Structure and variation in the wild-plant pathosystem:
Lactuca serriola–Bremia lactucae

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Abstract Over the past decade, extensive research on the wild-plant pathosystem, *Lactuca serriola* (prickly lettuce)–*Bremia lactucae* (lettuce downy mildew), has been conducted in the Czech Republic. Studies focused on pathogen occurrence and distribution, host range, variation in symptom expression and disease severity, interactions of *B. lactucae* with another fungal species (*Golovinomyces cichoracearum*) on *L. serriola*, variation in resistance within natural populations of *L. serriola*, the structure and dynamics of virulence within populations of *B. lactucae*, sexual reproduction of *B. lactucae*, and a comparison of virulence structure and changes in *B. lactucae* populations occurring in wild (*L. serriola*) and crop (*L. sativa*) pathosystems. The incidence of *B. lactucae* on naturally growing *L. serriola* and other Asteraceae was recorded. *Lactuca serriola* was the most commonly occurring host species, followed by *Sonchus oleraceus*. Over the duration of these studies, the incidence of *B. lactucae* in *L. serriola* populations varied between 45–87%. Disease incidence and disease prevalence were partly related to the size, density and different habitats of *L. serriola* populations. In addition to *B. lactucae* infection, infection by the lettuce powdery mildew fungus (*Golovinomyces cichoracearum*) was quite common, including co-infection. Variation in resistance to *B. lactucae* was studied by using ten isolates (NL and BL races with known virulence patterns) at a metapopulation level, i.e. 250 *L. serriola* samples representing 16 populations from the Czech Republic (CZ). Our comparisons revealed broad variation in host resistance among host populations and also intrapopulation variability. In the CZ populations, 45 resistance phenotypes were recorded, the most frequent were race-specific reaction patterns. Structural and temporal changes in virulence variation of *B. lactucae* populations on *L. serriola* were studied during 1998–2005. Altogether, 313 isolates of *B. lactucae* originating from the Czech Republic were examined for the presence of 32 virulence factors (v-factors), and 93 different virulence phenotypes (v-phenotypes) were recorded. A study of v-factor frequency showed that common v-factors in *B. lactucae* populations match some of the race-specific resistance genes/factors (*Dm* genes or R-factors) originating from *L. serriola*. The highest frequency was recorded by v-factors v7, v11, v15–17, and v24–30. In contrast, v-factors (e.g. v1–4, 6, and 10) matching *Dm* genes originating from *L. sativa* were very rare. This demonstrates the close adaptation of *B. lactucae* virulence to the host (*L. serriola*) genetic background. Temporal changes in virulence frequencies over the period were recorded. In many v-factors (v11, v14, v16, and v25–28), fluctuations were

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observed, some (v14 and v17) shifting to higher frequencies, and others (v5/8 and v23) decreasing. The occurrence of mating types was studied (1997–1999) in a set of 59 *B. lactucae* isolates. Both compatibility types (B1 and B2) were recorded; however the majority of the isolates (96%) were type B2. A comparative study of *B. lactucae* virulence variation between the wild (*L. serriola*) and crop (*L. sativa*) pathosystems showed major differences. Migration and gene flow between both pathosystems and the potential danger of wild *B. lactucae* populations for cultivated lettuce are discussed. This paper summarizes comprehensive and unique research on an oomycete pathogen (*B. lactucae*) that is shared between a crop (lettuce, *L. sativa*) and its close wild relative (prickly lettuce, *L. serriola*). The data demonstrate clear evidence about race-specific interactions, variation and changes in virulence, and coevolutionary relationships in the wild pathosystem *L. serriola–B. lactucae*. Conclusions contribute to the broadening and better understanding of gene-for-gene systems in natural host–pathogen populations and their relationships to crop pathosystems.

Introduction

Plant pathogens play a substantial role in the structure, dynamics, and evolution of natural plant communities. They may cause increased mortality, reduced fitness of individual plants, or dramatic shifts in the structure or composition of plant populations and communities. However, they may also help to maintain plant species diversity, and enhance the genetic diversity and structure of host populations (Gilbert 2002). The first detailed studies focusing on wild-plant pathosystem structure and function were published in the 1980s (Burdon 1987; Dinoor and Eshed 1984), and research on host–pathogen interactions in natural communities is a rapidly growing area of investigation in plant

pathology (Burdon et al. 2006). Most of these studies have focused on interactions between host plants and plant parasitic fungi. Investigations of interactions between host plants and oomycetes are still very rare (Lebeda 2002; Lebeda and Schwinn 1994), with one of the most extensively studied of such pathosystems being *Lactuca* spp.–*Bremia lactucae* (Lebeda et al. 2002, 2007c).

Bremia lactucae (lettuce downy mildew) is an oomycete pathogen of cultivated lettuce (*Lactuca sativa*) and many other species of Asteraceae (Lebeda et al. 2002) that is distributed worldwide. The most common wild host species of this pathogen is *Lactuca serriola* (prickly lettuce), and it can also be frequently found on *Sonchus* species (Lebeda et al. 2002; Lebeda and Petrželová 2004a; Lebeda and Syrovátko 1988; Petrželová and Lebeda 2004b). However, it is well documented that *B. lactucae* is highly host-specific and mostly limited to a single plant genus (Crute and Dixon 1981; Lebeda and Syrovátko 1988). Thus, except for certain *Lactuca* species, weedy growing Asteraceae cannot serve as a source of inoculum for cultivated lettuce (Lebeda and Syrovátko 1988) and vice versa.

The interaction between *L. sativa* and *L. serriola* and *B. lactucae* generally conforms to a gene-for-gene relationship (Crute 1992a, b), in which resistance is determined by dominant *Dm* resistance genes (or R-factors) in the hosts, matched by dominant avirulence factors in the pathogens (Hammond-Kosack and Jones 1997). Detailed analyses of the genetics of these host–parasite interactions (Crute and Johnson 1976; Farrara et al. 1987) made it possible to interpret the variability of virulence in *B. lactucae* individuals and populations in terms of virulence factors (v-factors) and virulence phenotypes (v-phenotypes; Lebeda 1981, 1982).

Several different mechanisms of resistance to *B. lactucae* have been identified in cultivated and wild lettuce (Lebeda et al. 2001a). Most of the resistance is considered to be race-specific (Lebeda et al. 2002, 2007b). This type of resistance has a big disadvantage as it does not provide durable protection against lettuce downy mildew and the introduction of new resistant cultivars is often followed by the appearance of new virulent pathogen races (Lebeda and Schwinn 1994; Lebeda and Zinkernagel 2003a). During the last few decades, lettuce resistance breeding has focused on searching for and utilizing novel sources of

resistance to *B. lactucae* from wild *Lactuca* species (Lebeda et al. 2002, 2007b). However, these new resistances could be quickly overcome by *B. lactucae* isolates from wild pathosystems (Lebeda 2002; Lebeda et al. 2002).

During the last few decades, studies of host resistance, variation and distribution of *B. lactucae* virulence phenotypes have focused on the population level and only on cultivated lettuce (*L. sativa*; e.g. Crute 1987; Lebeda and Zinkernagel 2003a). So far, there have been no studies of interactions between *Lactuca* spp.–*B. lactucae* in natural populations, especially from the viewpoint of host resistance, pathogen virulence, and their temporal and spatial dynamics (Lebeda 2002). In the Czech Republic, studies of the wild *L. serriola*–*B. lactucae* pathosystem were initiated at the beginning of the 1980s (Lebeda 1984, 1986; Lebeda and Boukema 1991; Lebeda and Syrovátko 1988). However, more detailed research focusing on the structure, spatial and temporal changes in this pathosystem, including interactions with the crop (*L. sativa*) pathosystem and coevolutionary studies, began only recently (Lebeda 2002; Lebeda et al. 2002; Lebeda and Petrželová 2004a, b; Petrželová and Lebeda 2003, 2004a, b, c).

In populations of *B. lactucae*, sexual reproduction has an important role in genetic recombination (Michelmore 1981) and is considered to be the major source of virulence variation (Crute 1992b; Lebeda and Schwinn 1994). *Bremia lactucae* is predominantly heterothallic, and two sexual compatibility types (mating types), designated B1 and B2, have been described (Michelmore 1981). Lebeda and Schwinn (1994) documented sexual reproduction in populations of *B. lactucae* occurring on lettuce (*L. sativa*), but studies focussing on pathogen isolates from *L. serriola* have been more limited (Lebeda and Blok 1990). These reports documented both mating types of *B. lactucae*.

The aim of this paper is to describe and analyze patterns of variation in interactions between naturally growing *L. serriola* populations and *B. lactucae*. This report includes both previously published and new data (collected between 1998 and 2006) about the structure and dynamics of this pathosystem from the viewpoint of host range, disease distribution and severity, and the occurrence of various types of symptoms, variation and spatial distribution of host resistance and pathogen virulence, temporal dynamics and microevolutionary shifts in *B. lactucae* popula-

tions on naturally growing populations of *L. serriola*. Of particular interest are the interactions between the wild- and crop (*Lactuca sativa*) pathosystems. Co-incidence of *B. lactucae* with *Golovinomyces cichoracearum* and its potential for competitive interactions is also considered.

Host range of *B. lactucae* in natural populations of Asteraceae plants

The natural incidence of *B. lactucae* on wild Asteraceae species was surveyed in two main areas of the Czech Republic (Fig. 1) during the period 1999–2006, with the main focus on populations of weedy *L. serriola* populations and associated Asteraceae plants. Field surveys usually took place between May and early September. Whenever possible, locations were visited repeatedly during the growing season. During the course of this study, *B. lactucae* was recorded on eight Asteraceae species (Table 1). It is evident that, in the Czech Republic, the most common host species of *B. lactucae* are *L. serriola* and *Sonchus oleraceus*; however sparse occurrence was also observed on *Arctium tomentosum*, *Carduus crispus*, *Cirsium arvense*, *Lapsana communis*, *Sonchus arvensis* and *Sonchus asper* (Table 1).

Bremia lactucae is an obligate biotrophic pathogen with a broad host range within the Asteraceae. On the lettuce crop (*L. sativa*), *B. lactucae* has a worldwide distribution (e.g. Achar 1996; Crute 1987; Datnoff et al. 1994; Lebeda 1979; Lebeda and Zinkernagel 2003a; Marlatt 1974; Sharaf et al. 2007; Trimboli and Crute 1983). It has also been recorded on more than 200 other Asteraceae species from about 40 genera of the tribes Lactuceae, Cynareae and Arctotideae (Crute and Dixon 1981; Koike and Ochoa 2007; Lebeda et al. 2002). However, information about the natural distribution and patterns of variation of *B. lactucae* populations on wild composites is very rare (Lebeda and Syrovátko 1988). Recently, *B. lactucae* was noted as a common disease on *L. serriola* in The Netherlands (Hooftman et al. 2007); however, only sporadic occurrence has been recorded in other European countries, such as Austria, France, Germany and Switzerland (Lebeda et al. 2001b), and Slovenia and Sweden (Doležalová et al. 2001). Detailed data on the distribution of *B. lactucae* within Europe are lacking. Only in the Czech Republic has

Fig. 1 Areas and locations (*by dots*) in the Czech Republic surveyed for the natural distribution of *Bremia lactucae* in the period 1998–2006

the natural occurrence of *B. lactucae* been studied more intensively during the last decade, with *L. serriola* and *Sonchus* species (especially *S. oleraceus*) found as its most common hosts (Lebeda et al. 2007a; Petrželová and Lebeda 2004b).

However, despite its broad host range, *B. lactucae* was found to be highly host-specific. Cross-inoculation laboratory experiments showed that pathogen popula-

tions occurring on wild Asteraceae mostly cannot serve as an inoculum source for cultivated lettuce, and inter-specific transmission was demonstrated only within the genera *Lactuca* and *Sonchus* (Lebeda and Syrovátko 1988). In another wild plant pathosystem, where the pathogen is the smut fungus *Microbotryum violaceum*, Carlsson-Granér (2006) recently showed that in a spatially fragmented metapopulation, the pathogen can alter its host species, which can increase disease spread. For cultivated lettuce, populations of *B. lactucae* on weedy-growing *L. serriola* plants, represent a very important danger to the race-specific resistance genes that originated from *L. serriola* and were introduced to lettuce (Lebeda 1984, 1989; Lebeda et al. 2002, 2007b). Our previous (Petrželová and Lebeda 2004b) and recent data (Table 1) documented the relatively high frequency of *B. lactucae* on *Sonchus* species. Recently, Vieira and Barreto (2006) suggested the possibility of lettuce (*L. sativa*) infection with *B. lactucae* originating from *Sonchus* spp. However, there is still no direct evidence for natural transmission of *B. lactucae* between the genera *Lactuca* and *Sonchus*, or vice versa.

Table 1 Distribution of *Bremia lactucae* in populations of *Lactuca serriola* and other Asteraceae plants occurring in the same plant associations (1999–2006)

Species	Number of populations		Proportion (%) of infected populations within evaluated Asteraceae species
	Observed	Infected	
Arctium lappa	3	0	0
Arctium tomentosum	82	2	0.33
Carduus acanthoides	22	0	0
Carduus crispus	49	1	0.16
Cirsium arvense	144	3	0.49
Cirsium canum	6	0	0
Cirsium oleraceum	7	0	0
Cirsium vulgare	18	0	0
Lactuca serriola	768	563	91.84
Lapsana communis	9	1	0.16
Sonchus arvensis	14	4	0.65
Sonchus asper	7	3	0.49
Sonchus oleraceus	64	36	5.88
Taraxacum spp.	50	0	0
Total	1,243	613	100

Variation in phenotypic expression of *B. lactucae* infection on naturally growing *L. serriola* plants

Large variation in phenotypic expression of *B. lactucae* infection on *L. serriola* plants was observed. During the disease survey, in addition to epidemiolog-

ical data (disease incidence, prevalence and severity) for each evaluated *L. serriola* population, the types of disease symptoms on leaves were also recorded. Generally, *B. lactucae* is described as a pathogen causing light green, yellow or (on older leaves) necrotic lesions visible on the upper surface of lettuce leaves. Those lesions are often surrounded by larger leaf veins, and under optimal conditions are covered with sporulation on the lower leaf surface. But it is known that there may be variation in lesion types and sporulation on infected lettuce leaves (Crute and Dixon 1981).

Disease symptoms on *L. serriola* may be divided into several groups according to the basic character of response, shape, abundance of lesions and intensity of sporulation (Fig. 2a,b). The first category of symp-

toms includes responses without any visible leaf necrosis (Fig. 2a). These may be characterized either by small, discrete chlorotic spots surrounded by veins that are variable in abundance on leaves according to the progress of infection, or by minute spots dispersed over the leaves with only a few conidiosporangiophores growing from each spot. Sometimes, dispersed sporulation over large parts or even the whole leaves with no obvious borders may be observed. However, there may be discrete chlorotic spots involving larger parts of leaves, with profuse sporulation on the lower leaf surface when conditions are suitable for asexual reproduction.

The second main type of macroscopic response is connected with leaf-tissue necrosis (Fig. 2b). Both

Fig. 2 Variation in expression of symptoms of *B. lactucae* on naturally infected leaves of *L. serriola*; **a** Symptoms without any visible leaf necrosis; *a1* both sides of the same leaf (*a–e* yellowing lesions well localized by main veins), *a→e* increasing percentage of leaf area with strongly sporulating lesions (on abaxial side) of *B. lactucae*; *f* and *g* frequent, small and localized lesions with unusual leaf discolouration, covered on abaxial side of the leaf by sporulating *B. lactucae*; *a2* abaxial side of the leaf (*h–k*: different examples of diffuse occurrence of sporulating lesions of *B. lactucae*); **b** Symptoms of *B. lactucae* infection are connected with the leaf-tissue (abaxial side) necrosis (*a→d*: an example of increasing percentage of necrotic lesions with reduced sporulation of *B. lactucae*)
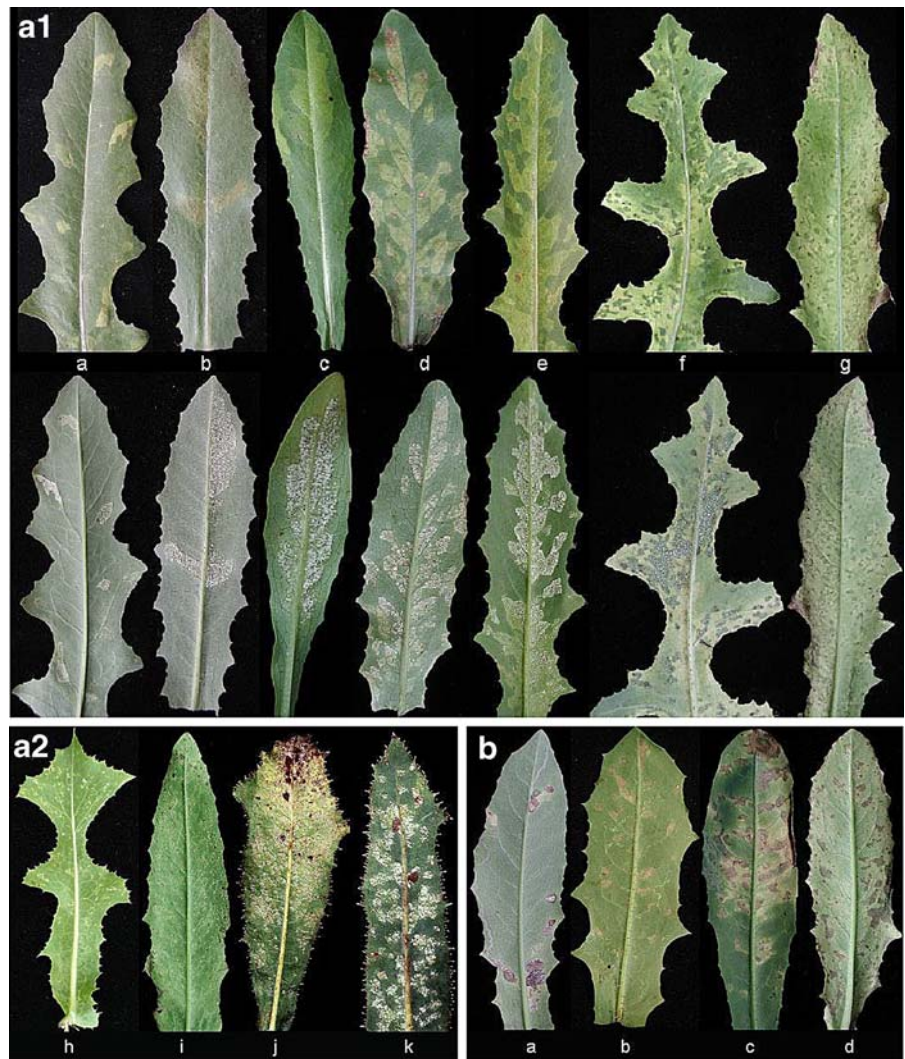
small necrotic spots or larger ones were observed. Both may be characterized by limited to profuse pathogen sporulation.

Symptoms of *B. lactucae* infection on *L. serriola* showed considerable variability in macroscopic disease expression. In contrast, on other evaluated wild-host composites, little or no variation was found; only typical lesions surrounded by bigger veins and covered with the sporulating pathogen were observed. The main reason for this difference may be the broad genetic diversity of *L. serriola* populations, characterized by the occurrence of a large number of race-specific resistance genes and/or factors (Kuang et al. 2006; Lebeda et al. 2002; Lebeda and Petrželová 2004b, 2007; Table 6 and Fig. 4), which may differ in their phenotypic expression. Despite the high variation in symptom expression found among evaluated *L. serriola* populations, our field observations showed that infected plants within individual populations generally displayed similar disease symptoms. However, no experimental data are available to compare whether different symptoms observed on naturally infected plants are directly linked to the presence of specific R-factors in *L. serriola*, or if it is a more complex phenomenon which involves interactions among host plant and pathogen populations and the environment (Cooke et al. 2006; Drenth 2004; Frantzen 2000; Zadoks and Schein 1979). These interactions could be very variable, substantially influenced by environmental factors, such as ambient temperature (Judelson and Michelmore 1992).

Natural distribution of *B. lactucae* and disease prevalence in populations of *L. serriola*

Disease incidence, seasonal and temporal dynamics of *B. lactucae* in natural populations of *L. serriola*

Two parameters were used to assess the distribution of *B. lactucae* in natural populations of *L. serriola*. Disease incidence was expressed as the percentage of occurrence of *B. lactucae* on surveyed sites and on populations of *L. serriola*. Disease prevalence was assessed in each host population by using a visual 0–3 scale (Lebeda 2002; Petrželová and Lebeda 2004b). *Bremia lactucae* was found frequently (ranging from ca 60 to 85%) in populations of *L. serriola* at surveyed

localities (Fig. 3). Disease was recorded at all developmental stages of the host plants. *Bremia lactucae* can be found throughout the growing season (April to September, in some extreme cases even up to October or November), as long as weather conditions are suitable for host plant growth. However, the highest disease incidence was recorded from June to August.

Disease prevalence in populations of *L. serriola*

Disease prevalence in infected *L. serriola* populations was surveyed, and some seasonal fluctuations within individual years were recorded. In some populations visited repeatedly during the growing season, it was possible to document the progress of infection; however, in other populations, no changes were observed. Also, the proportion of populations with different levels of disease prevalence fluctuated slightly among individual years. However, it appears that, under natural conditions, disease prevalence of *B. lactucae* infection mostly does not reach higher levels (Table 2).

Prevalence of *B. lactucae* in different types of habitats and populations of *L. serriola*

Possible influences of habitat type (Table 3) as well as of the size and density (Table 4) of host populations on the incidence and disease prevalence of *B. lactucae* were also considered (Petrželová and Lebeda 2004b). From these perspectives, there were some significant differences in the disease prevalence, which were most pronounced in urban areas (Table 3) with frequent occurrence of solitary host plants or small groups of plants, also in agricultural areas, or in habitats with moist substrates (Tables 3 and 4).

Interactions of *B. lactucae* with *G. cichoracearum* on *L. serriola*

The natural incidence of other fungal pathogens on *L. serriola* plants was also recorded. Only *Golovinomyces cichoracearum*, the causal agent of powdery mildew in Asteraceae (Braun 1995) was found to be of particular importance for incidence and prevalence of *B. lactucae* in the host populations. We focused on the co-incidence of both pathogens with results summarized in Table 5. It is evident that both pathogens are widely

Fig. 3 Fluctuation of *Bremia lactucae* infection in natural populations of *Lactuca serriola*. Figure shows among-year fluctuations in proportion of healthy (non-infected) and *B. lactucae* infected populations of *L. serriola* within all populations surveyed in a given year
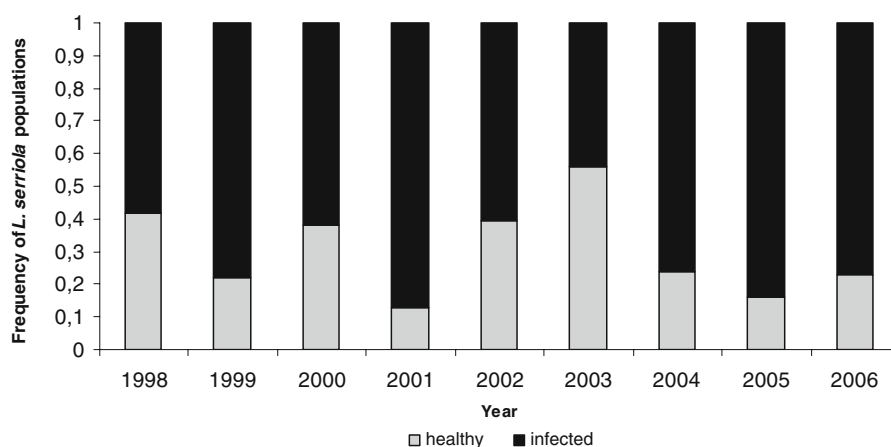

□ healthy ■ infected

Table 2 Temporal variation in *Bremia lactucae* prevalence in natural populations of *Lactuca serriola*

Year	Number of evaluated *L. serriola* populations	Disease prevalence/% of populations			
		0	1	2	3
1998	36	41.7*	41.7*	8.3	8.3
1999	77	22.1	63.6*	10.4	3.9
2000	136	38.2	38.2*	17.7	5.9
2001	132	12.9*	59.1*	20.4*	7.6
2002	53	39.6*	35.8*	18.9	5.7
2003	59	55.9*	35.6*	3.4*	5.1
2004	101	23.8	66.3*	9.9	0.0
2005	100	16.0*	55.0	21.0*	8.0
2006	109	22.9	57.8*	16.5	2.8
Mean		30.3	50.3	11.1	5.3
P		<0.000	<0.002	<0.000	<0.200

Table shows among-year variation in proportion of *L. serriola* populations differing in disease prevalence (proportion of individuals within a population diseased with *Bremia lactucae*). For simplification we used visual 0–3 scale for expressing different levels of disease prevalence (Lebeda 2002; Petrželová and Lebeda 2004b): *0* no symptoms of *B. lactucae* infection in a surveyed *L. serriola* population; *1* low disease prevalence; *2* medium disease prevalence; *3* high disease prevalence

Mean=average of disease prevalence for the whole period (1998–2006)

P level for observed vs. expected (average) chi-square test

Methodology for recording of field observations used over the study period was continuously supplemented with some new parameters (Petrželová and Lebeda 2004b). As a result, all data are not complete for all years in Tables 2, 3 and 4.

*P<0.05 for differences between two percentages (marked value of prevalence and mean value/under line/) used software: StatSoft, Inc. (2001). STATISTICA Cz, Version 6. Www. StatSoft.Cz

distributed on *L. serriola* under natural conditions; however, *B. lactucae* is more frequent. Nevertheless, the percentage of populations where both pathogens were recorded together was rather high (Table 5). Some differences were found in the incidence and prevalence of these pathogens during the growing season and among individual years (Table 5).

Generally, the dynamics of a pathogen on a weed is completely different than in a crop. In a dynamic weed population, pathogen epidemiology is likely to be intimately related to the ecology of its host, influencing the host's abundance, spatial distribution, and genetic diversity. Temporal variation in these factors affects the ability of the pathogen to reproduce and spread (Cousens and Croft 2000). Other important factors for the incidence of a pathogen in weed populations include general climatic conditions and specific microclimatic conditions at individual sites.

The first pre-condition of pathogen incidence is the presence of its host. Results summarized in this paper show that *B. lactucae* occurs in a patchy fashion wherever appropriate hosts grow and conditions are suitable for pathogenesis. Populations of *B. lactucae* and *L. serriola* are a good example of a host–pathogen metapopulation structure (McDermott and McDonald 1993; Thrall et al. 2001), where individual fragmented populations are linked together by the transport of spores and gene-flow.

Long-term research has been focused on the structure and dynamics of *B. lactucae* populations on *L. serriola* (Lebeda 2002). Within the genus *Lactuca*, *L. serriola* (prickly lettuce) is the most frequent weed species in Europe, especially within the

Table 3 Comparison of disease prevalence in populations of *Lactuca serriola* occurring in different types of habitats (1998–2006)

Habitat[a]	Total No. of observed populations	Disease prevalence[b]/% of populations			
		0	1	2	3
Along transport corridors (ditches, roadsides)	385	26.0	55.5	12.5	6.0
Agricultural areas (fields, field margins, field roads)	215	15.3*	60.0	19.1	5.6
Ruderal areas, dust-heaps, debris, building sites, piles of soil	114	22.8	55.3	17.5	4.4
Urban areas (pavements, lawns, parking sites)	61	59.0*	34.5	4.9*	1.6
Uncultivated areas, fallows	86	23.2	51.2	19.8	5.8
Moist with biological material (dunghills, compost-heaps)	22	9.1*	59.1	22.7	9.1
Mean		25.9	52.6	16.1	5.4
P		<0.000	<0.135	<0.025	<0.358

StatSoft, Inc. 2001. STATISTICA Cz, Version 6. Www.StatSoft.Cz

[a] Categorization according Petrželová and Lebeda (2004b)

[b] Categorization same as used in Table 2

P level for observed vs. expected (average) Chi-square test

*P<0.05 for differences between two percentages (marked value of prevalence and mean value/under line/)

last two decades as it has undergone a big population explosion connected to human activity (Hooftman et al. 2006; Lebeda et al. 2001b). It is a pioneer plant which colonizes disturbed areas (Feráková 1977; Lebeda et al. 2004) in various ruderal habitats, often along transport corridors (Lebeda et al. 2001b). It produces many achenes which can disperse over long distances, often generating large populations of hundreds or thousands of plants (Petrželová and Lebeda 2004b; Weaver and Downs 2003). However, our field observations showed that these habitats typically undergo a succession, where *L. serriola* is replaced by another species. For this reason, the natural distribution of *B. lactucae* on *L. serriola*

Table 4 Comparison of disease prevalence in populations of *Lactuca serriola* differing in the size and density (1998–2006)

Population size[a, c]	Total No. of observed populations	Disease prevalence[b]/% of populations			
		0	1	2	3
Individual plants[d]	89	34.8*	51.7	5.6*	7.9
Group of several dispersed plants[e]	211	29.4	53.1	12.3	5.2
Compact group of more plants[f]	364	22.8	56.9	15.9	4.4
Extensive and dense growth[g]	136	15.4	52.9	22.1*	9.6
Large areas with *L. serriola* (*Lactuca* fields)[h]	69	11.6*	60.9	18.8	8.7
Mean		22.8	55.1	14.9	7.2
P		<0.003	<0.904	<0.029	<0.588

P level for observed vs. expected (average) Chi-square test, * P<0.05 for differences between two percentages (marked value of prevalence and mean value /under line/; StatSoft, Inc. 2001. STATISTICA Cz, Version 6. Www.StatSoft.Cz)

[a] Categorization according to Petrželová and Lebeda (2004b)

[b] Categorization same as used in Table 2

[c] Approximate number of plants

[d] <5

[e] 5–10

[f] 11–50

[g] 51–100

[h] >100

Table 5 Temporal variation in the incidence/co-incidence of *Bremia lactucae* and *Golovinomyces cichoracearum* in populations of *Lactuca serriola*

Year	Total number of evaluated populations	Incidence of *B. lactucae* and *G. cichoracearum* (%)		Co-incidence of both pathogens
		Single infection		
		B. lactucae	*G. cichoracearum*	
1998	36	22.2	22.2	36.1
1999	77	37.7	19.5	40.3
2000	136	29.4	16.2	32.4
2001	132	48.5	5.3	38.6
2002	53	n.d.	n.d.	n.d.
2003	59	32.2	23.7	11.9
2004	101	49.5	13.9	26.7
2005	100	32.0	9.0	52.0
2006	109	45.0	11.0	32.1
In total[a]	750	38.8	13.5	34.7

n.d. not determined during the main season

[a] *L. serriola* populations (total number of evaluated populations, year 2002 excluded); % of incidence/co-incidence for the period 1998–2006

cannot be assessed only from the viewpoint of individual populations, but also at a larger geographical scale, i.e. metapopulation size.

In comparison to crops, weed populations mostly occur as mixtures of genotypes, and pathogen incidence is thus strongly dependent on the availability of susceptible host plants (Cousens and Croft 2000). Host genetic diversity has considerable influence on the occurrence of a pathogen and its variation, especially in pathosystems operating on a gene-for-gene basis (Burdon 1997). Thus, despite its persistence in the area during the studied period, it was not possible to predict the incidence of *B. lactucae* in particular sites due to the unpredictable dynamics of the host populations. Further, we observed a large discrepancy between disease incidence and disease prevalence (in the present study, expressed as the degree of infection in evaluated populations, Table 2). Although *L. serriola* populations with *B. lactucae* infection were widely distributed, the prevalence of infection in most populations was very low (Table 2). In natural populations such negative relationships between disease incidence and disease prevalence may be caused by higher levels of connectivity within the fragmented host metapopulation (Carlssson-Granér and Thrall 2002). Indeed, though *L. serriola* is distributed in a patchy fashion, it is very common in many plant associations.

The distribution of *B. lactucae* and its interaction with *L. serriola* must be also considered from the viewpoint of interactions with the physical environment. Differences in disease incidence during the main season of *B. lactucae* development were related to some extent with local temperature and rainfall in July and August. *Bremia lactucae* has a narrow optimal range of conditions for growth (Crute and Dixon 1981), preferring cool temperatures and relatively high humidity. Weather variables are considered crucial in the infection and epidemiology of *Bremia lactucae* on cultivated lettuce (Scherm and van Bruggen 1994; Su et al. 2004). A recent study (Mieslerová et al. 2007) demonstrated that in extremely dry and hot summer months (as was the case in 2003), more than 50% of populations were free of infection symptoms. In contrast, in the cool and wet Augusts of 2005 and 2006, a high frequency of infected *L. serriola* populations was observed (Mieslerová et al. 2007). The age of host plants can also influence disease severity (Petržeлová and Lebeda 2004b). From our empirical data, it is evident that plants initially infected at the early stages of development (leaf rosette formation or bolting) expressed higher degrees of infection under optimal conditions than plants infected as adults. This agrees with experimental data reported by Crute and Dickinson (1976). The success of a pathogen in a host population may also be influenced by its interactions and possible competition with other pathogens attacking the same host plants (Lindow 2006). Our recent research has focused on the co-incidence of *B. lactucae* and *Golovinomyces cichoracearum* (Mieslerová et al. 2007). *Golovinomyces cichoracearum*, in comparison with *B. lactucae*, has a shorter period of incidence during the growing season, and its epidemics start later (mostly in June or July), when *B. lactucae* is already widespread among *L. serriola* plants (Lebeda 2002; Petržeлová and Lebeda 2004b). However, where it did occur, *G. cichoracearum* was able to develop heavy and extensive infections within a few (3–4) weeks (Petržeлová and Lebeda 2004b). The peak of incidence of both pathogens was approximately the same, i.e. August. Both pathogens can co-occur within the same population of *L. serriola* and either of them may dominate, depending strongly on different environmental optima

for their growth and development, with humidity being the most pronounced parameter (Mieslerová et al. 2007). In relationship to disease severity and inter-actions of both pathogens, the phenomenon of induced resistance must also be seriously considered in natural plant pathosystems (Newton and Pons-Kühnemann 2007).

Host–pathogen interactions play an important role in plant populations and may have some impact on plant fitness and demography (Thrall and Burdon 2003, 2004). However, our knowledge of these ecological parameters in the *L. serriola–B. lactucae* interaction is still very limited. From our data, it is evident that there are differences in the expression of disease symptoms and disease intensity within and among individual plants inside populations and between populations, leading to some reduction in leaf surface (Fig. 2) and the number of leaves; however, we never observed the infection of repro-ductive (floral) parts of *L. serriola* plants. Recently, the first data about this phenomenon showed that the impact of inheriting *Bremia* resistance on reproduc-tive plant fitness is small (Hooftman et al. 2007). Nevertheless, it was observed that infected individuals had fewer leaves at the beginning of the bolting phase than did non-infected plants, and for individual seed weight there was a significant interaction between mainlines and *Bremia* infection. However, the total seed weight per head (capitulum) was not altered by *Bremia* infection, and it was concluded that *Bremia* infection did not affect these fitness components in any consistent manner (Hooftman et al. 2007).

Variation of resistance in populations of *L. serriola*

Research was focused on the determination of resistance variation within and among Czech popula-tions of *L. serriola* as well as on the evaluation of variation within other European populations of prick-ly lettuce (Lebeda and Petrželová 2004b, 2007). Samples of *L. serriola* were collected in 2001 within the framework of the EU project 'Gene-Mine' (for details see Lebeda et al. 2007a). In total, 250 individual plants from 16 Czech populations of *L. serriola* were screened following previously de-scribed methods (Lebeda and Zinkernagel 2003b) for resistance against 10 races of *B. lactucae* (NL1, NL5, NL12, NL14, NL15, NL16, BL17, BL18, BL21

and BL24) with known virulence patterns (van Ettekoven and van der Arend 1999) and were characterized by means of resistance phenotypes. Some basic results are given in Table 6 and Fig. 4.

A substantial proportion of the Czech *L. serriola* populations showed high levels of susceptibility to the *B. lactucae* isolates used (Table 6). Overall, 30% of the host individuals studied were completely suscep-tible, and this phenotype was widely distributed among studied populations (in 12 of 16 populations). A completely resistant phenotype was recorded in five populations, represented by 24 (9.6%) samples (Fig. 4). While most plants expressed intermediate levels of race-specific resistance, only four popula-tions showed relatively high levels of resistance. Most plants were susceptible to at least one to three races of *B. lactucae* (among them very often BL21 and BL24).

Despite their high levels of susceptibility, Czech populations of *L. serriola* were variable in terms of their resistance to ten races of *B. lactucae*. Presence of race-specific resistance was very common. Both inter- and intra-population variation of resistance were found. In total, 45 different resistance phenotypes were recognized in the studied populations of *L. serriola*. However, 80% of the individual plants evaluated were represented by only eight resistance phenotypes; the remaining phenotypes were generally rare. When compared to other European populations of *L. serriola*, Czech populations most closely resem-

Table 6 Variation of resistance within European metapopula-tions of *Lactuca serriola* (Lebeda and Petrželová 2004b; Lebeda et al. 2007a)

Populations	Number of *L. serriola* populations High level[a] of				Total
	Resistance	Susceptibility	Race-specific response	Intra-population variation in race-specificity	
CZ	4	9	0	3	16
D	0	6	1	9	16
NL	0	2	6	0	8
UK	0	0	10	0	10
Totally	4	17	17	12	50

[a] Relative variation in mean resistance of populations evaluated as a relative proportion of samples with prevalence of susceptibility, resistance and race-specificity to the used set of *B. lactucae* races
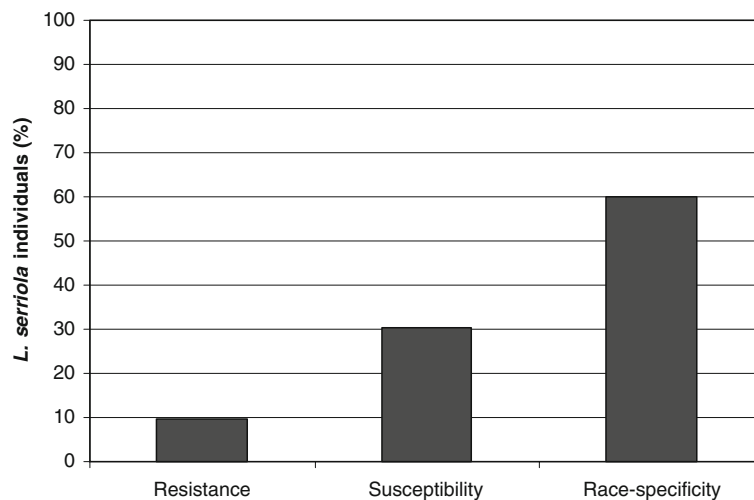
Fig. 4 Percentage of *Lactuca serriola* individuals (in total *n*=250), with different reaction patterns to ten races of *Bremia lactucae*, within the sampled Czech host populations (*n*=16; each population represented by ca 16 individual plants, from each plant tested ca 30 achenes/seedlings/). Three basic categories of reaction patterns were distinguished: *Resistance L. serriola* individual plants were resistant to all ten races of *B. lactucae* used; *Susceptibility L. serriola* individual plants were susceptible to all ten races of *B. lactucae* used; *Race-specificity* differential reaction patterns to races of *B. lactucae* used was recorded

bled resistance structures observed in their German counterparts (Table 6). However, while German populations expressed greater levels of intra-population variation, no population was completely resistant (Table 6) despite the widespread occurrence of resistant individuals among the populations. *Lactuca serriola* populations in the Netherlands also expressed a high level of intra-population variation; however, their responses showed higher levels of race-specificity (Table 6). Unlike the Czech, German and Dutch populations, disease responses of populations from the United Kingdom were much more uniform (Table 6).

During the last few decades, lettuce resistance breeding has focused on the identification and incorporation of novel sources of resistance to *B. lactucae* from wild *Lactuca* spp., especially from *L. serriola*, *L. saligna* and *L. virosa* (e.g. Beharav et al. 2006; Bonnier et al. 1992; Jeuken and Lindhout 2002; Lebeda et al. 2002, 2007b; Lebeda and Zinkernagel 2003b; Maisonneuve 2003). Mapping the distribution patterns of resistance and virulence can help us understand co-evolutionary dynamics in plant pathosystems (e.g. Carlsson-Granér 2006; Carlsson-Granér and Thrall 2002; Delmotte et al. 1999; Laine 2006; Thrall and Burdon 2003) and select appropriate resistance sources and crop-breeding strategies (Lebeda et al. 2002, 2007b). However, there is only limited information about the distribution of resistance to *B.*

lactucae in natural populations of its hosts (Lebeda and Petrželová 2004b, 2007). To date, most such studies have been based on evaluation of genebank germplasm samples (Lebeda et al. 2007b) which may not represent the structure of natural host populations. Only in Europe there has been extensive field collections aimed towards obtaining large population samples of *L. serriola* (Doležalová et al. 2001; Křístková and Lebeda 1999; Lebeda et al. 2001b, 2007a).

The interaction between *L. serriola* and *B. lactucae* in Europe is an exciting model for such studies (Lebeda et al. 2001b). First, *L. serriola* is a highly invasive species and recently, due to the increasing ruderalization of the environment, it has become quite a common weed in both agricultural and natural plant ecosystems (Hooftman et al. 2006; Lebeda et al. 2001b, 2004). Furthermore, it is closely related to the cultivated lettuce, considered to be its progenitor (Lebeda et al. 2001b, 2007b). It is also used very extensively as a source of resistance against *B. lactucae* in lettuce breeding, and many of its race-specific resistance genes have been introduced into commercial lettuce cultivars (Lebeda et al. 2002, 2007b). From these perspectives, it is probably the only plant pathosystem where we can precisely study the structure, dynamics and interactions between the wild- and crop-pathosystems (Lebeda 2002; Lebeda et al. 2007c) at both the individual and population levels.

Our recent research shows that Czech populations of *L. serriola* generally display a low level of resistance to *B. lactucae*, which correlates well with the high frequency of disease occurrence recorded during field observations (Petrželová and Lebeda 2004b; Table 2 and Fig. 3). However, the frequent occurrence of differential reaction patterns to the *B. lactucae* races tested (originating only from *L. sativa*) indicates that all the resistance recorded in these *L. serriola* populations is race-specific (Lebeda and Petrželová 2004b, 2007; Fig. 4), supporting previously reported results (Lebeda et al. 2002; Lebeda and Petrželová 2001). Both inter- and intra-population variation in race-specificity was recorded in the Czech metapopulation of *L. serriola*. When the spatial distribution of individual resistance phenotypes was assessed, they were randomly distributed over the study area with no obvious aggregation of populations with more resistant and/or susceptible phenotypes. One of the probable explanations for recorded resistance patterns over the Czech metapopulation of *L. serriola* is that host migration events among locally adapted populations play an important role in shaping resistance structure.

A completely different situation was found within other European metapopulations of *L. serriola* (Lebeda et al. 2007a). Although the same resistance phenotypes could be found in different countries, other European populations generally have completely different resistance patterns with an increasing frequency of race-specificity towards the Atlantic coast. Among-population variation in resistance is largely affected by the level of patchiness and connectivity of populations within the larger metapopulation (Carlsson-Granér and Thrall 2002). In the case of data summarized in this paper, this may be an explanation for greater differentiation, both among and within *L. serriola* populations observed in continental Europe, while the decrease in variation was most pronounced for populations in the United Kingdom, where only one form of the host, *L. serriola* f. *integrifolia*, is prevalent (Lebeda et al. 2004). These populations are spatially isolated from host populations in continental Europe, both by distance per se, but also by their island location (Lebeda and Petrželová 2005). In a previous study with a limited number of plant samples, it was concluded that populations of *L. serriola* in Britain were commonly homogeneous for the *B. lactucae* resistance phenotype. There was no

evidence for extensive resistance gene 'pyramiding' or population heterogeneity as defence strategies against *B. lactucae* in natural populations of *L. serriola* (Crute 1990). However, some variation in the level of field resistance was recorded (Crute 1990) which agrees with observed levels of field resistance in wild *Lactuca* spp. germplasm (Lebeda 1990).

In natural pathosystems, disease dynamics is the most important factor driving the diversity and distribution of host-resistance genotypes (Laine 2006). In the crop pathosystem, *L. sativa*–*B. lactucae*, geographic differences in virulence among pathogen populations from various countries and growing areas are relatively well known (e.g. Crute 1987; Lebeda and Zinkernagel 2003a) and the same should be expected in the wild pathosystem, *L. serriola*–*B. lactucae* (Lebeda 2002; Lebeda and Petrželová 2004a; Petrželová and Lebeda 2004c). However, for *L. serriola* we still lack sufficient data about relevant pathogen populations in much of Europe except for the Czech Republic, hindering our understanding of coevolutionary trends within this pathosystem. Thus, we can only suppose that, at the larger spatial scale, different *L. serriola* populations may have been exposed to differential selection pressures by *B. lactucae* and as a result evolved different patterns of resistance.

Temporal changes in variation of virulence in populations of *B. lactucae* occurring on *L. serriola*

Samples of *B. lactucae* from naturally infected wild populations of *L. serriola* and of cultivated lettuce (*L. sativa*) were collected and used for the virulence screening. Tests were carried out according to methods described previously (Lebeda 2002; Lebeda and Zinkernagel 2003b). Altogether, 313 isolates of *B. lactucae* from *L. serriola* were collected during the period of searching (23 in 1998, 31 in 1999, 78 in 2000, 43 in 2001, 8 in 2002, 19 in 2003, 51 in 2004 and 60 in 2005).

Virulence of isolates was examined by screening on a standard differential set (van Ettekoven and van der Arend 1999). More detailed characterization was made on 56 *L. sativa* and *L. serriola* genotypes (Lebeda and Zinkernagel 2003b) with well characterized patterns of race-specific resistance (*Dm*-genes or

R-factors). By use of both differential sets, 32 virulence factors (v-factors) were determined; however four of them (v32, v33, v41 and v42) are not included in the set of data (see Fig. 5).

Variation in virulence among natural populations of *B. lactucae* in the Czech Republic was studied from 1998 to 2005 and was analyzed at both individual and population levels. Results related to the analysis of virulence at the level of individuals have been partly (for the period 1997–2000) previously published (Lebeda and Petrželová 2004a; Petrželová and Lebeda 2004c). Variation in virulence and its temporal changes were quantified by relative frequencies of virulence factors (v-factors) in the tested isolates (Lebeda 1981, 1982; Fig. 5). With only a few exceptions (v18, v32, v33, v37, v41 and v42), most of the examined v-factors were recorded in populations of *B. lactucae* on *L. serriola* during the whole study period. Nevertheless, there were substantial differences in frequencies recorded for individual v-factors (Fig. 5). Medium to high frequencies were recorded for v-factors v5/8, v7, v11, v14–17 and v23–30. In contrast, factors v1–4, v6, v10, v12, v13, v35, v36 and v38 were detected in extremely low frequencies (Fig. 5). Frequencies of v-factors were not uneven with values varying by year. Fluctuations were most pronounced for factors v5/8, v11, v14, v16, v23 and v25–28, with v5/8 and v23 showing a rapid decrease within natural populations of *B. lactucae* (Petrželová and Lebeda 2004c). On the other hand, factors v14 and v17 increased.

Virulence patterns on the differential set recorded for individual isolates were described as virulence phenotypes (v-phenotypes). The complexity of recorded *B. lactucae* v-phenotypes varied broadly, with 6 to 33 *Lactuca* spp. differentials being infected by the tested isolates. The distribution of isolates based on the number of virulent responses showed a normal (Gaussian) distribution curve each year, with average virulence ranging from 9 to 15 virulent responses. In total, 93 different v-phenotypes were identified among 313 isolates tested in the period 1998 to 2005 (Table 7). Mean variation of v-phenotypes differed among years, ranging from 24.4% in year 2000 to 87.5% in year 2002. However, the basic spatial-distribution pattern of individual v-phenotypes was very similar among years. Each year, there was one or a few v-phenotypes prevailing in examined populations, and broad variation of other unique ones (Lebeda et al. 2007c). Some pathogen populations were also tested for the existence of intra-population variation. Different v-phenotypes were recorded even among pathogen isolates collected from *L. serriola* plants growing in close proximity in one host population (Lebeda and Petrželová 2004a).

A primary main focus for our long-term research on *Lactuca* spp.–*B. lactucae* pathosystems has been the determination of virulence variation within *B. lactucae* populations, documenting its temporal and spatial dynamics, and comparing pathogen populations in crop (*L. sativa*) and wild (*L. serriola*)
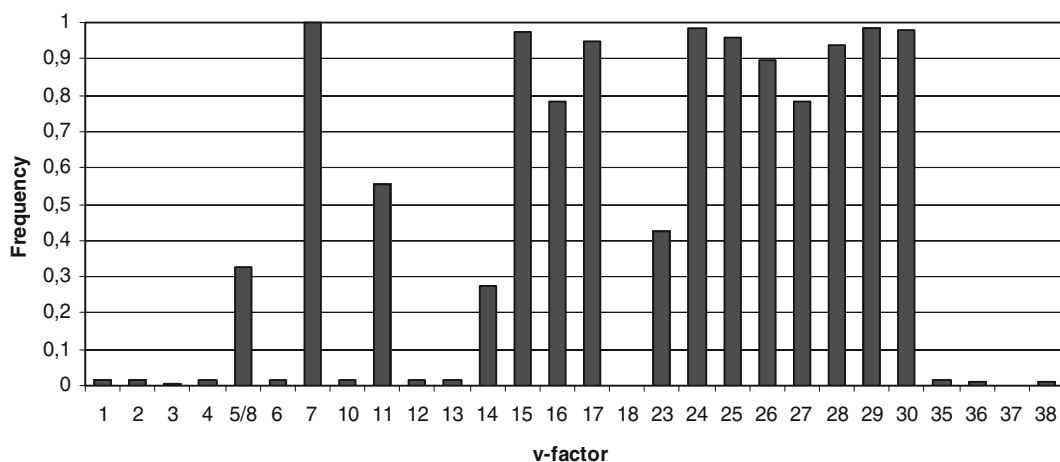

Fig. 5 Frequency of v-factors recorded in the sampled set of *Bremia lactucae* isolates (*n*=313) collected in the Czech pathogen populations on *Lactuca serriola* during the period 1998–2005

Table 7 Variation of virulence phenotypes in populations of *Bremia lactucae* on *Lactuca serriola* in the period 1998–2005

Year	No. of tested isolates	No. of determined v-phenotypes	Variation among studied isolates (%)
1998	23	12	52.2
1999	31	13	41.9
2000	78	19	24.4
2001	43	29	67.4
2002	8	7	87.5
2003	19	11	57.9
2004	51	16	31.4
2005	60	34	56.7
Total	313	93	29.7

pathosystems. At the population level, virulence can be thought of as the average ability of a pathogen population to overcome the diversity of resistance genes present in the corresponding host population (Thrall and Burdon 2003). From this viewpoint, *B. lactucae* populations occurring naturally on *L. serriola* showed highly complex patterns of virulence in relation to the *L. serriola* and/or *L. sativa* differentials with resistance derived from *L. serriola* (Lebeda 2002; Lebeda and Petrželová 2004a; Petrželová and Lebeda 2004c). Such complexity of pathogen isolates may have its origin in response to the heterogeneity of the host populations (Bevan et al. 1993), and it is supposed that, in gene-for-gene based pathosystems, broadly virulent isolates of pathogen are more likely to occur in highly diverse and resistant host populations (Thrall and Burdon 2003). Many papers have highlighted the importance of parallel studies of the structure and dynamics of the host and pathogen populations (e.g. Burdon and Jarosz 1991; Carlsson-Granér and Thrall 2002; Delmotte et al. 1999; Laine 2006; Thrall and Burdon 2000, 2003), which can bring new insights into the behaviour of the two interacting species.

Recently, we compared the resistance of Czech populations of *L. serriola* (Fig. 4) and the virulence of *B. lactucae* populations occurring within the same area. While *B. lactucae* isolates originating from naturally infected *L. serriola* generally showed high compatibility to *L. serriola* they were typically incompatible with *L. sativa* differentials, with the exception of those *L. sativa* genotypes carrying race-specific resistance genes derived from *L. serriola*

(Lebeda 1989, 2002; Lebeda and Petrželová 2004a). Interestingly, when samples from *L. serriola* populations were screened for resistance to races of *B. lactucae* with v-phenotypes generally able to overcome resistance in *L. sativa* they typically showed relatively low levels of race-specific resistance (Lebeda and Petrželová 2004b). This raises the question of why *B. lactucae* isolates with relevant virulence are not more common in natural populations of *B. lactucae*. Only a few such isolates were found in one year (1998) in a region where lettuce is frequently cultivated (Lebeda 2002). If we suppose that populations of *B. lactucae* on *L. sativa* and *L. serriola* are fully inter-connected, isolates with such v-phenotypes should have a selection advantage in *L. serriola* populations and would appear more frequently. However, it was not true in the populations we studied, and perhaps it occurs only under certain conditions. A logical explanation is that *B. lactucae* populations on *L. sativa* and *L. serriola* are highly isolated by their host specificity, and therefore co-evolution in the wild and crop pathosystems is operating independently.

From this viewpoint, *L. serriola* appears to be a good source of resistance genes for cultivated lettuce (Lebeda et al. 2007b), and cross-inoculation experiments with isolates from *L. sativa* may reveal new sources of resistance (Beharav et al. 2006; Lebeda and Zinkernagel 2003b). However, the occasional occurrence of isolates with combined v-phenotype structure recorded both in the wild (Lebeda and Petrželová 2004a; Petrželová and Lebeda 2004c) and crop pathosystems (Lebeda et al. 2007c) indicates that genotype and gene flow between both pathosystems is possible, which may increase variation in both pathogen populations, especially when both host species are grown in close proximity (Lebeda 2002; Lebeda and Petrželová 2004a). Furthermore, in lettuce cultivars with resistance derived from *L. serriola* (Lebeda et al. 2002, 2007b), the probability of an 'escape of virulence' from natural pathogen populations is much higher (Lebeda 1984), and it may increase when encountering wild and crop populations of pathogen that undergo sexual recombination (Lebeda and Blok 1990).

Despite the complexity of responses, broad variation in virulence of *B. lactucae* to *L. serriola* differentials was found, as seen in the variable distribution of v-factors among populations. From our data, it is evident that virulence structure in *B. lactucae* pop-

ulations occurring on *L. serriola* is very dynamic, undergoing both qualitative and quantitative shifts (Lebeda and Petrželová 2004a; Petrželová and Lebeda 2004c). In gene-for-gene systems, it is supposed that the genetic structure of host and pathogen populations follow each other in a dynamic interaction (Burdon 1997; Burdon et al. 1996), so a long-term decrease in particular v-factors (e.g. v5/8 or v23) in *B. lactucae* populations may be evidence of co-evolution taking place. However, increases in the frequency of other v-factors (e.g. v14 or v17) were less marked than the decreases, and may just be considered as evidence for year-to-year fluctuations over a longer time period.

On the level of individuals, no obvious changes in mean virulence were found during the study period, but there was a large variation in recorded virulence patterns (v-phenotypes) and their distribution over the pathogen metapopulation. Furthermore, great differences in the prevalence of individual v-phenotypes were observed, and many rare ones were recorded just once during our investigations. Similar population structures with uneven distribution of v-phenotypes, where just a few predominated have also been reported for other pathogens of wild plants (Bevan et al. 1993; Burdon and Jarosz 1991). It seems that populations of *B. lactucae* tend, on one hand, to higher diversity, but on the other hand to a higher prevalence of particular v-phenotypes. These v-phenotypes may have a selective advantage at a given time and place, which may be largely influenced by fluctuations in local variation in host resistance and the environmental conditions contributing to the establishment of infection in natural plant populations.

Comparison of *B. lactucae* virulence variation between wild (*L. serriola*) and crop (*L. sativa*) pathosystems

In comparison to *B. lactucae* isolates originating from *L. serriola*, isolates originating from cultivated lettuce generally displayed a highly complex response to *L. sativa* differentials and expressed a completely different virulence structure. A comparison of the frequencies of the most important v-factors (from the viewpoint of resistance breeding, see e.g. Lebeda et al. 2007b) in both pathosystems during the study period is illustrated in Fig. 6. Numerous v-factors were detected in both pathosystems; however, their

frequencies differed considerably. Many of the compared v-factors were more common in the crop pathosystem (e.g. v1–4, v6, v10, v12, v13, v36, v38). More or less equal frequencies were recorded for factors v7, v11 and v16, while differences between factors v5/8, v14 and v15 were more distinct (Fig. 6). In this case, it is very interesting that all complementary race-specific resistance genes to these v-factors originate from *L. serriola* (Lebeda et al. 2002). Factor v18 was recorded only on *L. sativa*, and factor v17 was found only on *L. serriola* (Fig. 6). Finally, factor v37 was not found in either pathosystem. In the crop pathosystem temporal shifts in frequencies of some v-factors were also recorded (Lebeda et al. 2007c).

Isolates with combined virulence (for *L. sativa* and *L. serriola*) structure were recorded only occasionally. Differences were also recorded in the dynamics of occurrence of v-phenotypes in both pathosystems. Most of the v-phenotypes found on *L. sativa* were unique and did not appear repeatedly in other pathogen populations or in subsequent years (Lebeda et al. 2007c; Petrželová and Lebeda 2004a).

Large differences in virulence were recorded between populations of *Bremia lactucae* occurring on *L. sativa* and *L. serriola*. Populations in crop and wild *Lactuca–B. lactucae* pathosystems have different structures of v-factors; individual v-factors occur in different frequencies and differences were recorded in their spatial and temporal population structure and dynamics. These findings show that individual *B. lactucae* populations substantially differ in their specificity to the host species and virulence to the host genotypes, respectively. However, we observed some unexpected overlaps in virulence structure between *B. lactucae* populations on *L. sativa* and *L. serriola* (Fig. 6).

Temporal shifts in virulence were recorded in both pathosystems. In the crop pathosystem, the changes are largely influenced by increasing usage of cultivars with newly introduced race-specific resistance genes (Lebeda and Zinkernagel 2003a). Characterization of the evolutionary forces driving the wild pathosystem, *L. serriola–B. lactucae*, will require more detailed studies of resistance patterns and their changes in *L. serriola* populations (Lebeda et al. 2001b, 2007c). The virulence data for both pathosystems are very unique and comparable results are not available for any other crop and wild pathosystems.
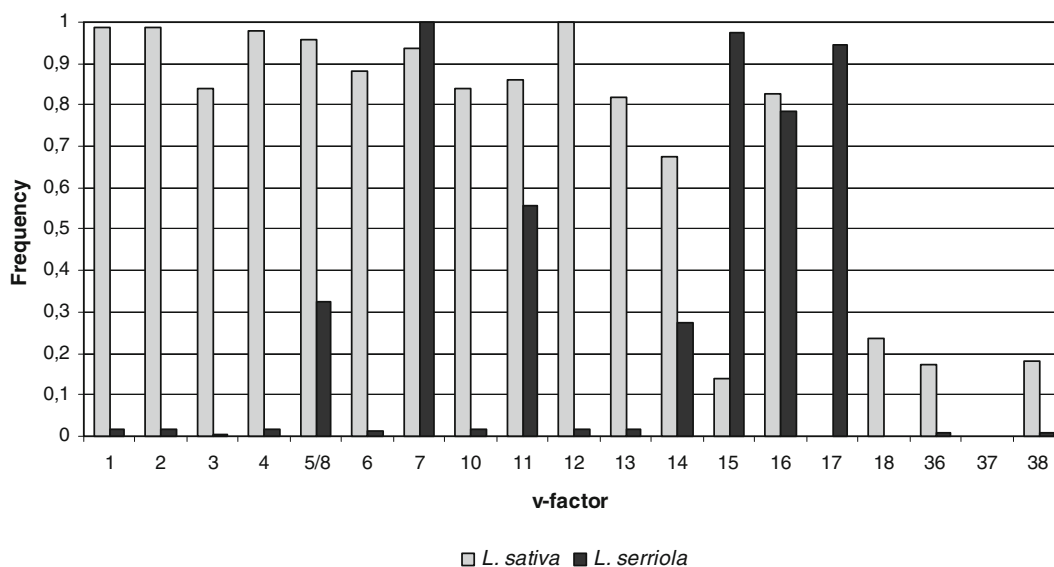
Fig. 6 Comparison of frequency of the most important v-factors recorded in the sampled set of *Bremia lactucae* isolates collected in the Czech pathogen populations on *Lactuca sativa* (*n*=93 isolates) and *Lactuca serriola* (*n*=313 isolates) during the period 1998–2005. v1, v2, v3, v4, v10, v12, v13, v14—v-factors matching *Dm* genes or R-factors in cvs of *L. sativa*. v7, v15, v16, v17, v23, v24, v25, v26, v27, v28, v29, v30—v-factors matching *Dm* genes or R-factors in *L. serriola*. v5/8, v6, v11, v18, v38—v-factors matching *Dm* genes or R-factors in cvs of *L. sativa* derived from *L. serriola*. v36, v37—v-factors matching *Dm* genes or R-factors in cvs of *L. sativa* derived from *L. saligna*. v35—v-factor matching *Dm* gene or R-factor in cvs of *L. sativa* derived from *L. virosa*. Relative frequencies of individual v-factors were expressed as the ratios between the number of isolates with given v-factor and the total number of isolates investigated for the presence of considered v-factor

Sexual reproduction and occurrence of mating types in *B. lactucae* populations on *L. serriola*

The occurrence of mating types was studied in a set of 59 *B. lactucae* isolates originating from 33 naturally infected and wild populations of *L. serriola* in the Czech Republic, including two isolates from Germany and France. The isolates were collected in the period 1997–1999. Both compatibility types were recorded; however, the majority of the isolates (96%) were determined as type B2, supporting the observation that sexual reproduction of *B. lactucae* on naturally growing *L. serriola* plants is rare (Petrželová and Lebeda 2003).

As was stated before, sexual recombination is considered to be important for generating considerable genetic variation in virulence in populations of *B. lactucae* on *L. sativa* (Crute 1992b; Lebeda and Schwinn 1994). However, our previous (Lebeda and Blok 1990) and recent results indicate that its importance for the pathogen populations on *L. serriola* is questionable (Petrželová and Lebeda 2003). From the practical viewpoint it is interesting that isolates of *B. lactucae* originating from *L. sativa*

and *L. serriola* are not completely compatible when pairing together to produce oospores under laboratory conditions (Petrželová and Lebeda 2003). This is additional evidence for at least some isolation of both pathosystems (*L. sativa* versus *L. serriola*–*B. lactucae*). We may assume that the possible danger of the natural formation of new virulent races of *B. lactucae* by the crossing of pathogen isolates from crop and wild lettuce is quite low; this is also supported by the results from virulence analyses (Fig. 6).

Conclusions and future developments

From the results summarized in this paper, it is evident that the wild pathosystem, *L. serriola*–*B. lactucae*, is very complex, variable and dynamic. The frequency of pathogen incidence in host populations is very high, but disease prevalence is rather low. Other host plant species have no substantial influence on *B. lactucae* epidemiology. Pathogen incidence is most strongly influenced by ecological factors, including host habitat, density of host populations, and climatic and microclimatic conditions. Host plants are

also frequently infected by powdery mildew (*Golovinomyces cichoracearum*); the frequency of co-infection by both pathogens is about 35%. We expect some competition for leaf niche, and the phenomenon of induced resistance may play a role in co-infection, but we lack clear experimental evidence to document this phenomenon.

Research showed that race-specific resistance is the dominant pattern in populations of *L. serriola*. A broad spectrum of resistance phenotypes (altogether 45) was detected in host populations occurring in the Czech Republic. In some populations, individuals with completely resistant or completely susceptible reactions were detected. Intra-population variability was rather common. Comparison of host populations from continental Europe with those from the UK showed substantial differences. The island populations were much more homogeneous with regard to variation in resistance, and reactions were always race-specific in inoculation studies.

From the viewpoint of virulence, the pathogen population is enormously variable, and most of the known v-factors were detected. However, there are substantial differences in the frequency of individual v-factors. In the pathogen population, v-factors that match R-factors originating from *L. serriola* prevail, but v-factors matching race-specific *Dm* genes from cultivated lettuce are very rare. At the individual level, we recorded many v-phenotypes, but only a few were common in *B. lactucae* populations. Comparative studies of the virulence structure of pathogen populations in wild and crop pathosystems clearly demonstrated completely different compositions of v-factors and v-phenotypes. It seems that there is no direct epidemiological linkage between both pathosystems. However, some unexpected overlaps in virulence structure were recorded, and these differences may be evidence for the existence of potential migration or gene flow between the pathosystems. This phenomenon, although rare, may be quite important and could influence the stability of resistance derived from *L. serriola* in cultivated lettuce.

The data obtained about this pathosystem demonstrate that *B. lactucae* belongs to a group of pathogens with high evolutionary potential (*sensu* McDonald and Linde 2002), and the wild host population is extremely variable from the viewpoint of resistance. The evolutionary forces operating in and between both pathosystems are not well known. Future

research on these pathosystems can contribute to our understanding of this exciting area of plant pathology and oomycete population biology. We propose that these pathosystems are quite suitable as model systems for the study of structure and variability from a spatial and temporal viewpoint, and that interactions between the wild and crop pathosystems are especially relevant for crop improvement and agricultural production.

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