenced by region but there was no effect of population size. The results suggest that populations on the slopes that grow on deep moist calcareous soils have higher prevalences – this might have been caused by greater probability of infection or lower probability of mortality of the infected plants. Low prevalences were common at dry and/or acidic localities on sandy soils.

Paper II.

Low effect of pathogen on population characteristics in pathosystem *Falcaria vulgaris*–*Pucinia sii-falcariae*: matrix model results

This study was conducted to find out how important is the effect of rust fungus disease at the population level in perennial plant *Falcaria vulgaris*. Matrix models were used for determining population characteristics like population growth rate, stable stage distributions and importance of the stages (their elasticities). Data on healthy and systemically infected plants in four populations over 4-5 years were used to calculate population characteristics for both complete populations and for only the healthy plants and their transitions. Differences between field and slope populations were compared. The growth rate of the healthy part of the population was significantly larger only in one case. Deterministic growth rates differed between population types with field populations growing in size and slope populations being around equilibrium or dropping in size. The slope populations had balanced amounts of plants projected in healthy and in infected stages, whereas field populations had most plants projected in the healthy stages. All populations relied on survival in healthy stages but for the slope populations stasis in infected stages was also important. For the field populations

growth and reproduction had the highest elasticity. The systemic infection was found to be only temporary in some cases, which was not known before.

Paper III.

Effect of systemic diseases on clonal integration: modelling approach

In this study, we used a spatially explicit model of clonal growth with disease spread implemented to test the hypothesis that systemic disease decreases the competitive ability of highly integrated clonal plants (integrators) when compared to less integrated plants (splitters) with the same parameters. In contrast to our expectations, the integrator was competitively stronger than the splitter in most cases and it lost only when the disease severity and infection rates were very high. Even a very small amount of resource sharing greatly increased the relative success of the integrator and larger integrators were competitively stronger than the smaller ones. Our results also indicate that although the same infection rate caused more systemic disease in the integrator than in the splitter population, the disease has only a limited potential to select for the splitter strategy.

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Are environmental factors influencing the prevalence of rust in pathosystem *Falcaria vulgaris – Puccinia sii-falcariae*?

Tomáš Koubek

Abstract

Environmental factors can influence both plant pathogens and their hosts. This study aimed to find out if environmental factors could be used to explain highly variable prevalence in populations of *Falcaria vulgaris* frequently infected by its systemic rust *Puccinia sii-falcariae*.

The data were collected for 40 populations in three regions in central Bohemia. The populations differed in prevalence from zero to 88% systemically infected plants and in size by three orders (12 to approx. 13000 individuals). For each locality, vegetation data were collected and used for computing of Ellenberg indicator values for light, temperature, continentality, moisture, soil reaction and nutrients; further data characterizing the locality were collected as well. The regression has shown that disease prevalence positively correlates with EIVs for soil reaction and moisture and with herb cover. The prevalences were further influenced by region but there was no effect of population size.

The results suggest that populations on the slopes that grow on deep moist calcareous soils have higher prevalences – this might be caused by greater probability of infection or lower probability of mortality of the infected plants. Low prevalences were common at dry and/or acidic localities on sandy soils. The fact that there was no correlation with population size is discussed.

Introduction

Plant epidemiology has always been of major interest to people because the impact of pathogens on crops directly affected their living. Interestingly the effects that weather has on disease development were known long before the causal agents were discovered (Colhoun 1973). When the fungi, bacteria, viruses and other organisms were determined as the cause of diseases of crops (de Bary 1861), it still took a long time to disentangle the effects of the environment (Jones 1924; Colhoun 1973; Coakley 1988) and genetic interplay between host and pathogen (Flor 1971; Thompson and Burdon 1992; Alexander et al 1996). Even with modern chemical and genetic methods of disease prevention and amelioration, the environmental factors still play important role in crop diseases epidemiology (Agrios 1997). Their impact could be also further altered with potential climate change (Eastburn et al 2011).

In contrast to the crops, the pathology of wild plants was less researched until the rise of population biology disciplines. Harper (1977) mentioned importance of including pathogens in plant population studies but the real interest started only after Burdon (1987) wrote his basic book on the subject. It facilitated research of plant pathology in a way that was either unknown or reserved for animal epidemiology only. Studies of sexually transmitted diseases in plants (Antonovics and Alexander 1992; Wennstrom and Ericson 2003), race specific resistance and coevolution of plant and pathogen (Frank 1992; Burdon et al 1996; Burdon et al 2002), temporal and spatial aspects of epidemiology including metapopulation dynamics (Burdon et al 1995; Thrall and Burdon 2003), use of fungi as biocontrol agents (Shishkoff and Bruckart 1996; Kluth et al 2003) or even positive effects of infection like mutualistic relationships of endophytes and grasses (Clay 1988) can be named as examples of the diverse array of subjects. Still, when talking about general population characteristics like disease incidence rate (proportion of newly infected plants) and prevalence (proportion of infected plants) the environmental variables interact in their effect with genetic factors to a large degree (Jarosz and Burdon 1988; Price et al 2004) although sometimes the genetic factors simply prevail (Meijer and Leuchtmann 2000).

Environmental factors can be important because they directly affect growth and reproduction of the host and the pathogen. The most important ones are: moisture acting as predisposition of the host health (Blodgett et al 1997; Warren and Mordecai 2010) or directly by facilitating fungal growth and penetration on the leaf surface (Huber and Gillespie 1992), temperature directly affecting fungal growth (Traperocasas and Kaiser 1992) and soil reaction that can influence belowground fungal pathogens (Myers and Campbell 1985). Light and

nutrient availability (or absence thereof) can predispose the host and make it more prone to infection (Colhoun 1973). These factors mostly act on the local scale while on the regional scale spatial arrangement of populations becomes important. Species traits like mobility and genetic structure of both pathogen and host are of importance at the regional scale (Laine 2004) and metapopulation structure of the pathogen is often shaping local disease prevalence (Burdon 1993; Thrall and Burdon 2003). The life history of the host is also one of the important factors and it interacts both with genetic and environmental factors (Barrett et al 2008). For example plants with strong lateral clonal growth tend to be hosts of pathogens with higher infection rate and disease fluctuations than non-clonal plants (Wennstrom 1994) and this can interact further e.g. with nutrient availability (Wennstrom 1999).

Yet from the practical point of view of population biology the aforementioned findings are often employed only by taking pathogen as a basic factor correlated with e.g. population size or densities of the host (Lienert and Fischer 2003; Colling and Matthies 2004). This can bring valuable insights into the population studies but it also may lead to simplistic explanations of the correlation if additional information is not available. When looking for reasonable data on interaction of plants and pathogens one should employ not only basic population characteristics but also some measure of spatial and environmental factors.

I started a study of a widespread but previously unresearched pathosystem *Falcaria vulgaris-Puccinia sii-falcariae.* It has locally high prevalence of the pathogen but in some places the rust is completely missing. This is even more striking because the disease did not seem to be locally abundant in particular area but the more and less diseased populations were intermingled. I wanted to connect the prevalence data not only with population size but also with other cues that would reveal more about the underlying processes. Direct measurements of environmental variables can be costly and/or take a long time to collect so I used indirect method of indicator values that builds on the fact that plant species specialize into living in distinct range of values of a particular factor (niche; Silvertown 2004). The most used and tested technique in central Europe is the method of Ellenberg, who tabulated values of environmental and vegetation factors for Central European flora (Ellenberg 1974; Ellenberg et al 1992). By collecting vegetation data and averaging the values for the individual species one can get very informative indicators if one can overcome the potential pitfalls (Ertsen et al 1998; Schaffers and Sýkora 2000; Wamelink et al 2002).

In this study I aimed to explain pattern of the disease prevalence with (i) Ellenberg indicator values as proxies for environmental data, (ii) population size and (iii) vegetation

characteristics such as plant cover, diversity and vegetation data that can characterize biotic interactions at the locality.

Methods

Study species

The studied plant was *Falcaria vulgaris* Bernh. (*Apiaceae*). It is a common species of dry grasslands and road verges of warm regions in central Europe. The original area of distribution spreads from Spain in the west to Near East in the south, central Sweden in the north and central Asia in the east (Meusel et al 1992). It is considered invasive in several parts of the world including USA (Korman et al 2010).

It has narrow leaves that form one or several rosettes that grow newly each year from the head of a substantial taproot. The taproot can split or produce multiple heads in disturbed habitats. The flowering stalk is between 40 and 90 cm high, usually branched with umbels forming hemispheric compound umbels. In autumn, the whole inflorescences can be released allowing a tumbleweed kind of seed dispersal within the locality. The seeds are small, formed in large amounts (several hundreds to several thousands per plant in studied populations, unpubl. data) with low numbers in only some exceptional years. Germination is generally low in the field but if successful, the seedlings grow very fast and root deep to reach for water (Ellenberg and Snoy 1957). The plant size varies considerably under different environmental conditions ranging from small non-flowering plants in dry conditions to large plants forming expansive populations in resource rich mesic habitats (pers. obs.).

The pathogen of interest was rust fungus *Puccinia sii-falcariae* J. Schröt. (*Pucciniaceae*) specific to *Falcaria vulgaris*. Some authors merge the species into larger, morphologically defined species *Puccinia bulbocastani* and regard the former species as *forma specialis* (Kokeš, personal information). The species is demicyclic (lacks uredia) and autoecious (has only one host plant). The infection is systemic and infected plants are recognizable at all stages. In early April, first leaves of infected host emerge early, grow upright and are fragile and etiolated compared to healthy plants, which have tougher leaves more or less horizontally or diagonally oriented. Orange spermogonia are formed soon after leaves emerge producing smelling nectar to attract insects (mostly flies) to fertilize the receptive hyphae with unrelated spermatia. In May, aecia develop predominantly on the underside of the leaf with occasional outbreak on the upper side. Although the aecial discs are generally larger than the spermogonia they usually cover smaller area of the leaf. The systemic infection later leads to

general leaf damage resulting in brown spots and premature leaf drying. In July the last stage of the fungal cycle – telia – appears as sparse black dots. The telial stage can be missing on systemically infected plants and can also appear on otherwise healthy plants that don't host any of the previous stages. This is usually considered outcome of successful infection by aeciospores (Marková, pers. communication) that may but may not necessarily lead to systemic infection. The cycle is finished in spring as the overwintering teliospores germinate into basidiospores infecting healthy plants. The differences in infection rates for the two kinds of spores (aeciospores and basidiospores) are however unknown. The pathogen is common and can be found in large proportion of *Falcaria* populations in the Czech Republic.

Study area and survey methods

The survey was conducted in May 2005 in central and north Bohemia (Czech Republic), generally in its warm regions. The localities of *Falcaria* were preliminarily identified using data from Czech National Phytosociological Database (Chytrý and Rafajová 2003) and then located by field search. This resulted in 40 localities in three distinct regions. Those were České středohoří in northern part of Bohemia, Prokopské údolí in Prague and Turbovický hřbet 25 km north of Prague; for exact locations see the Table 1. The geology of the localities was mostly comprised of Mesozoic alkaline bedrock such as marlite, claystone and a few Tertiary basalt outcrops in České středohoří. Minority of the localities was on sandy soils. Most of the habitats were broadleaved dry calcareous grasslands with dominant *Bromus erectus* and *Brachypodium sylvaticum* classified as *Bromion* alliance that are typical for deep soils with low productivity. Quite abundant were also mesic grasslands with dominant *Arrhenatherum elatius* (*Arrhenatherion* alliance) and related ruderal communities on fallow land. Study populations were defined as populations of plants separated from another population by 100 m or 20m and substantial physical barrier, usually formed by forest or shrub patches; in any case, most of the populations were well defined by the patches of fragmented grasslands hosting them.

At each of the localities, GPS coordinates were noted in the centre of the population. The numbers of infected and healthy *Falcaria* plants were counted either by counting all individuals if those were less than several hundreds clumped together or by method of transects as follows. The (usually elongated) population was divided in rectangular parts 50m long and in each of the compartments, 10 random positions were generated along a measure tape. All individuals were counted in transects 1m wide running perpendicularly to the measure tape at these positions. The numbers of individuals were then extrapolated within the

Table 1. Populations and their basic characteristics; regions: 1) Turbovický hřbet 2) České středohoří 3) Prokopské údolí; prevalence defined as momentary share of systemically infected plants in population

Number	Population name	Region	Latitude	Longitude	Individuals	Prevalence
1	Červená Píska	1	50.29421	14.543816	1892	0.547
2	Dlouhá stráň	1	50.30385	14.53068	12	0.167
3	Doubrava	1	50.287874	14.55715	2804	0.371
4	Chajda	1	50.290941	14.548853	483	0.880
5	Chlumín	1	50.266736	14.45523	223	0.013
6	Kafilerie 1	1	50.28529	14.54143	664	0.298
7	Kafilerie 2	1	50.28457	14.5448	1650	0.459
8	Kopeč	1	50.24771	14.41951	13875	0.762
9	Koridor	1	50.28873	14.548971	5929	0.577
10	Nad včelínem	1	50.293357	14.545501	396	0.051
11	Písčina u Tišic	1	50.265309	14.55155	8230	0.001
12	Písková cesta	1	50.297709	14.535072	1973	0.281
13	Pískovna	1	50.293254	14.540962	251	0.044
14	Plošina	1	50.294526	14.544642	1356	0.751
15	Pod dráty	1	50.298217	14.53635	90	0.000
16	Pod sadem	1	50.293888	14.544358	513	0.272
17	Posed	1	50.287688	14.558568	1543	0.190
18	Šípková stráň	1	50.289375	14.55072	2270	0.674
19	Stipa	1	50.30064	14.5363	75	0.293
20	Terásky	1	50.291256	14.548285	283	0.706
21	Třešňová cesta	1	50.31933	14.51954	116	0.707
22	U přejezdu	1	50.25543	14.50731	191	0.361
23	U strašáka	1	50.297813	14.53801	2880	0.372
24	V poli	1	50.290742	14.544374	1360	0.659
25	Za Kopčí	1	50.25067	14.42976	83	0.349
26	Za statkem	1	50.30112	14.53328	457	0.722
27	Hazmburk mez	2	50.43146	14.01956	5243	0.296
28	Hazmburk sady	2	50.42973	14.01789	2825	0.255
29	Hlinná	2	50.573147	14.11153	110	0.000
30	Hradiště	2	50.569345	14.113386	2223	0.000
31	U cesty	2	50.566511	14.10962	65	0.000
32	Vrutice	2	50.504626	14.303651	540	0.000
33	Butovické hradiště	3	50.041071	14.358389	130	0.769
34	Dlouhá populace	3	50.040489	14.361521	9017	0.587
35	K hradišti	3	50.041373	14.359217	255	0.314
36	Kovářovic mez	3	50.045117	14.363894	38	0.737
37	Kraj rezervace	3	50.046769	14.36413	628	0.361
38	Pod stromem	3	50.044691	14.363551	117	0.128
39	Trávník	3	50.03948	14.357959	3528	0.415
40	U zkamenělin	3	50.043583	14.360955	768	0.608

compartments giving reasonable estimate of the population size (approx. 20% of the population). Smaller, non-homogeneous or non-rectangular parts were counted individually or divided into smaller parts. The disease prevalence was defined as proportion of systemically infected of all *Falcaria* plants.

Two phytosociological relevés of 2×2 meters were done at each locality placed in the central part of the *Falcaria* population at least 5 m apart. One relevé was recorded in case the population was very small (14 times); once the population was very large and elongated so there were 3 relevés placed there. More than one relevé were used to control for heterogeneity within the localities. For each of the relevés date, cover of shrubs, herbs and mosses, aspect and slope were recorded. The relevés of all species and their abundances were recorded in Braun-Blanquet scale (r, +, 1, 2, 3, 4, 5).

Analysis

The vegetation data were entered into Turboveg (Hennekens and Schaminée 2001) and sorted and cleaned in Juice (Tichý 2002). The species abundance data were transformed into percentages using standard conversion (r = 1%, + = 2%, 1 = 3%, 2 = 13%, 3 = 38%, 4 = 63%, 5 = 88%). These values were used to calculate Shannon diversity index for each relevé and values of the index were averaged for localities with more than one relevé. The species data were then analyzed with detrended correspondence analysis (DCA) to extract the main gradients of species composition in CANOCO (Hill and Gauch 1980; ter Braak 1988); no data transformation was used.

Finally, the species data were transformed into presence/absence data that were used to calculate Ellenberg indicator values (EIV) for relevés (Ellenberg 1974; Ellenberg et al 1992). Using presence/absence data is recommended as it gives the same weight to abundant and scarce species and so it prevents the results to be overriden by dominant species with broad ecological amplitudes. The analysis was performed using Juice and tabulated EIVs of Czech flora used by CNPD. Values were averaged for localities with more then one relevé. It has been shown that grassland plots with size 4 m^2 are sufficient for calculation of EIVs (Otýpková 2006).

The variables entering the analysis were: region (encoded as three dummy variables), population size, Shannon diversity index, EIVs for light (L), temperature (T), continentality (K), moisture (F), soil reaction (R), nutrients (N), axis 1, 2 and 3 from DCA analysis, slope, total cover of shrubs, herbs and mosses and cover of herbs. The region was included as a block factor to subtract the effect of different macroclimate and bedrock differences. I also

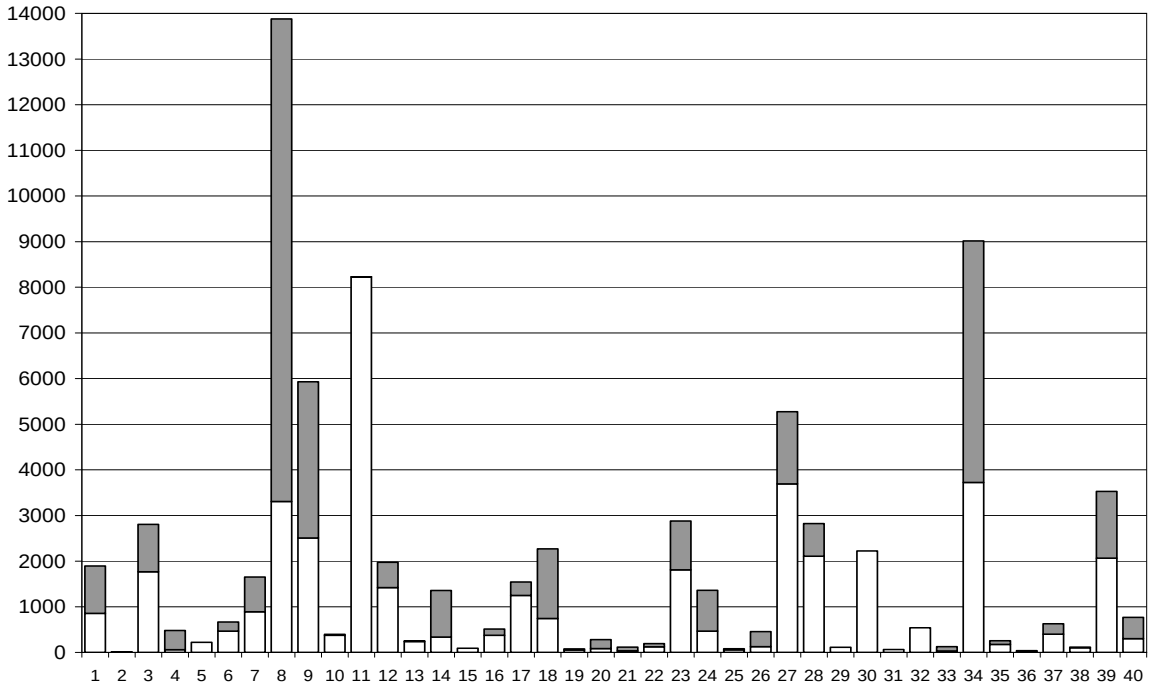

Fig. 1. Numbers of infected (grey) and healthy (white) plants in populations; see table 1. for population names.

added latitude, longitude and product of the two as a more general measure of geographic distance complementing the region. The data were analyzed using linear regression in SPSS (SPSS Inc., 11.5.1) with backward selection procedure; all variables entered the model and then they were excluded if their t-test resulting p value was over 0.1.

Results

The population sizes of the 40 chosen localities varied by 3 orders of magnitude (Table 1.) with the overall average population size being 1878 individuals. Overall mean prevalence of the disease was 0.448 but it also varied considerably from no infection up to 88% systemically infected plants (Fig. 1.).

The EIVs of individual localities varied mostly up to 1-2 units of the scale with exception of the nutrients that had range of 3-4 units; overall medians, ranges and inter-quartile ranges can be seen in Table 2. There were almost no differences between the regions in EIVs, only Prokopské údolí had a slightly more basic soil and was more continental than the other two regions. With Shannon index of 2.19 the relevés were generally of lower diversity and evenness. The mean cover values of 88% for all plants and 86% for herbs indicate that aboveground competition is in most cases not very strong. First three axes of the

Table 2. Medians, interquartile ranges and ranges for calculated Ellenberg indicator values, Shannon diversity index and cover values; for EIVs the characteristic describing the range included.

	Median	Interquartile range	Range	EIV characteristic
Light	7.28	0.42	1.34	medium light to light
Temperature	6.00	0.34	1.02	medium warm to warm
Continentality	4.68	0.50	2.19	sub-oceanic to intermediate
Moisture	4.05	0.67	1.55	fresh to dry
Soil reaction	7.50	0.52	2.04	weakly acidic to basic
Nutrients	4.89	1.82	4.36	nutrient poor to rich
Shannon div. ind.	2.24	0.64	1.48	
Total cover	90.75	17.88	37.50	
Herb layer cover	90.00	16.50	40.00	

DCA ordination of vegetation data were extracted explaining 4.8%, 3.4% and 2.9% of all the species variation.

The regression resulted in set of variables best describing relationship between disease prevalence at the population level and environmental data (Table 3. and Fig. 2.) The EIV for soil reaction was positively correlated with disease prevalence as well as the herb cover value. Ellenberg moisture was just marginally significantly (p=0.051) positively correlated with disease prevalence.

Region, which was included as a factor was also significant. Turbovický hřbet with various bedrock qualities differed from České středohoří that had more localities on basaltic outcrops with low disease prevalence. Prokopské udolí region was similar to Turbovický hřbet as most of the localities were also on claystone and marlite bedrock and had generally higher prevalence.

The variables with non-significant relationship with disease prevalence were slope, Shannon diversity index, sum of the cover of all vegetation layers, population size, vegetation composition (DCA axes), Ellenberg values for temperature, continentality, light and nutrients and latitude, longitude and their product.

Table 3. Results of the regression of the landscape data; B – unstandardized beta coefficient; adjusted R^2= 0.374

	B	**t**	**Sig.**
Constant	-2.535	-3.154	0.003
Moisture	0.172	2.019	0.051
Soil reaction	0.207	2.368	0.024
Herbs cover	0.008	2.919	0.006
Region (2)	-0.333	-3.344	0.002
Region (3)	-0.051	-0.511	0.612

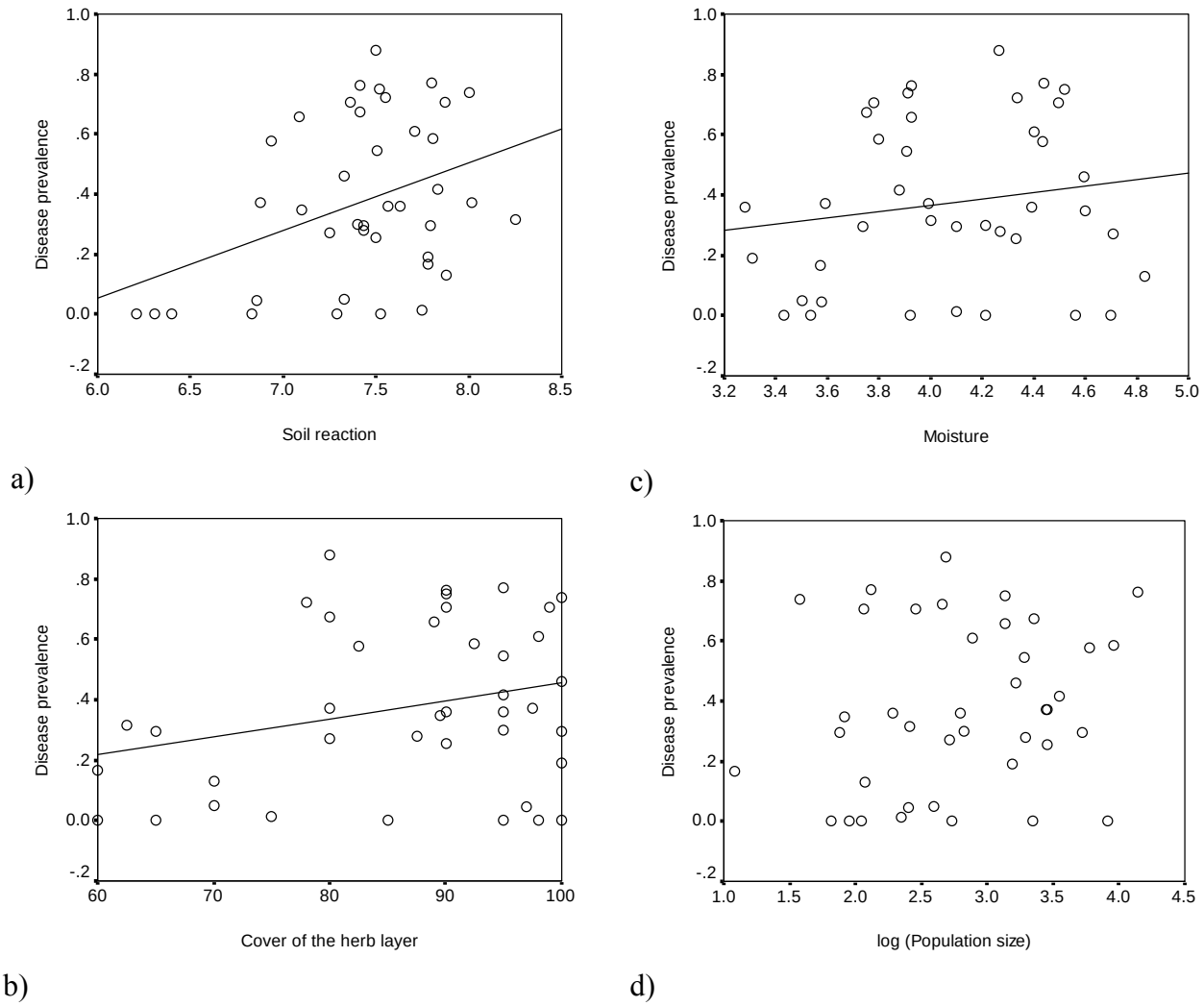

Fig. 2.: Scatter plots showing relationship between disease prevalence and a) Ellenberg indicator value for soil reaction (p=0.024) b) herb layer cover (p=0.006) c) Ellenberg indicator value for moisture (p=0.051) d) population size (n.s.); the whole model including a), b), c) and effect of the region had adjusted R^2=0.374

Discussion

The study found significant correlation between systemic disease prevalence of *Puccinia sii-falcariae* on *Falcaria vulgaris* and environmental factors represented by Ellenberg indicator values for soil reaction and moisture. There was also correlation with the cover of the herb layer and all three factors correlated with the disease prevalence positively (Fig. 2). Otherwise, only the effect of the region was significant. This implies that the infection is more prevalent in populations with more basic and moist soil and in populations with relatively larger cover of vascular herbaceous plants. The results can be attributed either

to direct effects of the environmental variables on the rust fungus or to indirect effects the variables have on host plants.

The moisture is the most obvious factor that could account for increased disease prevalence as it directly affects probability of successful infection for most fungi (e.g. Webb and Nutter 1997; Bradley et al 2003; Pinon et al 2006). However, it is important to notice that the localities with increased prevalence were often exposed ridges that hosted xeric type of vegetation and especially in summer the upper soil and the vegetation were very dry and so the infection favourable conditions would be limited to relatively shorter season in spring or in autumn. This discrepancy could have been caused by the character of the claystone/marlite soil type that sustains the soil moisture more efficiently than other types of soil. The host plant *Falcaria vulgaris* avoids competition while being able to grow on deep soils and reach for the water with its long taproot (Ellenberg and Snoy 1957). The claystone/marlite soil could thus lead to greater survival of infected plants and greater disease prevalence. The result is in accord with the notion of Schaffers and Sýkora (2000) that EIV for moisture is best correlated with lowest values of soil moisture in the summer.

The EIV for soil reaction was also well correlated with the disease prevalence in the regression. This could lead to a conclusion that soil pH or calcium content influence the pathogen directly. Studies showing such connection have however dealt with soil pathogens only (Myers and Campbell 1985; Smiley et al 1996). It is likely that this correlation is outcome of relation of soil calcium content with another environmental factor, again most likely soil type. This can be supported by the fact that the localities on basaltic bedrock (e.g. populations 29, 30; shallow ranker type of soil) with relatively high pH but low calcium content were in several cases disease free. Systemic disease symptoms often lead to increased effect of drought (Shishkoff and Bruckart 1996) and without access to the underground water in shallow or sandy soils are the infected plants more prone to die off.

The importance of the previous effects relies considerably on the relationship between the real environmental values and the indicator values. Several attempts have been made to test for reliability of EIVs. Ter Braak and Gremmen (1987) have tested the moisture value on Dutch vegetation data; in comparable manner, Ertsen et al (1998) have tested values for moisture, soil reaction, nutrient availability and salinity. Finally, Schaffers and Sýkora (2000) have performed a study to test values of moisture, soil reaction and nutrients. The studies conclude in concert that Ellenberg moisture is the most reliable and well correlated with lowest summer water content as well as average spring groundwater level, only ter Braak and Gremmen found that about one fifth of their studied species was inconsistent with other

species in the moisture value. Soil reaction correlated with pH in the study of Ertsen et al while Schaffers and Sýkora concluded that EIV for soil reaction is best related to soil calcium content and the correlation with pH can be nonlinear, especially at larger scales. The EIV for nutrients was the least reliable of the studied factors and the correlation with soil nutrients was weak but it fairly well related to aboveground biomass and the nitrogen content in it. In general, all the studies conclude that EIVs are well usable as proxies for certain environmental factors if used at the scale of a region.

Effect of the increasing herb layer cover on disease prevalence is rather unclear. On one hand, increased cover could lead to increased competition pressure in the aboveground. In several smaller plots with dominant *Elymus repens* and 100% cover were all *Falcaria* plants healthy indicating low competitive ability of the systemically diseased plants. However, this was rare and the effect was masked by within-locality variability. On the other hand, the median of the cover of the herb layer was 90%, which would indicate generally lower competition. It has been hypothesized that increased cover can increase air humidity in the growth so this might be one of the possible explanations, although mostly tested just for cultivated plants (Saindon et al 1995). Practically no infected plants in rather large and extremely dry population Písčina u Tišic support this theory.

The region was included as a factor to be subtracted from the data to extract more general patterns. The result reflected the fact that the Turbovický hřbet and Prokopské údolí significantly differed from České středohoří because disease prevalence was either low or zero in the latter region. This probably reflects general difference in any of factors like macroclimate, bedrock and soil differences or effect of localities disposition. I have included the latitude and longitude to control for effect of spatial configuration but those were not correlated with disease prevalence. This suggests that influence of the spatial factors is lower than effect of environmental factors.

There was no effect of population size on disease prevalence. Previous studies usually found correlation between disease prevalence or incidence and population size (e.g. Jennersten et al 1983; Burdon et al 1995; Colling and Matthies 2004). In the study of Burdon et al the pathogen was not systemic and so the dynamics of the disease was probably more variable and depended on larger and more predictable source of inoculum. There are other tendencies in the dataset that might shed light on the underlying processes though. With increasing of their size, the populations tend to have intermediate prevalence whereas in small populations there are either infested or almost clean ones. This could explain the non-significant result because the small populations are of two types in regard to age. The old

remnant populations with lot of infection could have accumulated homozygous individuals with low resistance because of inbreeding effects (Carr et al 2003) or might be close to large populations with high prevalence. The disease-free or low-infection populations are likely young (e.g. populations nr. 5 and 15) and they might accumulate more infection in the future. This could be rather similar to the study on *Ustilago violacea* on *Silene dioica* (Carlsson et al 1990) where young populations were disease-free, older had usually high disease incidence and oldest had intermediate levels of infection. Without good knowledge about population age there is no way to disentangle various effects acting in the relationship of population size and disease prevalence.

The method of indication values provided some informative results and it could be used to draw some general conclusions. The correlation of disease prevalence with moisture and soil reaction suggests that the most infected populations are on heavy calcareous soils that are rather suitable for *Falcaria vulgaris*. The perennial life history of the host plant probably causes greater survival of infected individuals, accumulation of the disease and thus greater disease prevalence in the populations on claystone or marlite soils. Low disease prevalence is then common in localities on sandy and dry soils where *Falcaria* faces environmental stress and infected plants probably die with higher frequency. Low air humidity at such localities might be also responsible for low success of infection by *Puccinia sii-falcariae*.

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Low effect of pathogen on population characteristics in pathosystem *Falcaria vulgaris–Pucinia sii-falcariae*: matrix model results

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Abstract

The diseases of wild plant species are thoroughly researched but their long-term effect at the population level is often unknown. To acquire such information one can use matrix models that allow for determining population characteristics like population growth rate, stable stage distributions and importance of the stages. I studied populations of common plant *Falcaria vulgaris* infected by systemic rust fungus *Puccinia sii-falcariae*. I followed healthy and systemically infected plants in four populations over 4-5 years to calculate population characteristics for both complete populations and for only the healthy plants and their transitions. *Falcaria* has a broad range of living conditions that might affect the disease effects and so I chose two types of populations to describe the differences in life cycle of expansive (field) and relict (slope) populations.

There was considerable variation both within and among populations. The growth rate of healthy part of the population was significantly larger than the growth rate of the whole population only in one year and locality. Deterministic growth rates differed between population types with field populations growing in size and slope populations either around equilibrium or dropping in size. The stable stage distributions changed substantially among individual years suggesting changes in conditions favourable for various transitions. The slope populations had balanced amounts of plants projected in healthy and in infected stages whereas field populations had most plants projected in the healthy stages. Finally, elasticities were computed for all matrix elements allowing identification of important phases of the life cycle. All populations relied on survival in healthy stages but for the slope populations stasis in infected stages was also important. For the field populations growth and reproduction had the highest elasticity.

The results suggest that although only minor effect of the disease on population growth was found, matrix modelling can still provide insights into the disease effects using stage distributions and elasticities. The systemic rust and its host *Falcaria vulgaris* coexist in dynamic relationship at the localities and the study found that the infection can be temporary which was not known before the study.

Introduction

The pathogens of the wild plants play substantial role in dynamics of their hosts as they often increase mortality (Alexander and Burdon 1984; Wennstrom and Ericson 1990; Burdon 1991; Wennstrom et al 1995), decrease biomass, fecundity and competitive ability (Clay 1990; Esquivel and Carranza 1996; Wolfe and Rissler 1999) and change growth patterns and allocation of resources (Wennstrom and Ericson 1991; Garcia-Guzman et al 1996; Pan and Clay 2002). In trees, pathogens are known to have caused significant regional declines of their hosts (Anagnostakis 1987; Hubbes 1999). The proximate effects are mostly negative but for instance in systemic pathogens the long-term effect can be negligible in terms of mortality (Wennstrom and Ericson 1994). Moreover, there is increasing body of works on pathogens in mutualistic relationships with their hosts (Clay 1988; Brem and Leuchtmann 2001; Pan and Clay 2004). Finally, negative effects on individuals need not necessarily lead to decrease at the population level – e.g. losses in the seedling phase can be compensated by better growth in later stages that are less density dependent due to former thinning (Alexander and Mihail 2000). In trees, mortality of old individuals can be compensated by greater growth of smaller stages that gain access to light (Davelos and Jarosz 2004). Such interactions can be resolved by acquiring detailed data on growth and survival of both infected and healthy individuals, which are scarce however. Without better knowledge of the whole host life cycle in the long-term, the definitive effect of disease on the host can be unknown or speculative.

Research on population biology of plants, particularly the endangered ones, utilizes array of techniques known as population viability analyses (PVA, Menges 2000) to describe the life cycle of a particular species and draw conclusions for its management. One of the most used techniques is matrix modelling (Caswell 2001) which provides several intuitive population characteristics like population growth rate, stable stage distribution, importance of life stages (using elasticities) or probability of survival over time (for review see Crone et al 2011). Matrix models have been used several times to evaluate characteristics of populations infected by pathogens (Antonovics and Alexander 1989; Frantzen 1994; Linders 1995; Emery 1997; Davelos and Jarosz 2004) but most of the early attempts suffered from insufficient data quality. The study of Frantzen (1994) could not use individuals as basic unit due to highly clonal behaviour of the host plant and thus his matrices were composed of cells of a spatial grid and were not comparable with the traditional matrices (the matrix did not represent life-cycle of the plant). Linders (1995) used system of powdery mildew (non-systemic foliar pathogen) infecting *Plantago lanceolata* with infected plants incorporated in separate stages

but he did not have data on the complete life cycle and so the matrix was partially dependent on estimated parameters. Davelos and Jarosz (2004) have used matrix modelling to compare whole populations that were either disease free, diseased or recovering thanks to spread of hypovirulence. The study found no difference in growth rate but stage distribution of diseased populations differed from the other types. The effect of the type of the population was however confounded with locality differences. To determine the definitive effect the disease has on the host population one would like to know the dynamics of the same population if it were not infected. This would allow for direct comparison of population characteristics and quantification of the effect of the pathogen.

In this study, I used the system of perennial plant *Falcaria vulgaris* (*Apiaceae*) and its systemic rust fungus *Puccinia sii-falcariae* to find out how large is the effect the pathogen has at the population level. The system was chosen for its commonness and for the fact that the prevalence of the disease is high in many populations – this allowed marking of sufficient amounts of infected plants. The plant is also non-clonal allowing for simple permanent marking and it excludes the escape of the plant from the disease via clonal growth. I followed individuals in four populations over 4-5 years and used the data for construction of population matrices.

The disease prevalence and severity are often affected by environmental conditions at the locality (Jarosz and Burdon 1988; Wennstrom and Hagner 1999) and at the same time conditions can alter the host plants vigour as well. Both these effects can then possibly lead to changes in growth rates of the healthy and the diseased plants and to changes in disease prevalence. I incorporated the effect of environment by choosing two populations from each type found in the area. In principle, there were localities of two types – they either resided on slopes of claystone ridges (probably relict populations) or grew expansively between fields and in road-verges in the surrounding area. From field experience, one could hypothesize that the slope populations will have lower growth rates than the field populations because of lower fecundity and slower growth.

I aimed to answer three basic questions using the presented dataset: i) how large are the differences in population characteristics between the infected populations of *Falcaria* and the healthy plants in the same populations; ii) which stages change their importance under influence of the pathogen and iii) are there differences between types of populations of *Falcaria* in different kinds of habitats?

Materials and methods

Study species

The studied plant was *Falcaria vulgaris* Bernh. (*Apiaceae*). It is a common species of dry grasslands and road verges of warm regions in central Europe. The original area of distribution spreads from Spain in the west to Near East in the south, central Sweden in the north and central Asia in the east (Meusel et al 1992). It is considered invasive in several parts of the world including USA (Korman et al 2010).

It has narrow leaves that form one or several rosettes that grow newly each year from the head of a substantial taproot. The taproot can split or produce multiple heads in disturbed habitats. The flowering stalk is between 40 and 90 cm high, usually branched with umbels forming hemispheric compound umbels. In autumn, the whole inflorescences can be released allowing a tumbleweed kind of seed dispersal within the locality. The seeds are small, formed in large amounts (several hundreds to several thousands per plant in studied populations, unpubl. data) and have low numbers in only some exceptional years. Germination is generally low in the field but if successful, the seedlings grow very fast and root deep to reach for water (Ellenberg and Snoy 1957). The plant size varies considerably under different environmental conditions ranging from small non-flowering plants in dry conditions to large plants forming expansive populations in resource rich mesic habitats (pers. obs.).

The pathogen of interest was rust fungus *Puccinia sii-falcaria*e J. Schröt. (*Pucciniaceae*) specific to *Falcaria vulgaris*. Some authors merge the species into larger, morphologically defined species *Puccinia bulbocastani* and regard the former species as forma specialis (Kokeš, personal information). The species is demicyclic (lacks uredia) and autoecious (has only one host plant). The infection is systemic and infected plants are recognizable at all stages. In early April, first leaves of infected host emerge early, grow upright and are fragile and etiolated compared to healthy plants, which have tougher leaves more or less horizontally or diagonally oriented. Orange spermogonia are formed soon after leaves emerge producing smelling nectar to attract insects (mostly flies) to fertilize the receptive hyphae with unrelated spermatia. In May, aecia develop predominantly on the underside of the leaf with occasional outbreak on the upper side. Although the aecial discs are generally larger than the spermogonia they usually cover smaller area of the leaf. The systemic infection later leads to general leaf damage resulting in brown spots and premature leaf drying. In July, the last stage of the fungal cycle – telia – appears as sparse black dots. The telial stage can be missing on systemically infected plants and can appear on healthy

plants that do not host any of the previous stages as well. This is usually considered outcome of successful infection by aeciospores (Marková, pers. communication) that may but may not necessarily lead to systemic infection. The cycle is finished in spring as the overwintering teliospores germinate into basidiospores infecting healthy plants. The differences in infection rates for the two kinds of spores (aeciospores and basidiospores) are however unknown. The pathogen is common and can be found in large proportion of *Falcaria* populations in the Czech Republic.

Localities and population data

The research was conducted each June-July in years 2004 to 2008 at four localities in the area of Turbovický hřbet ridge 25 km north of Prague. The geology of the ridge is comprised of Mesozoic alkaline bedrock, particularly marlite and claystone. Two of the localities – Červená píska and Doubrava were dry broadleaved calcareous grasslands of *Bromion* alliance located on the steep ridge. This type of vegetation typically grows on deep clay soils and hosts rich community of grasses and vascular plants. The dominant species are *Brachypodium pinnatum* and *Bromus erectus*. The other two localities were located in mesic type of grasslands from *Arrhenatherion* alliance located approximately 100 meters from the ridge in flat places between fields. The subsoil of the field localities was a mixture of calcareous and sandy soil and due to proximity of the fields it was probably enriched by nitrogen.

In year 2004, two localities were established but one was later destroyed so I continued with only one of them (Červená Píska – CP) in the next year. In year 2005, three other localities were established; for exact locations, codes, years of data collection and basic differences see the Table 1.

In the same year, I counted individuals at the localities to know the overall disease

Table 1. Summary characteristics of the localities and the respective populations; * – data on population size and disease prevalence were collected in year 2005, prevalence is defined as actual proportion of systemically infected in all individuals

Name	Code	Latitude	Longitude	Transition intervals	Individuals*	Prevalence*	Type
Červená Píska	CP	50.29421	14.543816	2004-5, 2005-6, 2006-7, 2007-8	1892	0.547	slope
Doubrava	DOU	50.287874	14.55715	2005-6, 2006-7, 2007-8	2804	0.371	slope
Koridor	KOR	50.28873	14.548971	2005-6, 2006-7, 2007-8	5929	0.577	field
U strašáka	STR	50.297813	14.53801	2005-6, 2006-7, 2007-8	2880	0.372	field

prevalence in the populations. I used transects 1 meter broad placed randomly along a measure tape. This way I counted approx. 20% of the individuals; the final numbers were then extrapolated. I recorded the numbers of healthy and systemically infected individuals.

Data on individuals

This dataset was collected to describe the life cycle of the species using matrix modelling. At each of the localities, two permanent plots were established covering main parts of the population of *Falcaria*. Their joint area was 20 to 40 square meters and they were marked with sticks and buried nails. Within the plots, I selected 100 healthy and 100 infected plants starting in one corner and marking all individuals across the plot. I preferentially marked equal numbers of plants in preliminary size groups to avoid low sample sizes in some of the future model stages; it has been shown that 20 individuals are already sufficient to estimate transitions in each stage (Münzbergová and Ehrlén 2005). From year 2006 onwards, I marked 20 new individuals each year to compensate for mortality, growth and transitions from healthy to infected status. Each plant was marked by a plastic and buried metal marker and measured. Each year I measured the length of the longest leaf of each non-flowering rosette and counted the number of leaves – these data were multiplied within rosette and summed for the plants yielding measure of size of the plants. The flowering rosettes produce less leaves and could not be measured like the vegetative rosettes. Identical method was used for healthy and infected plants. In the vicinity, amounts of roe deer can be rather high and they browse sometimes on leaves and green inflorescences. I recorded the proportion of inflorescence damaged for every recorded flowering individual.

Data on seed set and germination

Each September, when seeds of *Falcaria* were ripe, I randomly selected 10 flowering plants per locality outside the permanent plots, collected and counted their seeds and stored the seed for the sowing experiment. The amount of seeds lost to early fall-off or herbivory was estimated for each plant and used for correction of the final numbers. Amounts of the infected flowering plants were usually small or there were none at all in some years. I estimated the seed numbers but the amounts of the seeds were so small that sowing experiment was not feasible. Therefore, I used the germination of the seeds produced by healthy plants.

Small plots 20×20cm were established near the stable plots to study germination of sown seeds each year. In 2004, I used four plots with 200 seeds sown in each at Červená

Píska. As the resulting germination was rather low, the next year I used three new sowing plots with 500 seeds sown at each locality and the process was repeated in 2006 and 2007. The sowing took place in October and numbers of seedlings were counted next year at the same time as the plot data were collected. During winter the seeds naturally underwent low temperature phase, which is needed for breaking morphophysiological dormancy present in most *Apiaceae* (Vandelook et al 2009; Hawkins et al 2010). *Apiaceae* form only transient seed bank (e.g. Sheppard 1991) and the germination was generally low so the seed bank was not treated in the study.

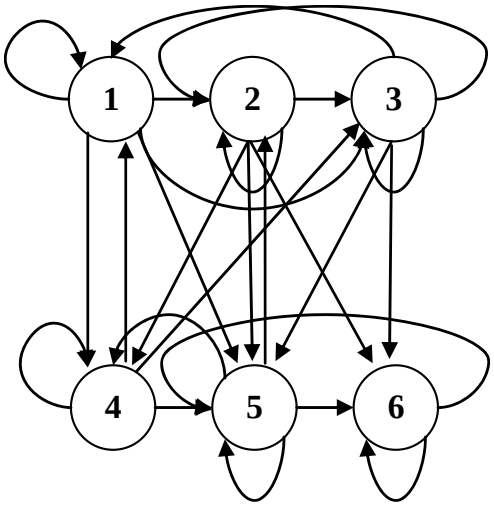


Fig. 1. Illustration of life-cycle graph

Stages construction

For the use of further analyses, I divided the plants in three size-based stages – juvenile and small vegetative plants, vegetative plants and flowering plants. The first stage was defined as plants with maximum size of 80 (e.g. plant with maximum 4 leaves with longest leaf 20 cm long). The second stage consisted of non-flowering plants larger than 80; the last stage was used for all plants that initiated flowering stalk without regard to the fact that some plants did not produce any seeds. All the stages were further divided into stage of infected and healthy individuals. This resulted into six stages altogether in fashion similar to the model of Linders (1995) only grouped by disease for visual clarity (Figs. 1. and 2.). This approach is valid because the infected plants can produce healthy seeds and there have been events of recovery from systemic disease observed. If those two were not true, there would be dead-ends in the lifecycle that would be equivalent to death of the individuals.

		t	
		healthy	**diseased**
t+1	**healthy**	**healthy part of the population**	**recovery events and seed production by diseased plants**
	diseased	**systemic infection events**	**diseased part of the population**

Fig. 2. Schematic representation of the structure of the matrix with diseased and healthy stages; each of the fields is further divided into 3 by 3 values representing transitions between size stages

Individual life-history rates

The rates of growth, stasis, retrogression, infection, recovery and reproduction were examined using the data divided into stages. Growth and retrogression were defined by shift to higher or lower size stage respectively; stasis meant staying in the same size stage. Reproduction was defined as progressing or staying in/to stage defined by flowering. Infection was defined as changing from healthy to infected stage and vice versa for recovery. The rate of infection was tested only for healthy plants and rate of recovery only on infected. All rates were tested one-by-one using logistic regression with stage in the previous year as covariate. The independent variables were year, locality and their two-way interaction. The year and locality were taken as categorical variables with identity contrasts. The analysis was performed in SPSS (SPSS Inc., 11.5.1).

Matrix models

The stage data can be summed into stage based matrices (Lefkovitch 1965) that represent the life cycle of particular population and further used for matrix modelling (Caswell 2001). I constructed matrix for each of the four transition intervals 2004-5 (only for Červená Píska), 2005-6, 2006-7 and 2007-8 and for all four localities resulting in 13 matrices altogether. The individual elements of the matrix are transitions between stages. Using the transition matrices, I computed basic characteristics of the populations. First, it was the finite rate of increase (lambda, λ) computed as the dominant eigenvalue of the matrix. Important parameter of each matrix element is its sensitivity (s_{ij}) defined as change of λ after change in the element (a_{ij}):

$$s_{ij} = \delta\lambda/\delta a_{ij}$$

The matrix elements are however not comparable in their ranges – e.g. reproduction has much larger values and its change is not comparable with other elements. Therefore, elasticity (e_{ij}) is used most often instead of sensitivity. Elasticities are sensitivities proportional to the change in the matrix element:

$$e_{ij} = (a_{ij}/\lambda)*(\delta\lambda/\delta a_{ij})$$

The elasticity is used as a measure of importance of the particular transition in the life cycle (Silvertown et al 1996; de Kroon et al 2000). Last parameter that can be derived directly from the matrix is the right eigenvector that represents stable stage structure of the population.

To compare population characteristics of infected and healthy parts of the populations I have constructed matrices for both the whole life cycle with all six stages and for only the healthy stages and transitions among them. This allows for calculation of hypothetical population characteristics of a healthy population that would live at the same locality if there were no disease. The healthy plants were divided into three size-based stages and transitions among them were calculated. For those 13 new matrices, I also computed all previous characteristics. The calculations were made in the Poptools 3.0.6 (Hood 2008) software and in MATLAB (Mathworks, Natick, Massachusetts, USA). The publications in which there are published respective scripts for MATLAB are cited along with the methods explanations.

Matrix model analyses

The use of population characteristics derived from matrices is constrained by the limited sample size. To estimate the error in the characteristics I used bootstrap techniques to calculate confidence intervals (Alvarez-Buylla and Slatkin 1994). To calculate 95% confidence interval of lambda I used a MATLAB script developed by Münzbergová (2006; 2007). The matrices were bootstrapped 2000 times. The bootstrap process included the raw transition data and the fixed data of numbers of sown and germinated plants.

To address the concern about the variability of the particular years and localities used for the study I used stochastic modelling. It enabled me to take the available deterministic matrices and combine them randomly to simulate natural stochasticity. The matrices computed by the bootstrap process were taken for each year and locality and combined to project the population for 200 years and to compute stochastic lambda using script developed by Münzbergová (2005).

Finally, I used life table response experiment (LTRE, Caswell 2001) analysis to find out what transition contributed the most to the effect of particular treatment on lambda. This is retrospective technique because it analyses past variation unlike the lambda and elasticity analyses that project the present state into future. The LTRE analysis results in positive or negative contributions of each transition to differences between the treatment levels – those were years for the whole matrices and populations for the healthy and whole matrices. The significances of the treatment levels were tested by permutation test with 2000 runs in MATLAB (Münzbergová 2007). In analyses with all populations and years, the transition 2004-5 in Červená Píska was excluded to keep the design balanced.

Results

Individual life-history rates

All the life-history rates differed among transition intervals (further also called years) and localities either as separate effects or in interaction except recovery rate that did not differ in years at all. The infection rate depended on locality with Červená Píska having bigger and Doubrava lower than average infection rate. The recovery rate depended on locality and stage – the two slope localities had much higher rates of recovery especially in the two adult stages than the plants in field populations where recovery from systemic infection was rather rare. The probability of reproduction in the next year differed among years and localities but not in interaction. The mortality of plants depended on locality, year and their interaction with Doubrava having the highest mortality in all stages while the other three localities were similar with most plants dying only in small plants stage. There were several transitions in which no plants have died in some years – this had destabilising effect on the results of some of the later analyses (e.g. by increasing elasticity). Stasis, growth to higher and retrogression to lower stage were all differing among localities and years and they also depended on the stage in previous year.

Growth rates

The growth rates or lambdas were computed for all 26 constructed matrices and their confidence intervals were calculated with bootstrap procedure. The difference in growth rate (lambda, Fig. 3.) between the whole populations and their healthy part was not substantial in most cases. Doubrava was the only locality where lambda of the whole populations was below zero all three years and the healthy part of the population showed more or less the same growth rate of 0.7 which means 30% decrease each year. The other slope locality Červená

Table 2. Results of logistic regression of individual life-history rates; significant P is in bold

	Infection rate			Recovery rate			Reproduction			Mortality		
	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P
Stage previous year	0.038	1	0.846	8.305	1	**0.004**	0.075	1	0.785	1.104	1	0.293
Year	4.463	2	0.107	1.182	2	0.554	7.874	2	**0.02**	23.3	2	**<0.001**
Locality	16.6	3	**0.001**	8.879	3	**0.031**	18.08	3	**<0.001**	56.03	3	**<0.001**
Locality × Year	16.35	6	**0.012**	2.069	6	0.913	10.06	6	0.122	17.79	6	**0.007**

	Stasis			Growth			Retrogression		
	χ^2	df	P	χ^2	df	P	χ^2	df	P
Stage previous year	8.263	1	**0.004**	62.89	1	**<0.001**	74.19	1	**<0.001**
Year	2.208	2	0.332	8.894	2	**0.012**	22.13	2	**<0.001**
Locality	31.62	3	**<0.001**	16.58	3	**0.001**	0.953	3	0.813
Locality × Year	17.58	6	**0.007**	26.13	6	**<0.001**	45.66	6	**<0.001**

Píska had growth rates varying around equilibrium (λ=1) with lambda of healthy plants considerably larger only in the first transition interval (2004-5). The field localities had growth rates in most cases above one with Koridor increasing in the consecutive years until lambda 1.5 while U strašáka started at this value in the first year and dropped to approximately equilibrium values in the following years. The growth rates of the healthy parts of the populations were usually moderately bigger than of the whole population for the field populations.

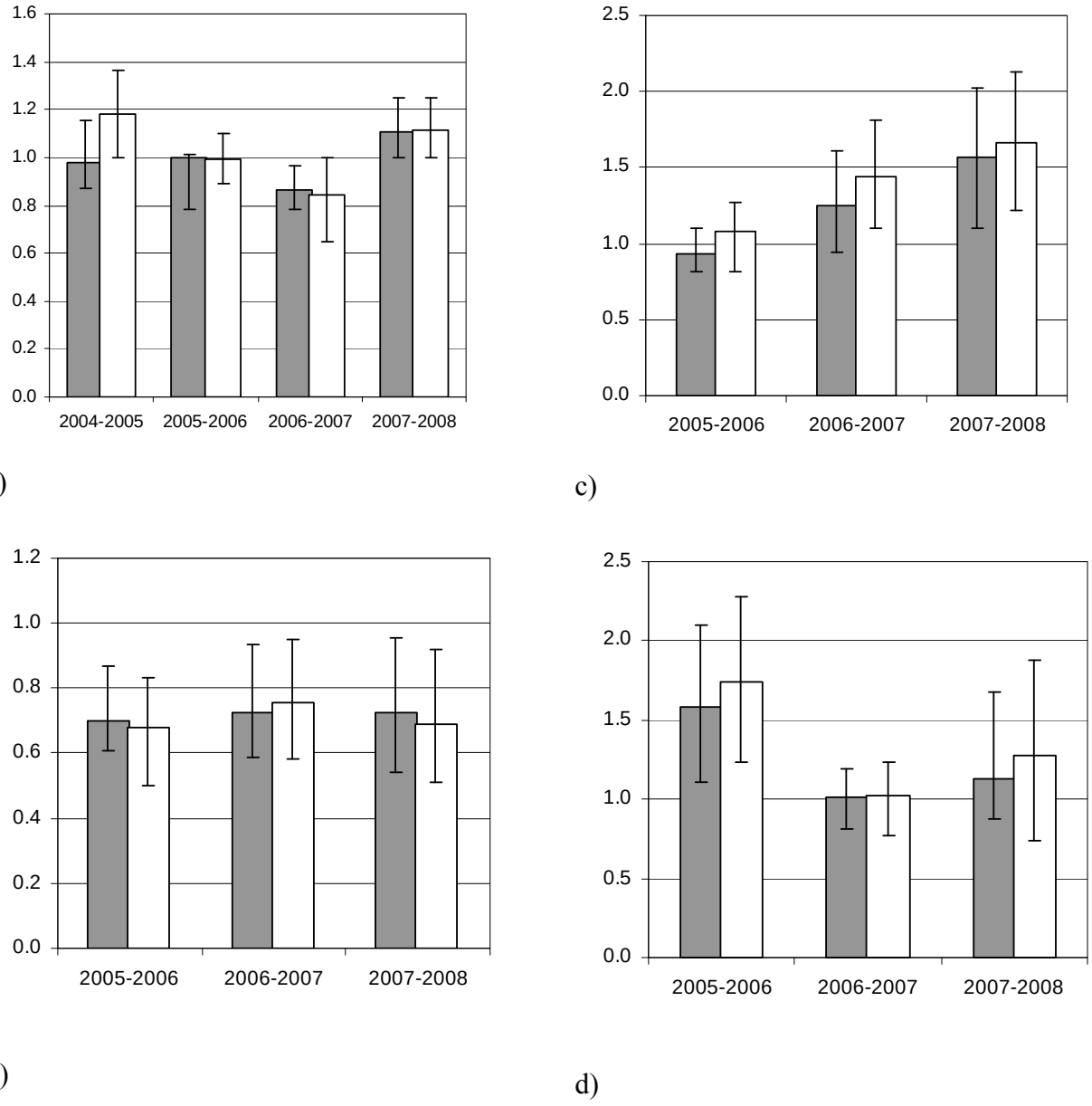
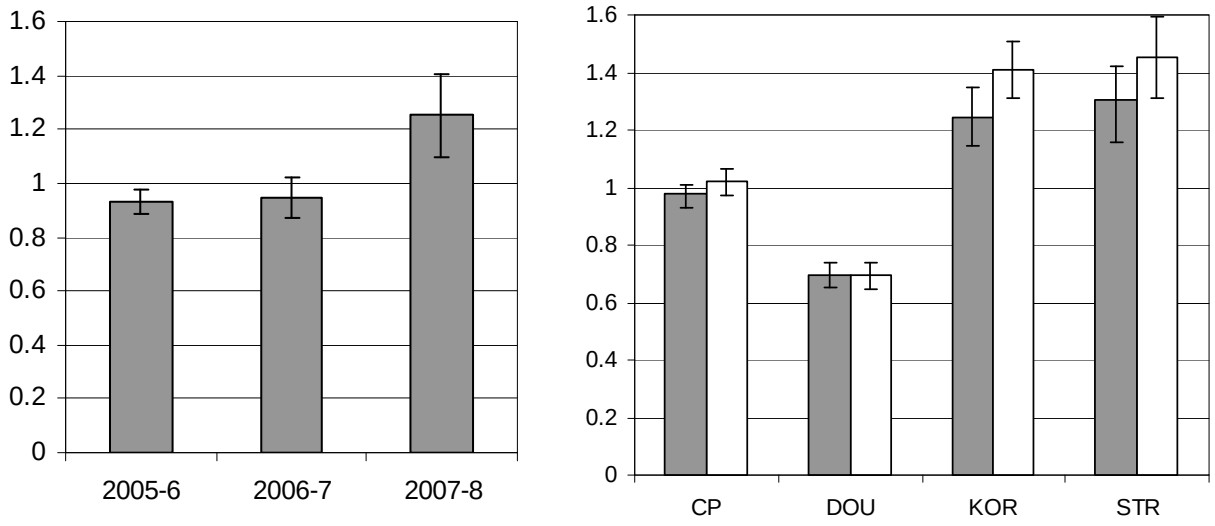


Fig. 3. Population growth rates (λ) and their 95% confidence intervals for the whole population (grey) and for the healthy part of the population (white) in all transition intervals for: a) Červená Píska, b) Doubrava, c) Koridor and d) U strašáka



a) b)

Fig. 4. Mean stochastic growth rates and standard deviations of the 2000 runs for a) transition intervals of whole populations and b) whole (grey) and healthy (white) populations within localities

Using the stochastic matrices I can show that the year 2008 was favourable for *Falcaria* populations with lambda 1.2 compared to the previous years when overall growth rate was just below one (Fig. 4a). The differences among localities (Fig. 4b) more or less copied the results of the deterministic matrices as the two slope populations had projected growth below $\lambda=1$ and the field populations had projected growth of 1.2. The stochastic lambdas of the healthy part of the population of were moderately higher for Červená Píska, Koridor and U strašáka but not for Doubrava.

Stage distributions

The stable stage distribution (SSD) was computed for single matrices and for stochastic matrix projections. The SSDs from single year projections show that the results for different localities in one year can be more similar than results from one population in different years (Att. 1). The transition 2007-8 favoured growth and survival of the healthy plants whereas the first three transition intervals resulted in comparable values in the healthy and in infected stages except for U strašáka where high proportion of small healthy individuals is projected. The pattern of distribution of plants into size stages did not differ considerably in the stable stage distributions obtained from healthy plants.

The stochastic models show more general picture of the populations and overall values in years. The slope populations had higher proportions of infected plants projected while the

Table 3. Stable stage distributions as predicted by stochastic model a) for localities separately and together, b) for years

a)

Stage	CP	DOU	KOR	STR	Overall
1	0.28	0.45	0.41	0.54	0.42
2	0.21	0.14	0.35	0.17	0.21
3	0.03	0.02	0.10	0.04	0.05
4	0.29	0.26	0.04	0.10	0.18
5	0.19	0.12	0.09	0.11	0.14
6	0.00	0.00	0.01	0.03	0.01

b)

Stage	2006	2007	2008
1	0.20	0.50	0.42
2	0.09	0.13	0.32
3	0.01	0.03	0.11
4	0.47	0.23	0.04
5	0.21	0.10	0.10
6	0.02	0.00	0.01

field populations had larger proportions in stages of healthy plants (Table 3a). The overall values of the transition intervals projected steady decrease of infected stages in favour of the healthy part of the population (Table 3b). The stochastic projection of all the years and localities shows the overall stage structure for *Falcaria* in the studied area – 42, 21 and 5 percent of the population were attributable to the healthy stages and 18, 14 and 1 percent were projected in infected stages resulting in overall projected prevalence of the disease being 32.38%. This could be compared with the weighted average of prevalence from the survey in 2005 that was 48.63%. Finally, one can compare the values of prevalence for localities representing outcome of processes in the past with the stochastic prevalences (Table 4). The surveyed values for Doubrava were close to the projected result while the rest of the localities had projected prevalences lower than the surveyed values indicating change in infection or growth rates in the study interval.

Elasticities

The elasticities for all transitions of both whole populations (Att. 2) and the healthy part matrices (Att. 3) were computed. The highest elasticities were mostly those in stasis and growth stages, only for Doubrava the retrogression into smaller stages had higher values of elasticity. In Červená Píska, the stasis transitions were the most important. At this locality in two years, there were stages in which no plant changed its state between years resulting in transition of exactly one – such transitions tend to overrule the outcome of the projection because they produce high elasticities. All other localities had highest

Table 4. Comparision of surveyed prevalences and values from stochastic model

locality \ timescale	survey 2005	stochastic 2005-8
Červená Píska	0.55	0.48
Doubrava	0.37	0.39
Koridor	0.58	0.13
U strasaka	0.37	0.24

elasticities in stages of stasis and growth in the healthy part of the matrix and in some years reproduction was also important, especially in field populations. This pattern was generally similar to the matrices constructed of the healthy transitions, only they less depended on stasis in flowering stage and reproduction. The transitions representing infection and recovery from the systemic disease never had any considerable elasticity. Finally, systemically infected plants were important for the life cycle in some years but predominantly in the slope populations. In the table 5, values of elasticities generated by stochastic modelling are presented.

Table 5. Stochastic elasticities for localities, transition intervals and for projection of all types together; elasticities over 0.05 bold and over 0.1 shaded

0.1420	**0.0509**	**0.0579**	0.0040	0.0179	0.0003
0.0806	**0.1308**	0.0041	0.0023	0.0032	0.0003
0.0204	0.0171	**0.0651**	0.0255	0	0
0.0153	0.0055	0.0008	**0.1610**	0.0481	0
0.0136	0.0167	0.0022	0.0369	**0.0765**	0.0002
0	0	0.0006	0	0	0.0002

a) Červená Píska, all transition int.

0.3045	**0.0784**	**0.0501**	0.0115	0	0.0026
0.0830	**0.0858**	0.0047	0	0.0021	0
0.0335	0	0.0397	0	0.0200	0.0012
0.0094	0.0054	0	**0.1141**	0.0350	0.0006
0.0149	0.0059	0.0015	0.0380	**0.0510**	0.0008
0	0	0.0007	0	0.0039	0.0020

b) Doubrava, all transition intervals

0.0968	0.0158	**0.1851**	0.0010	0	0.0012
0.1020	**0.1222**	0.0097	0	0.0011	0
0.0984	**0.0946**	**0.2543**	0	0	0
0.0009	0.0003	0	0.0030	0.0014	0.0000
0.0007	0.0015	0.0002	0.0010	0.0064	0.0004
0	0	0.0011	0	0.0004	0.0006

c) Koridor, all transition intervals

0.1363	0.0153	**0.1966**	0.0014	0.0005	0.0068
0.1000	**0.0632**	0.0031	0	0.0036	0.0005
0.0995	**0.0835**	**0.1916**	0.0144	0	0
0.0081	0.0017	0	0.0164	0.0102	0.0007
0.0080	0.0039	0.0005	0.0046	0.0112	0.0016
0.0024	0.0023	0.0008	0	0.0039	0.0074

d) U strašáka, all transition intervals

0.1573	0.0379	**0.0573**	0.0090	0.0014	0.0101
0.0909	**0.1112**	0.0063	0	0	0
0.0124	**0.0503**	**0.0707**	0	0	0
0.0067	0.0022	0	**0.1772**	0.0405	0.0009
0.0033	0.0054	0.0005	0.0391	**0.0740**	0.0047
0.0018	0.0018	0.0014	0	0.0103	0.0156

e) all localities, transition 2005-6

0.3620	**0.0514**	**0.1092**	0.0042	0.0063	0.0013
0.0739	**0.0653**	0.0045	0.0024	0.0024	0.0006
0.0760	0.0264	**0.0603**	0.0104	0	0.0006
0.0076	0.0014	0.0004	**0.0677**	0.0180	0.0001
0.0129	0.0045	0.0010	0.0096	0.0162	0.0004
0	0	0.0010	0	0.0014	0.0007

f) all localities, transition 2006-7

0.0689	0.0298	**0.1848**	0.0007	0.0031	0.0001
0.0881	**0.0942**	0.0024	0	0.0020	0
0.1176	0.0462	**0.2687**	0.0056	0.0161	0
0.0033	0.0043	0	0.0068	0.0048	0
0.0079	0.0109	0.0010	0.0061	0.0252	0.0005
0	0.0001	0.0002	0	0.0003	0.0003

g) all localities, transition 2007-8

0.1847	**0.0507**	**0.1268**	0.0033	0.0045	0.0027
0.1001	**0.1045**	0.0062	0.0005	0.0025	0.0007
0.0678	0.0451	**0.1232**	0.0109	0.0088	0.0008
0.0084	0.0035	0.0003	0.0468	0.0183	0.0003
0.0093	0.0094	0.0015	0.0153	0.0332	0.0016
0.0007	0.0009	0.0014	0	0.0026	0.0028

h) all localities, all transition intervals

Červená Píska population overall elasticities are largest for stasis in stages 1-5, growth from and into small healthy stage and for reproduction. The same transitions are important for Doubrava except for stasis in stage 3, which is not as important here. The field populations had similar patterns of the most important transitions – they relied on growth and stasis in of healthy plants as well as reproduction. The values for transition intervals show increasing importance of reproduction and growth in 2006-7 and 2007-8 compared to 2005-6 with most elasticity in stasis. The overall values show that in long term *Falcaria* relies most on stasis of small and fertile stage and on generative reproduction.

Life-table response experiments

The LTRE analysis revealed significant contributions of various transitions to population growth rate for the whole populations and for healthy parts of the populations. When decomposing variability within locality using years as treatment, only Červená Píska and Koridor showed significant results (Table 6a). For Červená Píska transition from 2006-7 showed negative and 2007-8 positive contribution to growth rate corresponding to the growth rates in the respective times. This could be attributed to negative effect of stasis in small infected plants stage in the first and positive effect of reproduction in the second (fine transition data not shown). At Koridor, the significant contributions were negative for 2005-6 and 2007-8 mainly due to contribution of transitions among infected plants. The overall lambda (Table 6b) got negative contributions from stasis in flowering stage at Doubrava and from small to flowering transition at Koridor. The healthy part of the population was

Table 6. Results of LTRE; a) differences among years within localities for whole population matrices, b) differences among localities for whole population and healthy part matrices; significant contributions in bold

a)

	Červená Píska		Doubrava		Koridor		U Strašáka	
transition	contribution	p	contribution	p	contribution	p	contribution	p
2004-5	-0.001	0.097						
2005-6	0.001	0.082	0.001	0.339	**-0.003**	**0.044**	-0.004	0.059
2006-7	**-0.001**	**0.003**	-0.002	0.787	0.005	0.922	0.005	0.154
2007-8	**0.001**	**0.003**	0.001	0.535	**-0.003**	**0.046**	-0.001	0.666

b)

	whole population		healthy part	
population	contribution	p	contribution	p
CP	0.003	0.531	-0.004	0.460
DOU	**-0.001**	**<0.001**	**0.002**	**<0.001**
KOR	**-0.004**	**<0.001**	**0.003**	**0.002**
STR	0.002	0.439	-0.001	0.080

positively influenced by stasis in flowering stage at Doubrava and by regression into small plants stage from both flowering and vegetative plants stages at Koridor.

Discussion

The presented study of long-term effects of systemic rust on perennial plant has found only minor differences between the whole population and the hypothetical healthy population at the same locality. There was only one year for one population in which the lambda of the healthy portion of the population was significantly higher than lambda of the whole population. Differences in stable stage distributions and elasticities between infected and healthy matrices were small or negligible as well. There was significant difference in all characteristics between the two types of populations – the field populations were having higher lambdas and relied more on growth and reproduction.

Effect of infection on population characteristics

There are several possible explanations for the non-significant difference in the healthy and whole population growth rates. First, there was considerable variation in the population characteristics. Although measures were taken to keep sufficient numbers of plants in all stages, in some years there were all plants in certain stage within the locality marked but still the numbers were low. This could have caused greater variability and skewed distributions of some approximations of lambdas and SSDs. On the other hand, the difference between healthy and diseased growth rates at some localities was consistently positive and in long-term this can lead to significantly larger populations of the healthy plants. Second, although three transitions at four localities is above-average dataset (Menges 2000; Crone et al 2011), it is still possible that the three selected years were in a way adverse. By extending the study one could come across a year that is exceptionally favourable for the healthy plants (or detrimental for diseased plants) like it probably was in case of Červená Píska and transition 2004-5. This could be analogous with result of Thrall and Jarosz (1994) who found that *Microbotryum violaceum* increased mortality of *Silene alba* only in years with mild winters.

In systemic pathogens the population level effects are often low (Wennstrom and Ericson 1991; Roy 1993; Wennstrom and Ericson 1994; Davelos and Jarosz 2004). It has been proposed that prolonged coevolution of plant and its systemic pathogen would lead to decrease of its aggressiveness as long as there is low chance of multiple infections (Frank 1992; Jarosz and Davelos 1995). Wennstrom (1994) also suggested that lower aggressiveness of disease is more typical for plants with weak or no lateral growth – *Falcaria vulgaris* forms

extensive taproot in the studied localities and clonal growth has not been recorded. Both proposed mechanisms support the notion that the selected system evolved towards tolerance of the disease rather than resistance (Roy and Kirchner 2000). This is further supported by the rather striking finding of this study that systemically infected plants are able to recover from the disease. There were few similar reports (Alexander and Antonovics 1988; Wennstrom and Ericson 1994) in which authors proposed that if the systemic infection does not reach the root before leaves die off in the summer, the infection does not become systemic. Although recovery was never high enough to drive the life cycle of the host, it did functionally offset the infection rate. Together with non-zero seed set of the infected flowering plants this probably lowered the effect of the disease at the population level.

Effect of disease on transitions importance

There was no considerable effect of the infection on importance of life cycle transitions evaluated as differences in the pattern of the elasticities. This might have been caused by the method, as transitions of healthy and whole populations were not directly comparable due to different number of stages. Davelos and Jarosz (2004) found no difference in lambda of infected and healthy populations but they found differences in SSDs and elasticities. It would not be correct to copy their method though, as it would mean combining infected and healthy plants into mixed stages – the results would then depend on the proportion of infected plants to great extent. After all, the result reflects the fact that the healthy plants are not influenced by the infected plants to any considerable extent. The transitions representing infection and recovery events were indeed having low elasticities. This was likely caused by their position in the life cycle. As the infected plants only rarely produce new healthy or potentially reproductive plants, their overall contribution to the growth rate is inevitably small which consequently leads to low elasticity of the whole loop of infection and recovery.

Differences among localities

The effect of locality and locality type was the predominant influence in the dataset. Localities differed in individual life-history rates, growth rates, stable stage distributions and also in the pattern of elasticities. *Falcaria vulgaris* populations on the calcareous ridge were stable or declining and relied largely on stasis and tended to accumulate infected plants as indicated by stochastic stage distribution approximations. Field populations were growing in size regardless of the disease and relied mostly on growth, survival in flowering stage and reproduction. These attributes cause projected stage distribution dominated by healthy plants.

Indeed, the population U strašáka was expansive population with lots of flowering plants in the first year but then it regressed because of low germination in the following years. It was likely caused by succession at the locality and because of progress of the disease. These findings indicate that environmental conditions play important role in plant-pathogen interactions although we cannot rule out the possibility that race-specific resistance was higher in the field populations. Unfortunately, we did not have means to determine genetic and resistance structure of the populations, which is otherwise subject of many plant-pathogen studies (Burdon et al 1996).

When I summarized the whole matrices SSDs to compute stochastic prevalence value, the only population that fitted the real values from field survey prior to the study was Doubrava. This is a sign that the population Doubrava was in equilibrium state. It also corresponds with Doubrava being the only population showing stable (negative) growth rates over all three years. All the other populations showed considerable variability in the growth rates over the scope of the study. Besides, the prevalence in year 2005 was result of trends in years prior to the survey while the stochastic projection summarizes the overall trend of the four studied years (Caswell 2001). The difference in prevalence between Doubrava and Červená píska is consistent with result of the infection rate analysis though – because Doubrava had below and Červená Píska above average infection rate. The results of LTRE showed that year and population differences can be attributed to single transitions. Interestingly, the same transition of stasis in reproductive stage was responsible for decrease in the whole and increase in the healthy partial matrix for Doubrava.

The result of the local population dynamics investigation is the more interesting as it sheds light on the prevalence data at the landscape level (Paper I. of the thesis). Although prevalences of the four studied populations did not follow the general pattern of higher prevalence on slopes (Table 1), the results of matrix modelling corresponded with the general notion of Paper I. that populations on calcareous soils tend to accumulate infected plants while populations on sandy dry soils tend to grow more rapidly thus prominently hosting healthy individuals.

Caveats

In study of such extent, some simplifications are inevitable and it is important to be aware of their potential effect on the general results. To determine the fecundity of infected plants I used the seed production numbers from flowering infected individuals whenever they were available. Germination of the seeds from the infected plants was unavailable and so I

used the germination of seeds from healthy plants. This is a possible shortcoming because the seed quality can be influenced by the infection (Jarosz et al 1989). However, I observed that the seeds developed on infected plants only if the systemic infection was not fast enough to grow into the flowers so the seeds should be comparable to seeds of the healthy plants although their numbers were smaller. This could have lead to lower survival and seedset in the seeds from infected plants but I believe that the difference is generally rather low.

Second, there is not a separate stage of seedlings, which is often incorporated in population studies and the germination was too low to follow survival of the sown seeds. On the other hand each year there were several seedlings observed and newly marked and their size was comparable with sizes of the small juveniles. However, larger plants regressing into small plants stage could have had higher survival than seedlings and juveniles. This could have led to undervalued mortality in the small plants stage and could have caused overvalued lambda and change in SSDs. Therefore, I always used the population characteristics for comparison and not for projection of the real population sizes.

Conclusions

This study shows that matrix models can help us to determine the overall effect of disease at the population level. In this particular system, it has been shown that the systemic disease can have relatively low effect on population characteristics in the populations of long-lived perennial plants despite the rather high prevalence. This is further accompanied by the novel finding that the observed systemic infection is sometimes not sustained until the next year. Finally, the study suggests that the effect of the disease is largely influenced by the environmental conditions.

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Attachments

Attachment 1 The stable stage distributions of all populations and transition intervals in percentages of all plants in a) the whole populations and b) healthy population parts; proportions over 0.2, 0.4 and 0.6 highlighted with shades of grey for whole populations and over 0.25 and 0. 5 for healthy population parts.

a)

		CP	DOU	KOR	STR
2004-5	1	0.15			
	2	0.16			
	3	0.01			
	4	0.24			
	5	0.44			
	6	0.00			
2005-6	1	0.20	0.21	0.10	0.66
	2	0.15	0.06	0.04	0.13
	3	0.01	0	0.01	0.05
	4	0.28	0.46	0.71	0.08
	5	0.14	0.23	0.14	0.04
	6	0.22	0.04	0.00	0.03
2006-7	1	0.26	0.61	0.40	0.61
	2	0.05	0.07	0.23	0.09
	3	0	0.02	0.09	0.02
	4	0.66	0.26	0.06	0.12
	5	0.03	0.04	0.20	0.16
	6	0	0	0.02	0.00
2007-8	1	0.33	0.59	0.40	0.34
	2	0.30	0.24	0.34	0.27
	3	0.31	0.05	0.14	0.04
	4	0.01	0.05	0.01	0.08
	5	0.04	0.06	0.11	0.16
	6	0	0	0.00	0.11

b)

		CP	DOU	KOR	STR
2004-5	1	0.44			
	2	0.53			
	3	0.03			
2005-6	1	0.26	0.73	0.63	0.77
	2	0.69	0.27	0.29	0.16
	3	0.05	0	0.08	0.07
2006-7	1	0.90	0.86	0.54	0.85
	2	0.10	0.10	0.32	0.13
	3	0	0.03	0.14	0.02
2007-8	1	0.35	0.65	0.43	0.49
	2	0.33	0.31	0.40	0.45
	3	0.32	0	0.17	0.06

Attachment 2 Matrices for the whole populations and the respective elasticities; probability of transition from t (columns) to t+1 (rows); elasticities over 0.05 bold and over 0.1 shaded; lambdas of the populations are indicated in brackets in the same order as matrices

		transitions						elasticities					
		1	2	3	4	5	6	1	2	3	4	5	6
2004-2005	1	0.43	0.11	5.29	0.06	0.02	0	**0.0860**	0.0243	**0.0585**	0.0189	0.0091	0
	2	0.29	0.71	0.08	0	0	0	**0.0938**	**0.2498**	0.0015	0	0	0
	3	0	0.02	0.67	0	0	0	0	**0.0606**	**0.1287**	0	0	0
	4	0.14	0.03	0	0.59	0.15	0	0.0082	0.0020	0	**0.0543**	0.0260	0
	5	0.14	0.13	0.17	0.18	0.79	0	0.0087	0.0085	0.0006	0.0173	**0.1433**	0
	6	0	0	0.08	0	0	0	0	0	0	0	0	0
2005-2006	1	0.47	0.07	1.218	0	0.02	0.38	0.0026	0.0003	0.0002	0	0.0001	0.0024
	2	0.27	0.64	0.27	0	0	0	0.0030	0.0055	0.0001	0	0	0
	3	0	0.02	0.45	0	0	0	0	0.0027	0.0023	0	0	0
	4	0.07	0.07	0	0.72	0.38	0	0.00001	0	0	0.0001	0.00002	0
	5	0.07	0.18	0.09	0.08	0.57	0	0.00002	0.00004	0	0.00003	0.0001	0
	6	0	0	0.09	0	0	1.00	0	0	0.0024	0	0	**0.9780**
2006-2007	1	0.72	0.63	2.447	0	0.17	0.25	**0.1874**	0.0315	0	0	0.0047	0
	2	0.06	0.27	0.13	0.02	0	0.50	0.0184	0.0176	0	0.0193	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0
	4	0.06	0.04	0.13	0.81	0.54	0	0.0178	0.0024	0	**0.6530**	0.0182	0
	5	0	0.06	0.13	0.02	0.20	0.50	0	0.0038	0	0.0192	0.0067	0
	6	0	0	0	0	0	0	0	0	0	0	0	0
2007-2008	1	0.28	0.19	0.675	0	0.21	0	0.0324	0.0199	0.0722	0	0.0028	0
	2	0.37	0.69	0	0	0.07	0	0.0469	**0.0794**	0	0	0.0010	0
	3	0.07	0.04	1.00	0.08	0	0	0.0457	0.0243	**0.6538**	0.0022	0	0
	4	0	0	0	0.46	0.21	0	0	0	0	0.0025	0.0035	0
	5	0.02	0.04	0	0.34	0.50	0	0.0023	0.0037	0	0.0013	0.0060	0
	6	0	0	0	0	0	0	0	0	0	0	0	0

a) Červená Píska (0.98, 1.00, 0.86, 1.11)

		transitions						elasticities					
		1	2	3	4	5	6	1	2	3	4	5	6
2005-2006	1	0.44	0.47	0.25	0.04	0	0.14	**0.1217**	0.0385	0	0.0248	0	0.0086
	2	0.12	0.29	0.14	0	0	0	0.0486	0.0352	0	0	0	0
	3	0	0	0.18	0	0	0	0	0	0	0	0	0
	4	0.04	0.06	0	0.52	0.29	0.14	0.0089	0.0039	0	**0.2582**	**0.0697**	0.0069
	5	0.04	0.06	0.07	0.08	0.43	0.29	0.0144	0.0063	0	**0.0645**	**0.1699**	0.0224
	6	0	0	0.05	0	0.05	0.43	0	0	0	0	0.0378	**0.0598**
2006-2007	1	0.57	0.41	2.282	0.03	0	0.62	**0.5499**	0.0448	**0.0876**	0.0122	0	0
	2	0.04	0.33	0.16	0	0.03	0	0.0384	0.0397	0.0066	0	0.0017	0
	3	0.02	0	0.26	0	0	0.10	**0.0947**	0	**0.0539**	0	0	0
	4	0.04	0.04	0	0.55	0.58	0.20	0.0074	0.0008	0	0.0458	0.0068	0
	5	0.02	0.04	0.05	0.03	0.08	0	0.0042	0.0010	0.0005	0.0029	0.0011	0
	6	0	0	0	0	0	0.10	0	0	0	0	0	0
2007-2008	1	0.38	0.29	2.415	0.02	0	0.05	**0.2068**	**0.0646**	**0.1199**	0.0010	0	0
	2	0.15	0.35	0.06	0	0	0	**0.0815**	**0.0802**	0.0029	0	0	0
	3	0.02	0	0.39	0	0.13	0	**0.0726**	0	**0.1423**	0	0.0502	0
	4	0	0.06	0	0.30	0.13	0	0	0.0198	0	0.0220	0.0105	0
	5	0.02	0	0	0.20	0.38	0	0.0314	0	0	0.0293	**0.0651**	0
	6	0	0	0	0	0	0	0	0	0	0	0	0

b) Doubrava (0.70, 0.73, 0.72)

		transitions						elasticities					
		1	2	3	4	5	6	1	2	3	4	5	6
2005-2006	1	0.57	0.19	2.342	0	0	0.03	**0.1979**	0.0272	**0.0975**	0	0	0.0001
	2	0.14	0.52	0.21	0	0	0	**0.1248**	**0.1886**	0.0221	0	0	0
	3	0	0.10	0.61	0	0	0	0	**0.1197**	**0.2219**	0	0	0
	4	0.14	0.07	0	0.83	0.39	0.08	0	0	0	0	0	0
	5	0	0.12	0.03	0.06	0.60	0.85	0	0	0	0	0	0
	6	0	0	0.08	0	0	0	0	0	0.0001	0	0	0
2006-2007	1	0.38	0.03	3.679	0	0	0.34	**0.0944**	0.0045	**0.2111**	0	0	0.0047
	2	0.29	0.69	0.09	0	0.02	0	**0.0836**	**0.1135**	0.0058	0	0.0034	0
	3	0.08	0.09	0.65	0	0	0	**0.1342**	**0.0869**	**0.2373**	0	0	0
	4	0.04	0	0	0.54	0.15	0	0.00009	0.0000	0	0.0002	0.0002	0
	5	0.13	0.13	0.06	0.08	0.73	0.57	0.0025	0.0014	0.0003	0.0002	0.0070	0.0006
	6	0	0	0.15	0	0.02	0.43	0	0	0	0	0.0014	0.0028
2007-2008	1	0.08	0.03	4.042	0.04	0	0.06	0.0146	0.0043	**0.2558**	0.0002	0	0.0001
	2	0.75	0.66	0.06	0	0	0	**0.0970**	**0.0720**	0.0026	0	0	0
	3	0.17	0.11	0.83	0	0	0	**0.1634**	**0.0950**	**0.2942**	0	0	0
	4	0	0.03	0	0.35	0.06	0	0	0.0001	0	0.0001	0.0001	0
	5	0	0.17	0.06	0.42	0.89	0.38	0	0.0001	0.0000	0.00001	0.0002	0
	6	0	0	0	0	0.03	0.63	0	0	0	0	0.0001	0.0001

c) Koridor (0.93, 1.25, 1.57)

		transitions						elasticities					
		1	2	3	4	5	6	1	2	3	4	5	6
2005-2006	1	0.57	0.21	11.91	0	0	1.10	**0.1393**	0.0099	**0.2216**	0	0	0.0139
	2	0.21	0.45	0.06	0	0	0	**0.1052**	0.0424	0.0021	0	0	0
	3	0.04	0.13	0.78	0	0	0	**0.1313**	**0.0935**	**0.2183**	0	0	0
	4	0.07	0.03	0	0.67	0.43	0.13	0.0004	0.00003	0	0.0005	0.0002	0.00004
	5	0	0.08	0.08	0.33	0.44	0.25	0	0.0002	0.0001	0.0007	0.0005	0.0002
	6	0.04	0.08	0.06	0	0.07	0.44	0.0085	0.0036	0.0010	0.0000	0.0011	0.0054
2006-2007	1	0.68	0.31	9.393	0.03	0.02	0.36	**0.2508**	0.0164	**0.1017**	0.0019	0.0020	0.0009
	2	0.09	0.33	0.13	0	0.02	0.05	**0.0822**	0.0450	0.0036	0	0.0049	0.0003
	3	0	0.06	0.56	0.03	0	0	0.0000	**0.0647**	**0.1305**	0.0415	0	0
	4	0	0.03	0	0.54	0.35	0.10	0	0.0026	0	**0.0690**	**0.0571**	0.0004
	5	0.09	0.11	0.07	0.18	0.45	0.35	0.0407	0.0074	0.0009	0.0167	**0.0537**	0.0011
	6	0	0	0	0	0.02	0.25	0	0	0	0	0.0028	0.0009
2007-2008	1	0.14	0	8.406	0.05	0	0	0.0349	0	**0.2516**	0.0029	0.0000	0
	2	0.39	0.58	0.08	0	0.08	0	**0.0728**	**0.0848**	0.0017	0	0.0073	0
	3	0.05	0.03	0.53	0.02	0	0	**0.1634**	**0.0704**	**0.2205**	0.0200	0	0
	4	0.09	0.05	0	0.39	0.08	0	0.0133	0.0057	0	0.0130	0.0057	0
	5	0.11	0.15	0.05	0.17	0.44	0.14	0.0051	0.0052	0.0003	0.0017	0.0091	0.0021
	6	0	0.03	0.10	0	0.13	0.86	0	0.0005	0.0003	0	0.0014	0.0066

d) U strašáka (1.58, 1.01, 1.13)

Attachment 3 Matrices for the healthy part of the populations and the respective elasticities; probability of transition from t (columns) to t+1 (rows); elasticities over 0.2 bold and over 0.4 shaded; lambdas of the populations are indicated in brackets in the same order as matrices

		transitions			elasticities		
		1	2	3	1	2	3
2004-5	1	0.60	0.13	5.29	0.1196	0.0326	0.0837
	2	0.40	0.85	0.11	0.1163	**0.2984**	0.0026
	3	0	0.02	0.89	0	0.0862	**0.2606**
2005-6	1	0.54	0.09	1.22	0.0968	0.0421	0.0393
	2	0.31	0.85	0.33	0.0814	**0.5994**	0.0158
	3	0	0.03	0.56	0	0.0551	0.0702
2006-7	1	0.76	0.70	2.66	**0.7962**	0.0794	0
	2	0.06	0.30	0.17	0.0794	0.0450	0
	3	0	0	0	0	0	0
2007-8	1	0.2889	0.2	0.6747	0.0338	0.0225	0.0738
	2	0.3778	0.72	0	0.0495	0.0908	0
	3	0.0667	0.04	1	0.0468	0.0270	**0.6559**

a) Červená Píska (1.18, 0.99, 0.84, 1.11)

		transitions			elasticities		
		1	2	3	1	2	3
2005-6	1	0.48	0.53	0.29	**0.4453**	0.1872	0
	2	0.13	0.33	0.16	0.1872	0.1803	0
	3	0	0	0.21	0	0	0
2006-7	1	0.61	0.44	2.32	**0.6145**	0.0526	0.0896
	2	0.04	0.36	0.18	0.0449	0.0488	0.0077
	3	0.02	0	0.24	0.0973	0	0.0446
2007-8	1	0.39	0.31	2.42	**0.2838**	0.1101	0.1088
	2	0.15	0.38	0.06	0.1076	0.1316	0.0025
	3	0.02	0	0.39	0.1113	0	0.1443

b) Doubrava (0.68, 0.75, 0.69)

		transitions			elasticities		
		1	2	3	1	2	3
2005-6	1	0.67	0.24	2.35	0.1997	0.0320	0.0926
	2	0.17	0.65	0.24	0.1246	**0.2195**	0.0231
	3	0	0.12	0.68	0	0.1157	0.1928
2006-7	1	0.45	0.04	3.69	0.0972	0.0045	**0.2085**
	2	0.35	0.79	0.11	0.0805	0.1053	0.0067
	3	0.10	0.11	0.81	0.1325	0.0827	**0.2821**
2007-8	1	0.08	0.04	4.04	0.0130	0.0051	**0.2403**
	2	0.75	0.82	0.06	0.1092	0.1099	0.0033
	3	0.17	0.14	0.88	0.1362	0.1073	**0.2757**

c) Koridor (1.08, 1.44, 1.66)

		transitions			elasticities		
		1	2	3	1	2	3
2005-6	1	0.64	0.26	11.92	0.1356	0.0113	**0.2215**
	2	0.24	0.55	0.06	0.1105	0.0521	0.0026
	3	0.04	0.16	0.90	0.1223	0.1018	**0.2423**
2006-7	1	0.74	0.35	9.40	**0.3621**	0.0273	0.1094
	2	0.10	0.39	0.14	0.1367	0.0863	0.0048
	3	0	0.06	0.60	0	0.1142	0.1593
2007-8	1	0.17	0	8.42	0.0394	0	**0.2546**
	2	0.49	0.74	0.09	0.0860	0.1217	0.0021
	3	0.06	0.03	0.62	0.1685	0.0881	**0.2396**

d) U strašáka (1.74, 1.02, 1.28)

Effect of systemic diseases on clonal integration: modelling approach

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Abstract

Systemic disease spread has been suggested as a possible disadvantage of clonal plant integration. As connected ramets have higher risk of being infected, disease should cause a selective pressure against clonality. Since experimental tests of this hypothesis are not easy to perform, we chose a modelling approach, by which we could easily separate different factors influencing the process. We used a spatially explicit model of clonal growth with disease spread implemented and we tested the hypothesis that systemic disease decreases the competitive ability of highly integrated clonal plants when compared to less integrated plants with the same parameters.

In contrast to our expectations, the integrator was competitively stronger than the splitter in most cases and it lost only when the disease severity and infection rates were very high. We think that the larger the integrated network is, the better the plant utilizes its translocation ability. Even a very small amount of resource sharing greatly increased the relative success of the integrator and larger integrators were competitively stronger than the smaller ones. Our results also indicate that although the same infection rate caused more systemic disease in the integrator than in the splitter population, the disease has only a limited potential to select for the splitter strategy. This is caused not only by the advantages of the clonal integration but also by the fact that there is only a small range of infection rates at which there is sufficient difference in disease impact between the strategies.

Co-authorship statement: The co-author Tomáš Herben agrees that the presented paper will be part of the submitted thesis of Tomáš Koubek who performed major part of obtaining the data and writing of the manuscript.

Keywords: Clonal plants, Disease spread, Resource translocation, Systemic pathogens, Spatially explicit modelling

Introduction

Clonality is an ability to form new individuals (ramets) vegetatively and stay interconnected for some time. It provides many benefits that include support of daughter ramets (Marshall and Price 1997), translocation of carbohydrates, mineral nutrients and water from resource-rich to resource-poor patches (e.g. Alpert 1996; Stuefer et al 1996; Hutchings and Wijesinghe 1997) or information transport that allows early defence against parasites (Gomez and Stuefer 2006).

On the other hand, the very same connectedness of the ramets may handicap the genet. The ramet specialisation could be disadvantageous if the connection was interrupted externally (e.g. by trampling or herbivory, Kelly 1995) and spread of systemic pathogens could be facilitated. Infection spread in the clonal network has indeed been mentioned as a possible important exogenous negative effect of ramet connectedness (Cook 1985; Pitelka and Ashmun 1985; Eriksson and Jerling 1990; Kelly 1995; Fischer and van Kleunen 2001; Stuefer et al 2004). Diseases such as viruses can spread through the plant vascular system (Cheng et al 2000) while fungal pathogens are able to grow their hyphae along vascular vessels through intercellular spaces (Mendgen and Hahn 2002). As the infection effects are in most cases negative, clonal integration should decrease the fitness of interconnected ramets if infection risk is high enough.

There are ways to escape the systemic disease effects in clonal plants. (i) Clonal growth can in case of fungal pathogens be faster than the growth of the mycelia and thus the plant can simply outgrow the infection (Wennström and Ericson 1992; Frantzen 1994; D'Hertefeldt and van der Putten 1998; Piqueras 1999); however, this does not apply for viral infections, which spread much quicker. (ii) Another defence mechanism is splitting into independent ramets or clonal fragments so the plant can spread the risk of being infected (Eriksson and Jerling 1990; Piqueras and Klimeš 1998). If the mortality of the ramets becomes independent, the genet persistence is likely to be increased. (iii) Some plants are even able to detach infected ramets or tissues deliberately as a reaction to an infection but this has been rarely reported as an effective way of protection against systemic pathogens (McCrea and Abrahamson 1985). Although there are some ways to avoid the infection spread and its effects, there are still many host-pathogen systems where the transmission of the infection is successful and threatens the host plant.

Clonality has appeared and also disappeared many times in the evolution of plants (Klimeš et al 1997) and we can only hypothesize what were the causes of the switches. There

are many possible causal agents such as changes in the environmental conditions or changes in the species interactions (van Groenendael et al 1996). Systemic diseases might have influenced the evolution and persistence of clonality as well. Stuefer et al (2004) have proposed that strong selection pressures of pathogen might have caused evolution of early genet fragmentation. Consequently, this could have lead to evolution of early splitting genotypes or even to giving up clonality entirely.

While the conceptual matter is rather clear, closer analysis of the effect of systemic infections on clonality is difficult to make as it would require examination of disease-related fitness changes in plants that differ in the degree of splitting. As clonal plants are usually obligate integrators or splitters, no direct competition experiment can be performed to test this unless we use artificial spacer severing. However, spacer severing is known to have side effects in some species (Kelly 1995). On the other hand, the fitness change can be tested by a simulation study that allows easy manipulation of plant traits (such as fragmentation) independently of any other traits.

Therefore we used a spatially explicit simulation model of a clonally growing plants of Herben and Suzuki (2001, see also Herben 2004) with disease infection and spread implemented. The model has sufficient realism to be parameterized for real plants and can therefore be used to test specific hypotheses on the role of clonal growth parameters (see Wildová et al 2007). We used a simple resource reducing disease with fast spread in our simulations. Disease effects differ considerably between systems and they often change the parameters of the host such as the resource translocation or generative reproduction. We wanted to see the net effects of the systemic disease on these parameters and so we did not use a disease with any complex behaviour or influence.

First, we tested the hypothesis that ramets in interconnected networks have lower competitive abilities than ramets in less connected networks due to disease spread. Furthermore, we tested how the results change with varying resource availability and with different incidence and severity of the disease. Finally, we tested how our hypothesis applies to different sets of characteristics of the clonal plant such as amount of the resource shared or size of the clonal fragments. In order to examine net effects of these processes, we assumed that the infected and non-infected plants differ only in the amount of produced (or acquired) resource and they are completely identical in all other respects.

Methods

The model

In this study we used a time-tested model of clonal growth (Herben and Suzuki 2001; Herben 2004; Wildová et al 2007; Herben in this issue). The model runs on a continuous plane with toroidal boundaries. The simulation plane is homogeneous; any heterogeneity is generated by the ramets themselves. The model uses traits of ramet growth, allocation, competitive ability, and spacer (rhizome) architecture to simulate long-term population dynamics. The model represents ramets of fixed sizes. These ramets acquire "resource" for rhizome growth. This resource may be anything limiting for the plants that is either gathered by the ramet from the environment (e.g., water, nutrients) or synthesized by it (photosynthate) and whose accumulation is density-dependent. The rate of resource acquisition by a ramet is determined by competition with neighbouring ramets; in each time step, the number of ramets in the neighbourhood determines the amount of resource accumulated within each ramet. This amount can be positive or negative; the latter if the effect of neighbouring ramets is strong. The resource is put into the node bearing the ramet. Resource levels at each node change by resource acquisition by the ramet attached to that node, and by its consumption for growth. Any resource not used is left at the node for the next time step. If resource at a terminal node is zero or negative, the node loses the capacity for further growth and dies.

Rhizomes grow by adding nodes only at terminal positions. A new node is always added to a terminal node if the quantity of resource available at the existing terminal node is sufficient. When a new node is added; the original ramet always dies and a new ramet is formed at the newly added terminal node (i.e. only replacement growth is modelled – the original ramet “moves” to the new node). Therefore, ramets are by definition attached to all growing terminal nodes and to no other nodes. If a new node is added, the length and angle of the growth of the internode are independent of the amount of resource in the rhizome and the density of ramets or rhizomes in its neighbourhood. Resource at the original node is reduced by a fixed cost (which is one of the model parameters) when an internode is added. After a new node is added, part of the resource accumulated at the maternal node is passed to the daughter node.

Nodes may also be added to a rhizome by terminal branching (i.e., by adding two terminal nodes to one in a single time step). Branching processes take place only if the available quantity of resource at that node is sufficient and if the branching is not constrained by architectural rules - the architectural constraint determines the minimum number of nodes

between branching points. Branching angle is independent of the resources of the rhizome and of its neighbourhood. If a node bearing a branch dies, the branch becomes independent and the rhizome fragments into two. The length of the whole rhizome system is controlled by a process, which removes internode from the basal part in each step as it reaches a specified maximum age. This is an important characteristic of the model plants as it enables formation of strategies with different degree of splitting.

At the beginning, the system is initiated by a fixed number of seedlings. Further, in each time step, there is an additional seedling recruitment defined as a Poisson distributed variable with a mean of $f \times n$, where f is the mean ramet fertility and n is the number of existing ramets. The mean ramet fertility was set to 0.05, which creates approx. 20 to 30 seeds of both strategies together in every step in equilibrium stage (compared to approx. 10 to 20 ramets created by clonal growth). No difference in fertility was assumed between infected and non-infected ramets.

Translocation modelling

The nodes in one interconnected system share specified proportion of their unused resource. Resource translocation takes place at all nodes, no matter whether terminal or not, or whether they bear a ramet or not. Translocation is driven by the resource available at potential donor nodes. Each donor node searches for potential sinks up to specific distance (sharing range), both basipetally and acropetally; all relevant branches in the acropetal direction are considered for translocation. Branches in the basipetal direction are not considered as this would include first basipetal and then acropetal translocation (Kemball and Marshall 1995). Three types of sinks are distinguished (i) terminal ramets (ii) non-ramet nodes in acropetal position and (iii) non-ramet nodes in basipetal position – strengths of these sinks were set to 1:1:0.5 respectively. There is no translocation cost included.

Modelling of the disease

In each step, specified proportion of ramets is infected with a "disease"; the ramets to be infected are chosen randomly. The infection rate is constant in the particular run and does not depend on the number of infected ramets in the population. As soon as the disease infects one ramet of an interconnected system, it spreads instantly through the whole fragment and infects all connected ramets (similarly to the virus particles). The disease either causes instant death of the whole fragment or it reduces the accumulated resource of all its ramets by a constant

proportion (which is a parameter of the disease). Infected plants produce the same amounts of seeds as healthy plants and the disease is not carried by the seed.

Parameterisation

The model was parameterised to represent a clonally growing plant with ramets with little variation in size. The simulation plane was assumed to represent an area sufficiently large to cover reasonably large rhizome systems of this plant. To minimise arbitrariness in the choice of parameter values, basic parameter values were selected to approximate values from a stand of grass ramets in short-turf grassland in an area of 0.5 x 0.5 m in size. We used some data on architectural and growth parameters from a previously studied mountain grassland system (Herben et al 1993; Hara and Herben 1997; Pecháčková et al 1999; Suzuki et al 1999) but some of the parameters were changed. We switched off secondary ramet formation and activation of sleeping buds to avoid ramet loss at the basipetal end when the adjacent internode reaches its maximum age. This means that the plant cannot be taken as a particular plant species but still the parameters are more or less realistic.

Simulation experiments

The experiments were done with two strategies called splitters and integrators. The splitter was defined by internode lifetime of 10 steps and by the same sharing range (previously mentioned distance of translocation). The lifetime of the integrator's internode was 20 or 30 steps and the same were its sharing ranges. The two types of integrator strategy were chosen to see if bigger difference between the splitter and the integrator would change the results. All other parameters were the same for both strategies in each run. The parameters whose interactions with disease effects were tested and their values are shown in Table 1.

(i) In order to interpret the results correctly we have made single-strategy runs for only the splitter and the integrator with sets of parameters identical for both strategies. The run was

parameter	**description**	**values tested**	
		basic comp.	int. invasion
resource availability	relative amount of resource available to plants, the number is arbitrary	1, 3, 5	3
disease severity	amount of resources consumed by the disease (value 1 kills plant immediately)	1, 0.75, 0.5, 0.25	1, 0.75
proportion of resources shared	maximum amount of resource a ramet can translocate to other ramets	0, 0.05, 0.1, 0.25, 0.5	
infection rate	proportion of ramets of each species randomly infected by disease	0, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1	

50 steps long and started with 100 individual ramets. (ii) The first competition experiment (further called basic competition exp.) started with 100 randomly distributed ramets of each strategy and the plants grew and produced seeds for 200 steps. Results of this simulation covered the main parameter/competition response space of both strategies. As the integrator gained dominance in most runs, we performed another simulation to trace integrators relative competition strength. (iii) The second competition experiment was done with an invasion approach (further will be called integrator invasion experiment). It started with 100 ramets of the splitter that grew freely for 100 steps (such population more or less reached equilibrium). After 100 steps, 10 ramets of the integrator were added. The simulation finished after another 100 steps, which is 200 steps in total.

Results

Single-strategy runs

Ramets of both strategies were infected with the same rates in all simulations and thus any differences were caused mainly by differences in connectedness. Proportion of infected ramets (Fig. 1.) was quite low for infection rates between 0.0005 and 0.01 but it grew rapidly for values 0.05 and 0.1. It was always higher in integrator than in splitter but the difference decreased as the infection rate increased. The proportion of integrator to splitter ramets was over three at infection rate of 0.0005 and it decreased gradually to 1.5 at infection rate of 0.1; that was with approx. 65% infected integrators and 45% infected splitters.

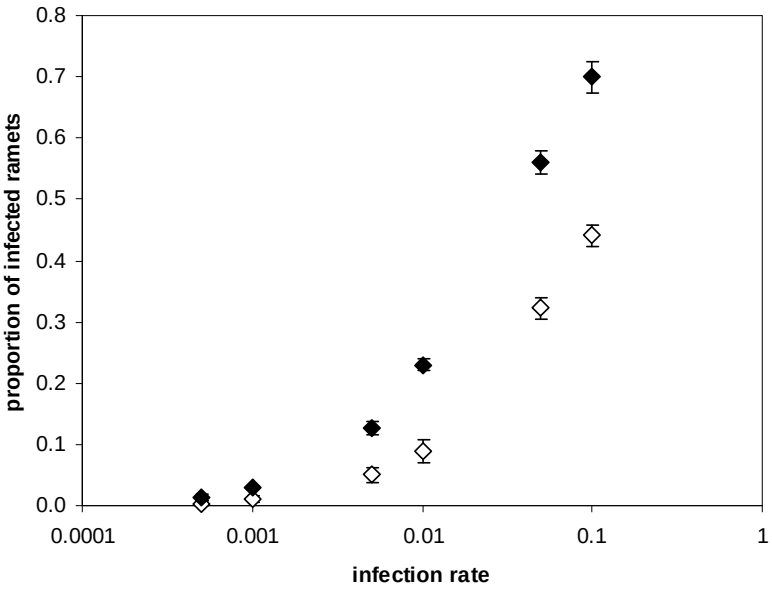
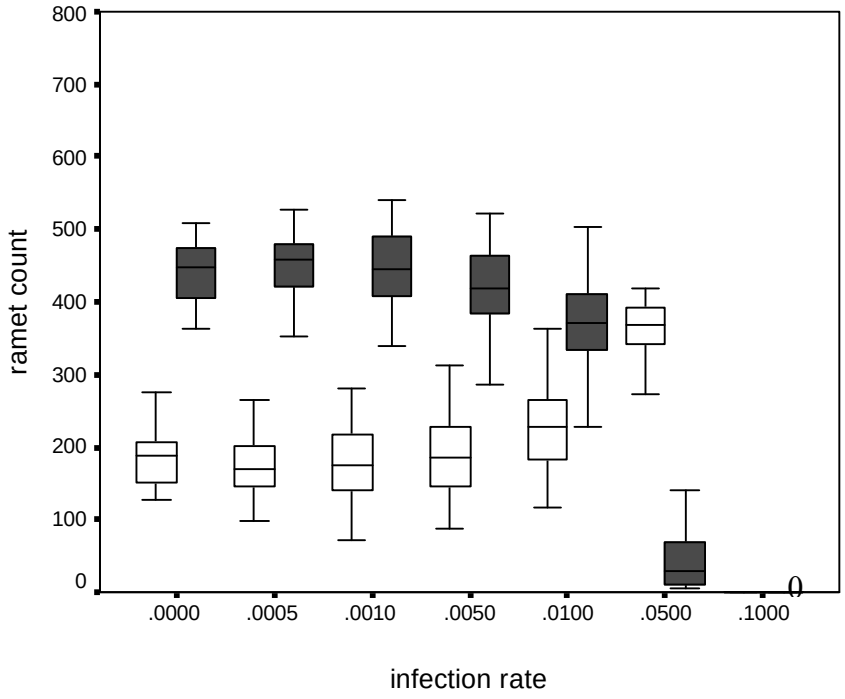


Fig. 1. Proportions of infected splitter (empty) and integrator (filled) ramets in single strategy runs after 50 steps; disease severity 0.75, proportion shared 0.25, resource availability 3, integrator 30 ramets long. The data shown are means and standard deviations.

a)



b)

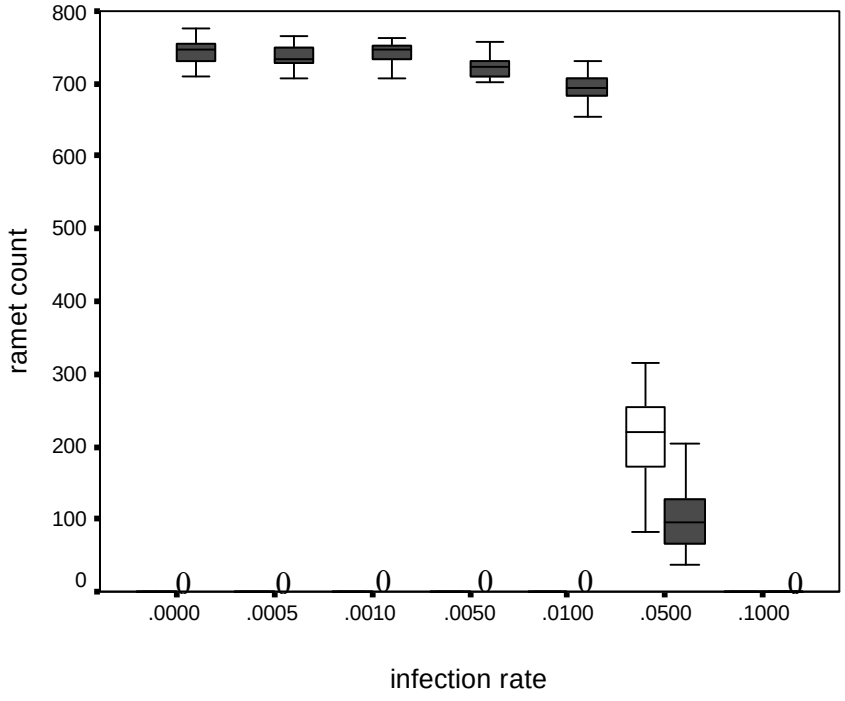


Fig. 2. Results of a the basic competition experiment with equal initial numbers (100 ramets) of splitters (white) and integrators (grey); 200 steps, resource availability 3, disease severity 1, integrator 30 ramets long; a) proportion of resource shared 0, b) proportion of resource shared 0.25. The data shown are medians, quartiles and minimal/maximal adjacent values.

Basic competition experiment

The integrator (with sharing range both 20 and 30 nodes) was competitively superior to the splitter in almost all parameter settings. Interestingly, when there was no translocation, the integrator was slightly better than the splitter (Fig. 2a). With higher proportion of resources shared, the integrator was able to take advantage of integration of its nodes and it was much stronger than without translocation (Fig. 2a, b). Only when two other factors were simultaneously at high values – the severity of the disease was 1 (meaning that the infected plant was killed instantly) and the infection rate was as high as 0.05 (5% of ramets randomly infected in each step) – the splitter was able to outcompete the integrator. The effect of different levels of resource availability was not very strong. However, when the resource was relatively scarce, the integrator was even a little more successful than with higher resource levels since it could translocate the deficient resource (data not shown).

Integrator invasion experiment

This simulation was carried out with a smaller range of parameter values (see Table 1). The integrator was generally successful in invading the splitter population. When translocation was set to zero, the final amount of integrator ramets was in most cases at its initial values (average of 10 ramets, no invasion success); nevertheless with increasing infection rate its success became a bit higher as an outcome of the model configuration. To see the net result of the invasion for all other levels of translocation parameter, we visualised the results as values of the level minus average of the zero-translocation level.

If the disease severity was set to instant death (1), the integrators success decreased with increasing infection rates. If the disease was milder (0.75), the integrators strength decreased with increasing infection rate only in integrator with sharing range 20 nodes. The success of the integrator sharing resources 30 nodes far was slightly higher than that of the one sharing on shorter distances and it was even stable across the infection rates gradient when disease severity was 0.75 (Fig. 3.). Increasing the proportion of shared resources did not affect the integrator's success much, though when the ramets shared potentially as much as half of its resources the integrator appeared to be a little less successful in invading the population of the splitter (data not shown).

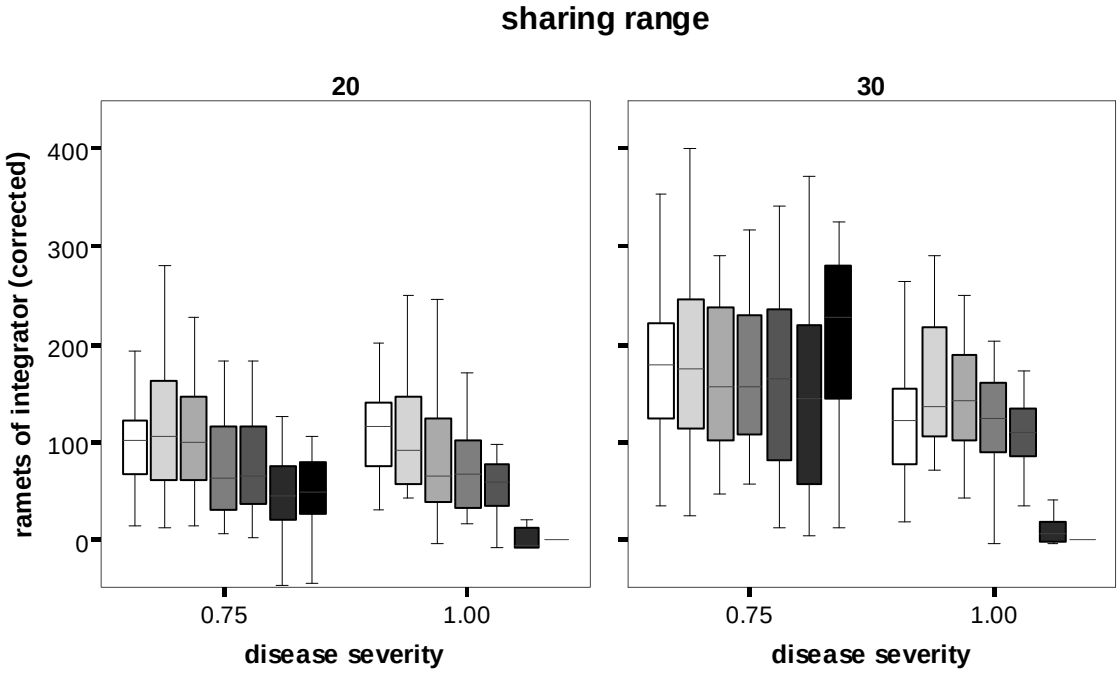


Fig. 3. Results of the integrator invasion experiment - ramet counts of the integrator after invading the splitter population (corrected by zero-translocation values); resource availability 3, proportion shared 0.1. The colours represent infection rates – 0 (□), 0.0005 (□), 0.001 (□), 0.005 (□), 0.01 (□), 0.05 (□) and 0.1 (■).The data shown are medians, quartiles and minimal/maximal adjacent values.

Discussion

Effect of systemic disease on performance of splitters and integrators

The results of our simulations suggest that systemic growth reducing diseases have limited potential to create selective pressure against the integration in clonal plants. It is important to note that integration has almost invariably positive effects on growth of the modeled plants, namely by enabling better allocation of resources to growth and competition (Stuefer et al 1994; Hutchings and Wijesinghe 1997; Oborny et al 2000; Herben 2004) and this effect is much stronger in plants that are integrators. Therefore if a systemic disease is to have a negative effect, it must balance these positive effects - given differences in infection probability of large clonal fragments relative to the small ones.

If you look at the results of the single-strategy simulations (Fig. 1.) it is obvious that there is not much disease effect when the infection rates are low. In the range between 0.0005 and 0.01, the integrator has to cope with much more infection than the splitter but a majority

of the population (almost three quarters of integrator ramets) stays unaffected. At the other side of the continuum (infection rate 0.1) both strategies must deal with the disease because more than 45% of ramets of the splitter and 65% of ramets of the integrator are infected. Importantly, the relative difference between the strategies is however not as high at this infection rate. The "operational space" where the disease makes an effective difference between the two strategies (around infection rate of 0.05) is hence quite narrow. There is a whole array of possible infection rates in the field that differ between and among species and habitats and are highly variable due to environmental factors as well (Burdon 1987). The probability that some clonal host-disease system will fit into this operational space is small and therefore we think that the effect of disease spread on clonal integration is rather restricted by this simple fact.

The basic competition experiment has shown that although the disease infects a higher proportion of ramets of the integrator than of the splitter the difference in proportion of infected integrators and splitters is high enough to compensate for the advantage due to integration only at infection rate 0.05 and lethal disease (Fig 2b). However, combination of lethal effects and high infection rate is probably quite uncommon in systemic diseases. First, the effects of systemic diseases are generally milder because genotypes favouring early death would have selective disadvantage (Clay and Kover 1996; Garcia-Guzman and Burdon 1997); for example Alexander and Antonovics (1988) found no difference in mortality of *Silene alba* plants systemically infected by *Microbotryum violaceum*. Second, the infection rates at which our splitter wins are quite high – 0.05 means 5% of ramets infected in each step which is approximately 30 – 50% of infected the fragments of integrator in 10^{th} time step. High disease levels are generally occurring more in systems with non-systemic pathogens (Burdon 1987; Ericson et al 2002) and systemic pathogens rather stay at lower disease levels although this largely depends on particular system, population sizes, spatial population structure and other environmental conditions (Wennström and Ericson 1991, 1994; Colling and Matthies 2004; personal observations). However, limited pathogenicity in systemic pathogens can have various causes and remains to be examined.

Apart from the main result that the populations of integrator suffer from the disease less than those of the splitter in most cases, there were some other trends observed. In the integrator invasion experiment we examined differences in the integrator success in different parameter settings. As the infection rate increased, the integrator was generally less successful in invading the splitter. This was not the case only when the integrator was translocating through 30 internodes and disease severity was set to 0.75. With this "milder" disease setting,

the integrator survived even if it was infected and it could outcompete the splitter, which had comparable amount of infected ramets. Because the effect of the disease was defined as a decrease in resource, it caused general decrease of the competition in the simulation plane and increased the integrator's advantage gained by translocation among more ramets (Fig. 3). These findings support the main results as increase in the length of the interconnected system does not pose a greater threat due to more extensive disease spread but rather increases the positive effect of long distance translocation.

Limitations of the approach

One of our models properties was the maximum age of an internode that enables the splitting process. This property caused the integrator to have some advantage even at zero translocation where it should have been equal to the splitter. Under severe competition the plants probably weren't able to grow new terminal ramets in each step; the last terminal internode eventually reached its maximum age and was removed. The attached node and ramet were then removed as well because a node had to be by definition attached to an internode. This process probably influenced the splitter slightly more than the integrator. It was an inevitable consequence of the fragmentation of the network and we chose not to correct this in the model but rather to take the zero-translocation level as a control compared with the other levels. Although the integrator had some initial advantage, with just a little translocation (5% of unused resource) it was much stronger (data not shown) and we think that this confirms its competitive superiority regardless the initial conditions.

We are deliberately using a few simplifications in our model as otherwise the interpretation would be difficult because of too many parameters operating at the same time. First, there was no translocation cost employed in the model. This gives some advantage to the integrator as it translocates over larger distances and thus it would need more resource to cover the costs than the splitter. However, earlier examination of the effects of translocation cost has shown that it has only additive effects on the model behaviour (Herben 2004); therefore it is likely that introducing translocation cost would help the splitter, but would not alter the overall picture. Moreover, this effect would depend on the numerical value of the cost, which may be difficult to estimate from the field data. The experimental data on *Potentilla anserina* (van Kleunen and Stuefer 1999) for example show that the translocation cost is small or absent and is outweighed by translocation benefits.

The ramets of one clonal fragment got all infected instantly although in systems with fungal pathogen this may not be true (Wennström and Ericson 1992; Frantzen 1994;

D'Hertefeldt and van der Putten 1998; Piqueras 1999). However, our preliminary tests with modelling slow infection (behaving more like pathogenic fungi) showed responses comparable to no infection (data not shown). Therefore we chose more virus-like disease for our experiment as viruses are known to spread to clonally connected ramets quite easily (Cheng et al 2000).

Further, we did not model any vertical transfer in our simulations and one can hypothesize that this could have had negative effects on the integrator strategy as well. It always has larger amount of ramets infected even when it is more successful and so its offspring would be infected more often. The vertical transfer of various diseases has been documented in many of plant host-pathogen systems (e.g. Kover and Clay 1998, Brunt et al 1996) but even in the documented systems it is often successful only in rather small proportion of seeds. We used no vertical transfer to see the net effect of disease on integration and the seed production was implemented only to create new disease-free individuals of both strategies to pronounce the general disease effects.

Natural host-pathogen systems often show various deviations from general view of pathogens only as sinks of resources. The infected plants are often castrated and particularly in grass – fungal endophyte systems the pathogen markedly changes allocation of resources to clonal growth and reproduction (Nus 1990, Garcia-Guzman and Burdon 1997; Pan and Clay 2002, 2004). Such effects may affect evolution of integration differently from simple resource reduction as it was used in this paper. One can speculate that increased allocation into the clonal growth would give even more advantage to the integrator (more clonal growth would enhance the translocation benefits) but our model cannot test such a hypothesis. To model systems like this one should look for quite a different approach and change several parameters of the infected plants at the same time. The problem is that one would need to have realistic values of the resource allocation, the seed production and of the translocation parameters and those are not easy to get.

Conclusions

Our results suggest that the advantage of translocation probably overrules the negative selective pressures of resource-reducing diseases on clonal integration. The general message of this paper is that the systemic diseases may not play a significant role in the evolution of clonality. This does not necessarily mean that the infection by systemic disease never lead to selection against clonal integration in some particular host – pathogen system but it only shows that this effect is constrained by the effect of the advantage of clonal integration. We

also showed that modelled disease has only a narrow space in which it can act as a major evolutionary force. It is therefore necessary to examine infection rates in many different field systems to determine how strong an effect the particular pathogen might have on its host and what selection pressure it may exert towards fragmentation.

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Conclusions

The thesis proved that traditional population biology methods including analysis of environmental factors, thorough exploration of the host plant's life cycle or modelling are able to answer important questions of plant pathology. The two papers on system of *Falcaria vulgaris – Puccinia sii-falcariae* have shown that environmental factors and life history of the host plant interact to large extent and impact population characteristics of the studied populations. The highly prevalent disease has only small impact in chosen populations suggesting that the system might have evolved towards tolerance of the disease by the host species and/or lower aggressiveness of the pathogen. The otherwise permanent systemic infection can be only temporary in this species supporting the hypothesis. The clonal life history was investigated in the third paper deviating slightly from the other two studies. The previously unresearched possibility of selection against clonality under influence of systemic disease was studied by a model of clonal growth. The results show, that positive effect of clonality would probably alleviate the negative effects of disease in most cases and that the systemic disease has only small selective influence in our model setting. Although often complicated to study, the systemic pathogens are important group of organisms that influences plant populations. As such, further research in this direction is needed and anticipated.

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2005: International botanical congress, Vienna, poster: Population biology of plants infected by systemic fungal pathogens

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