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Diversity of defence mechanisms in plant–oomycete interactions: a case study of *Lactuca* spp. and *Bremia lactucae*

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Abstract Plant pathogenic oomycetes, including biotrophic downy mildews and hemibiotrophs/necrotrophs such as *Phytophthora* and *Pythium*, cause enormous economic losses on cultivated crops. Lettuce breeders and growers face the threat of *Bremia lactucae*, the causal agent of lettuce downy mildew. This pathogen damages leaf tissues and lettuce heads and is also frequent on wild Asteraceae plants. The interactions of *Lactuca* spp. with *B. lactucae* (abbr. lettuce–*Bremia*) display extreme variability, due to a long co-evolutionary history. For this reason, during the last 30 years, the lettuce–*Bremia* pathosystem has been used as a model for many studies at the population, individual, organ, tissue, cellular, physiological and molecular levels, as well as on genetic variability and the genetics of host–parasite interactions. The first part of this review summarizes recent data on host–parasite specificity, host variability, resistance mechanisms and genetics of lettuce–*Bremia* interactions. The second part focuses on the development infection structures. Phenotypic expression of infection, behaviour of *B. lactucae* on leaf surfaces, the process of penetration, development of primary infection structures, hyphae and haustoria are discussed in relation to different resistance mechanisms. In the third part, the components of host resistance and the variability of defence responses are analysed. The role of reactive oxygen species (ROS), antioxidant enzymes, nitric oxide (NO), phenolic compounds, reorganization of cytoskeleton, electrolyte leakage, membrane damage, cell wall disruption, hypersensitive reaction and plant energetics are discussed in relation to defence responses. In general, the extreme variability of interactions between lettuce and *Bremia*, and their phenotypic expression, results from diversity of the genetic background. Different mechanisms of resistance are conditioned by an orchestra of defence responses at the tissue, cell, and molecular levels. The various events responsible for defence involve a complex interaction of the processes and reactions mentioned above. This review also provides an overview on the timing of pathogen development, host pathological anatomy, cytology and physiology

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of lettuce–*Bremia* associations. The significance of these factors on the expression of different resistance mechanisms (non-host and host resistance, race-specific and race non-specific resistance, field resistance) is discussed.

Keywords Cytoskeleton · Genetics · Host-and non-host resistance · Hypersensitive reaction · Infection structures · Lettuce · Lettuce downy mildew · Nitric oxide · Phenolic compounds · Photosynthesis · Plant energetics · Reactive oxygen species · Specificity

Abbreviations

ATP	adenosine triphosphate
BAP	benzylaminopurine
CKs	cytokinins
dai	days after inoculation
EHM	extrahaustorial membrane
ER	endoplasmatic reticulum
H	intercellular hypha
HA	haustorium
hai	hours after inoculation
HR	hypersensitive reaction
IH	intracellular hypha
IMD	irreversible membrane damage
MTs	microtubules
NADPH	reduced nicotinamide adenine dinucleotide phophate
NO	nitric oxide
NOS	nitric oxide synthase
PAL	phenylalanine ammonium lyase
PAs	phenolic acids
POX	peroxidase
PSII	photosystem II
PTIO	2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide
QTL	quantitative trait loci
ROS	reactive oxygen species
TEM	transmission electron microscopy
3-MeOBAPR	6-(3-methoxy-benzylamino)purine-9-β-ribofuranoside

Introduction

Aspects of plant defence against pathogens have been studied in different model pathosystems, including interactions between plants and oomycetes (Glazebrook 2005; Göker et al. 2007; Hardham 2007; Hulbert et al. 2001; Kamoun 2006). One of the most important pathosystems in terms of economic loss is the *Lactuca* spp.–*Bremia lactucae* (abbr. lettuce–*Bremia*) pathosystem (Crute 1992a; Lebeda et al. 2007, 2008). This paper highlights several aspects of defence mechanisms in this pathosystem from the viewpoint of phenotypic expression, cytology, physiology, biochemistry and biophysics.

Lettuce is the common name used for about 100 species of the genus *Lactuca*; they are prevalently distributed in Asia and Africa, but occur also in Europe, North and Central America (Lebeda et al. 2004). Only one species, cultivated lettuce (*Lactuca sativa*), is grown as a crop worldwide. Lettuce ranks as one of the earliest domesticated vegetables (8,000 years ago; Lebeda et al. 2007). The centre of *Lactuca* spp. biodiversity is in southwestern Asia, in the Tigris–Euphrates region, and lettuce probably originates as a food plant from this region. Systematic breeding of cultivated lettuce started in the 19th century and nowadays it is an extremely variable species, both morphologically and genetically (Lebeda et al. 2007).

Many diseases of lettuce have been described (Davis et al. 1997), but only a few are important enough to be considered in crop protection and resistance breeding programmes (Lebeda et al. 2007). One such disease is lettuce downy mildew, caused by the biotrophic oomycete *B. lactucae* (Lebeda et al. 2002). The breeding of lettuce for resistance to *B. lactucae* started in 1920s and now is considered as a priority among the vegetable features. Several different mechanisms of resistance to *B. lactucae* have been identified within cultivated and wild lettuce (Lebeda et al. 2001b). Because of limited durability of race-specific resistance (Lebeda et al. 2002, 2007; Lebeda and Zinkernagel 2003a), the search has focused on field resistance (Grube and Ochoa 2005) and new sources of resistance in wild *Lactuca* species (Lebeda et al. 2002, 2007).

This paper deals with the complexity of pathological processes induced in host *Lactuca* spp. plants following infection by *B. lactucae*. The main aim was to analyse critically the recent knowledge in this area, within a wider context of plant–oomycete interactions and with the main focus on the lettuce–*Bremia* pathosystem.

Host–parasite specificity

General aspects of specificity in plant–oomycete interactions have been at the centre of mycological and phytopathological research during the 1990s and early 2000s (Clark and Spencer-Phillips 2004; Glazebrook 2005; Göker et al. 2007; Grenville-Briggs and van West 2005; Hardham 2007; Holub and Cooper 2004; Latijnhouwers et al. 2003; Lebeda and Schwinn 1994; Lipka et al. 2005; Lucas et al. 1995; Mauch-Mani 2002; O'Connell and Panstruga 2006). For the lettuce–*Bremia* pathosystem, a detailed survey of host–parasite specificity can be found in Lebeda et al. (2001b, 2002). The enormous variability in the specificity of interactions can be explained by the lengthy coevolution of the host–parasite association between *Lactuca* spp. and *B. lactucae* (Lebeda 2002; Lebeda et al. 2002).

Taxonomy, host range and specialization of *B. lactucae*

It has become apparent that taxa in the Peronosporaceae are polyphyletic or paraphyletic (Thines et al. 2006; Voglmayr 2008; Voglmayr et al. 2004). As a result of molecular phylogenetic investigations, six new genera have been described in the Peronosporaceae, with features revealed by scanning electron microscopy (Thines 2007). The genera *Bremia, Protobremia, Paraperonospora, Plasmoverna, Basidiophora, Benua* and *Plasmopara* form a dense cluster due to the uniting aspects of similarities in the morphology of their haustoria (vesicular to pyriform) and ultimate branches (Göker et al. 2007; Thines 2007; Voglmayr 2008).

Bremia lactucae, a pathogen of cultivated and wild lettuce (Lebeda et al. 2002), has been reported to infect plants of more than two hundred species from about 40 genera of the Asteraceae (Crute and Dixon 1981; Lebeda et al. 2002). New host species continue to be reported (e.g. Koike and Ochoa 2007). Based on cross-inoculation experiments and morphological observations, the specialization of *B. lactucae* into 11 formae speciales was accepted (Lebeda et al. 2002; Skidmore and Ingram 1985). These experiments showed a high specificity of the formae speciales, with each almost exclusively limited to an individual host genus (Lebeda et al. 2002). However, several previous (Lebeda and Syrovátko 1988) and recent (Vieira and Barreto 2006) experiments have suggested the possibility that infection of lettuce (*L. sativa*) by *B. lactucae* originates from *Sonchus* spp. and *vice versa*. *Sonchus oleraceus* was the most common weed hosting *B. lactucae* outside of *Lactuca* spp. (Lebeda et al. 2008). From about 100 wild species described within the genus *Lactuca* (Lebeda et al. 2004), only 14 are reported as natural hosts of *B. lactucae* (Lebeda et al. 2002). *Lactuca serriola* is considered to be the most common weed host in central Europe (Lebeda et al. 2008; Petrželová and Lebeda 2004). Specificity of the interactions between wild *Lactuca* spp. and *B. lactucae* is still not completely understood. Comprehensive data are available only for *L. serriola* (primary lettuce gene pool), *L. saligna* and *L. virosa* (secondary and tertiary gene pools, respectively; Lebeda et al. 2002, 2007).

Molecular phylogenetic studies, using nuclear large subunit rDNA sequences with D1/D2 regions (Voglmayr et al. 2004) and internal transcribed spacer rDNA (Choi et al. 2007), revealed the presence of several highly supported clades within the *B. lactucae* complex. These lineages match partially to formae speciales (Skidmore and Ingram 1985). Most importantly, the genetic distance of isolates originating from *Lactuca* spp. and those from other hosts was clearly demonstrated, suggesting a lack of interbreeding. Therefore, infected wild Asteraceae plants other than *Lactuca* are unlikely to serve as a source of inoculum for infections in *Lactuca* spp. populations (Lebeda et al. 2002, 2008; Voglmayr et al. 2004). Previous individual (Lebeda 1986) and recent population (Lebeda 2002; Lebeda et al. 2008) studies, however, have shown that isolates of *B. lactucae* from *L. serriola* are significantly more pathogenic to *L. serriola* than to *L. sativa*. Data from these phenotypic studies demonstrate the possibility of very close genetic affinity between *Lactuca* host species and *B. lactucae* and *vice versa*, and this must be considered in the planning of experiments focused on research of resistance mechanisms.

Resistance mechanisms in *Lactuca* spp.

In most interactions, the resistance of *Lactuca* spp. to *B. lactucae* is considered as host-resistance, according to the phenotypic, tissue and cellular expression. Only *L. saligna* appears to differ in several features, thus raising the possible existence of non-host resistance (basic incompatibility; Lebeda et al. 2002). Recent

studies with individual plants (Beharav et al. 2006; Lebeda and Zinkernagel 2003b) and populations (Petrželová et al. 2007) of *L. saligna* showed a high degree of resistance to all *B. lactucae* races originating from lettuce, and also those from *L. serriola* (Lebeda 1986; Lebeda and Boukema 1991). Moreover, studies at the tissue, cellular and physiological levels (Lebeda et al. 2001b, 2002, 2006; Lebeda and Pink 1998; Lebeda and Reinink 1994; Sedlářová and Lebeda 2001; Sedlářová et al. 2001b, 2007a, b) confirmed that the mechanism of resistance in *L. saligna* differs significantly from the mechanisms known in *L. sativa, L. serriola* and *L. virosa* (Lebeda et al. 2002).

Host resistance (basic compatibility) is a better known phenomenon in this pathosystem as it has been studied since the beginning of the 20th century from many perspectives. The most common three categories of host resistance are reviewed below, i.e. race-specific resistance, race non-specific resistance and field resistance (Lebeda et al. 2001b, 2002).

Race-specific resistance with its characteristic phenotypic expression and intensively studied genetics can be found in cultivars of *L. sativa* (e.g. Lebeda et al. 2007). The specificity is determined by dominant resistance genes and/or factors in the host (*Dm* genes and/or R-factors) which are matched by pathogen dominant factors of avirulence (Crute 1992b; for more details see Genetics of *Lactuca* spp.–*Bremia lactucae* interactions). Race-specificity is well documented also in wild *Lactuca* spp. (Table 1) and a few closely related genera (Lebeda et al. 2002). Recently, it was found as a common phenomenon in wild populations of *L. serriola* where enormous diversity of this type of resistance was described (Lebeda et al. 2008; Lebeda and Petrželová 2004).

Race non-specific (non-differential) resistance is conferred by several genes and characterized by effectiveness against a spectrum of *B. lactucae* races. *Lactuca* spp. genotypes with this type of resistance posses a certain level of non-specific resistance according to phenotypic expression (Lebeda et al. 2002). The presence of race non-specific resistance is not well-documented for *L. sativa* (Lebeda et al. 2001b). It has only been reported in some accessions of *L. serriola* (PI 281876 and PI 281877) for which the genetic background is not well known, and the presence of some major genes and modifiers is predicted (Lebeda et al. 2002).

Field resistance is a complex epidemiological phenomenon (Lebeda et al. 2002), expressed by reduced susceptibility of mature plants grown in the field with natural infections of *B. lactucae* (Grube and Ochoa 2005). A search for sources of field resistance in *L. sativa* located a high level of this resistance in cvs Iceberg and Grand Rapids (Crute and Norwood 1981). Recent studies suggested simple inheritance of this trait, but the single gene models did not fit the data obtained (Grube and Ochoa 2005). Field resistance also is expected in wild *Lactuca* spp., with direct evidence existing for some *L. serriola* accessions (e.g. PI 281876; Lebeda et al. 2002).

Genetics of *Lactuca* spp.–*Bremia lactucae* interactions

Nowadays, more than 45 host race-specific resistance genes/factors (*Dm*/R) and complementary pathogen virulence (*v*) genes (factors) are predicted in the lettuce-*Bremia* pathosystem (Lebeda et al. 2006). Many of these are used for phenotypic screening of *B. lactucae* isolates and characterization of their virulence (Lebeda and Petrželová 2008). The number of host resistance genes is expected to increase further with continuation of extensive phenotypic characterization of *Lactuca* germplasm (Beharav et al. 2006; Lebeda and Petrželová 2004; Lebeda and Zinkernagel 2003b) and molecular investigations (Kuang et al. 2004, 2006; Michelmore, pers. comm.). At least 15 *Dm* genes have been characterized in lettuce spp. (*Dm*1, *Dm*2, *Dm*3, *Dm*4, *Dm*5/8, *Dm*6, *Dm*7, *Dm*10, *Dm*11, *Dm*12, *Dm*13, *Dm*14, *Dm*15, *Dm*16 and R18). These genes occur in distinct clusters within the lettuce genome, at least five clusters being recognized (Witsenboer et al. 1997). Some of these *Dm* genes originate from *L. serriola* (Lebeda et al. 2002), others were described and subjected to preliminary genetic characterisation (Bonnier et al. 1994), and numerous others are to be expected (Lebeda and Petrželová 2004). Some recently released R-factors (R36 and R37), which are located in *L. sativa* originate from *L. saligna* (Michelmore et al. 2005). Other resistance gene candidates (RGCs) are proposed and used for evolutionary studies to explore the diversity of *Lactuca* spp. germplasm (Kuang et al. 2004, 2006). However, the specificities of the genes need to be established and their effectiveness against given pathogen races must be demonstrated. This poses a

Table 1 Generalized overview on variability in formation of *B. lactucae* infection structures and reactions of *Lactuca* spp. tissues at 48 h after inoculation in various categories of resistance (compiled according to Lebeda et al. 2001b, 2002, 2006; Lebeda and Pink 1998; the data were obtained on leaf discs derived from adult plants)

Category of resistance	*Lactuca* spp. genotype	Genetical background/ resistance gene (factor)[a]	Response to *Bremia lactucae*[b]	Relative degree of infection structure development and tissue response[c]					
				Primary vesicle	Secondary vesicle	Hyphae	Haustoria	Hypersensitive reaction	Subepidermal necrosis
Non-host?	*L. saligna* (LSA/6)	?	−	3	2	0	0	1	1
Race-specific	*L. sativa* (Cobham Green)	R 0 (?)	+	4	4	4	4	1	0
	L. sativa (Dandie)	*Dm* 3	+	4	4	2	1	1	1
	L. sativa (Valmaine)	*Dm* 5/8	−	3/4	1	1	0	2	0
	L. sativa (Mariska)	R 18	−	2	2	2	2	4	3
	L. serriola (PIVT 1168)	R ?	−	2	1	0/1	0	4	2
	L. saligna (CGN 5147)	R ?	−	3	1	1	0	1	0
	L. virosa (LVIR/57/1)	R ?	(−)	3/4	3	1/2	1/2	4	2/3
Race-non-specific	*L. serriola* (PI 281876)	R ? (+modif.?)	(−)	4	4	2	2	4	2
Field	*L. sativa* (Iceberg)	nR?	+	3	3	3	3	1	0

? This category is still questionable for *L. saligna* (see discussion in: Jeuken and Lindhout 2002; Lebeda et al. 2001b, 2002)

[a] ? Not known or unspecified, *R* race-specific resistance factor, *Dm* race-specific resistance gene; *modif.* modifier gene(s); *n* more R-factors

[b] Categories of phenotypic expression of *Lactuca* spp. response to *B. lactucae*: − incompatible (no sporulation); *(−)* incompletely incompatible (very limited sporulation occurring mostly at the cutting edges of leaf discs); + compatible (profuse sporulation); a field resistance cannot be distinguished by screening either on cotyledons or leaf discs

[c] Relative degree of occurrence of pathogen infection structures and plant tissue response compared to susceptible control (details are given in Lebeda et al. 2002): *0* none recorded, *1* very low frequency, *2* low frequency, *3* medium frequency, *4* high frequency. Significant differences in frequency and timing are specific for given genotype-race interaction, usually *L. sativa* genotypes vary from other wild *Lactuca* spp.

barrier to the rapid engineering of durable resistance. Currently, much effort in lettuce resistance breeding is focused on deployment of non-durable R-genes (Lebeda et al. 2007; Pink 2002). Thus, breeders have to look for new sources in wild *Lactuca* spp. (Beharav et al. 2006; Lebeda et al. 2002; Lebeda and Zinkernagel 2003b), but there is still a lack of genetic and molecular data on variation and resistance in other wild *Lactuca* spp. (e.g. *L. virosa, L. saligna*; Kitner et al. 2008; Lebeda et al. 2002, 2007).

The first detailed genetic studies dealing with *L. saligna* resistance against *B. lactucae* were performed by Jeuken and Lindhout (2002) as a QTL analysis on plants of a *L. saligna* (resistant) × *L. sativa* (susceptible) cross. The phenotype of the F2 population showed a continuous range of resistance categories from completely resistant to completely susceptible, providing evidence that both qualitative and quantitative resistance were involved. Subsequent QTL mapping revealed a qualitative gene (R39) and three QTL (RBQ1, RBQ2 and RBQ3) accountable for the quantitative resistance. Some additional studies implied that resistance in *L. saligna* was quantitatively expressed and might be race non-specific. The current general view on *L. saligna* non-host resistance is that it is not explained by accumulation of race-specific resistance genes (*Dm* genes) but instead by resistance mechanisms based on QTL (Jeuken and Lindhout 2002).

Pyramiding of resistance genes in lettuce cultivars (Crute 1992b) forms a selection pressure that alters the structure of pathogen populations (Lebeda and Zinkernagel 2003a) and initiates the boom and bust cycle. On the other hand, gene-flow from cultivated to natural *Lactuca* spp.–*B. lactucae* populations and *vice versa* must also be considered (Lebeda 2002; Lebeda et al. 2008). An hypothesis of Hooftman et al. (2007) attributed the expanding distribution of prickly lettuce (*L. serriola*) in Europe to enhanced plant fitness by hybridisation with lettuce (*L. sativa*). However, this ecological study revealed that introgression of an important crop trait, downy mildew resistance, from lettuce into *L. serriola* hybrids was insignificant for plant reproductive fitness. In contrast, effectiveness of some resistance traits introduced to lettuce from wild *Lactuca* spp. (esp. *L. serriola*) might be broken by pathogen populations present in wild plant pathosystems (Lebeda 2002; Lebeda et al. 2008).

Development of *Bremia lactucae*

Symptoms of lettuce downy mildew

Description of downy mildew symptoms on lettuce (*L. sativa*) can be found elsewhere (e.g. Crute and Dixon 1981; Davis et al. 1997). Symptoms typically appear as areas of chlorotic tissue, mostly delimited by the main veins, which is accompanied by profuse sporulation on the abaxial side of leaves in compatible interactions. In field conditions, the air-borne asexual conidia are the most important means of disease spread throughout the growing season. Intensity of sporulation as well as viability of conidia is influenced substantially by environmental factors (Judelson and Michelmore 1992; Nordskog et al. 2007) and by concentration of primary inoculum (Crute and Dickinson 1976).

Broad variation of phenotypic expression of *B. lactucae* infection was reported in both susceptible and resistant *Lactuca* spp. genotypes (Table 1; for review see Lebeda et al. 2001b, 2002, 2008). The phenotype of non-host resistance (e.g. in some *L. saligna* accessions and most Asteraceae species) is characterized by a lack of symptoms (Crute and Dickinson 1976; Lebeda and Reinink 1994; Lebeda and Syrovátko 1988; Sedlářová et al. 2001b). Nevertheless, expression of macroscopic chlorosis (Crute and Dickinson 1976), necrosis (Lebeda and Reinink 1994; Norwood et al. 1981) or sub-epidermal necrosis (Lebeda and Reinink 1994; Lebeda et al. 2006) was also recorded. Expression of host resistance symptoms varies according to the ontogenetic stage of the host, as it was found to differ between the cotyledons and adult plants within the same interaction (Crute and Dickinson 1976; Lebeda and Reinink 1991; Lebeda et al. 2006). In lettuce–*Bremia* interactions a wide array of symptoms occurs, ranging from no visible symptoms to an extensive necrotization (incompatibility), and from limited sporulation (incomplete resistance) to profuse sporulation without any other visible symptoms (full compatibility; Lebeda et al. 2002). These categories of symptoms are highly specific and conditioned by race-specific resistance *Dm* genes in many cases. For example, *Dm*7 conditions reduced sporulation and necrosis in some genotype–race interactions (Crute and Johnson 1976). Wild *Lactuca* spp., e.g. *L. serriola* and *L. virosa*, have also been reported to express a broad spectrum of symptoms (Lebeda and Pink 1998; Norwood et al. 1981). There

are many intermediate phenotypes between the extremes with a substantial influence of experimental and environmental conditions (for overview see Lebeda et al. 2001b, 2002).

Characteristics of leaf surface: influence on conidial germination and appressorium formation

The characteristics of plant leaf surfaces, referred to here as the indumentum and including the number and character of trichomes, thickness and composition of waxes, number and position of stomata, determine success or failure of pathogen spore deposition and subsequent ingress by infection structures. Spores deposited on the leaf surface face several obstacles to gaining host nutrients: cuticle, cell wall and plasma membrane (Lebeda et al. 2001b).

The cuticle is known as a 'two-step' barrier comprising an internal and external layer. The internal cuticle on the inner periclinal walls of epidermal cells functions primarily in water exchange regulation (Pesacreta and Hasenstein 1999). More important from the pathogen perspective is the external cuticle. Its structure and function have been documented in many plants but experimental data are lacking for *Lactuca* species. Study of indumentum characteristics revealed substantial differences among *Lactuca* species (Lebeda et al. 1999), but there are no data relating the indumentum pattern to *B. lactucae* germination. However, evidence does exist for a relationship between leaf epidermal characteristics in cultivars of potato (*Solanum tuberosum*) and expression of resistance or susceptibility to *Phytophthora infestans* (Mahajan and Dhillon 2003).

There are several crucial steps required prior to the start of oomycete pathogenesis similar to fungal pathogens, i.e. adhesion of spores to the plant surface, and the formation of germ tubes, appressoria and penetration pegs (reviewed in Latijnhouwers et al. 2003). Attachment of germinating spores is mediated through secretion of an extra-conidial matrix. As soon as a germ tube emerges from the *Hyaloperonospora arabidopsidis* (*H. parasitica*) conidium, an 'adhesive coctail' composed of proteins, glycoproteins and β-1,3-glucans is released (Carzaniga et al. 2001). Recently, Hardham (2007) brought together microscopic and molecular data relating to early stages of the infection process in *Phytophthora*, *Pythium* and *Hyaloperonospora* spp.

The mechanisms of retention and adhesion of *B. lactucae* conidia to host leaf surfaces have not been elucidated in detail. The pre-penetration phase was studied by Andrews (1975) who reported the possibility that *B. lactucae* absorbs nutrients (e.g. glucose) from the leaf surface. Many papers deal with *B. lactucae* spore germination, penetration and the further development of infection structures (reviewed in Lebeda et al. 2001b). Conidia start germination mostly at 1–3 h after inoculation on both non-host and host plants (summarized in Lebeda et al. 2002). Germ-tube length is a highly variable parameter (compare Fig. 1a and g; Lebeda and Pink 1998) and does not relate directly to the host resistance, i.e. to *Dm* gene expression, but seems to be specific for each host genotype–parasite race interaction (Lebeda et al. 2001b, 2006). In general, significantly shorter germ tubes develop on wild *Lactuca* spp. than on lettuce (*L. sativa*) genotypes (Lebeda and Pink 1998; Lebeda et al. 2006).

The germination of *B. lactucae* conidia (Fig. 1a) is affected by environmental conditions, such as temperature (Sargent 1976; Sargent and Payne 1974). The peripheral cytoplasm of conidia in the 'dormant' stage contains lipid droplets which are dispersed during the phase of activation (preceding germination). This phase is followed by activation of dictyosomes and endoplasmatic reticulum. Mobilisation of reserves for development of the germ tube tip is accompanied by increased lipolytic activity in mitochondria and esterase activity in vacuoles (Duddridge and Sargent 1978).

Formation of oomycete appressoria, non-pigmented swellings of germ tube tips that differentiate penetration pegs, is not synchronised with germination and may be induced by topological features of the leaf surface (Latijnhouwers et al. 2003; Lebeda et al. 2001b). Significant differences in the frequency of appressorial formation were found between cotyledons (higher frequency) and leaf discs of adult plants (Lebeda and Reinink 1991). The influence of leaf surface character on appressorial formation was demonstrated in cv. Iceberg (genotype with high level of field resistance), where frequency of appressorial formation was significantly lower than on lettuce cultivars with ineffective race-specific resistance (Lebeda and Reinink 1991). A comparative study showed a higher incidence of *B. lactucae* appressorial development on lettuce (*L. sativa*) plants compared to wild relatives (*L. serriola,*

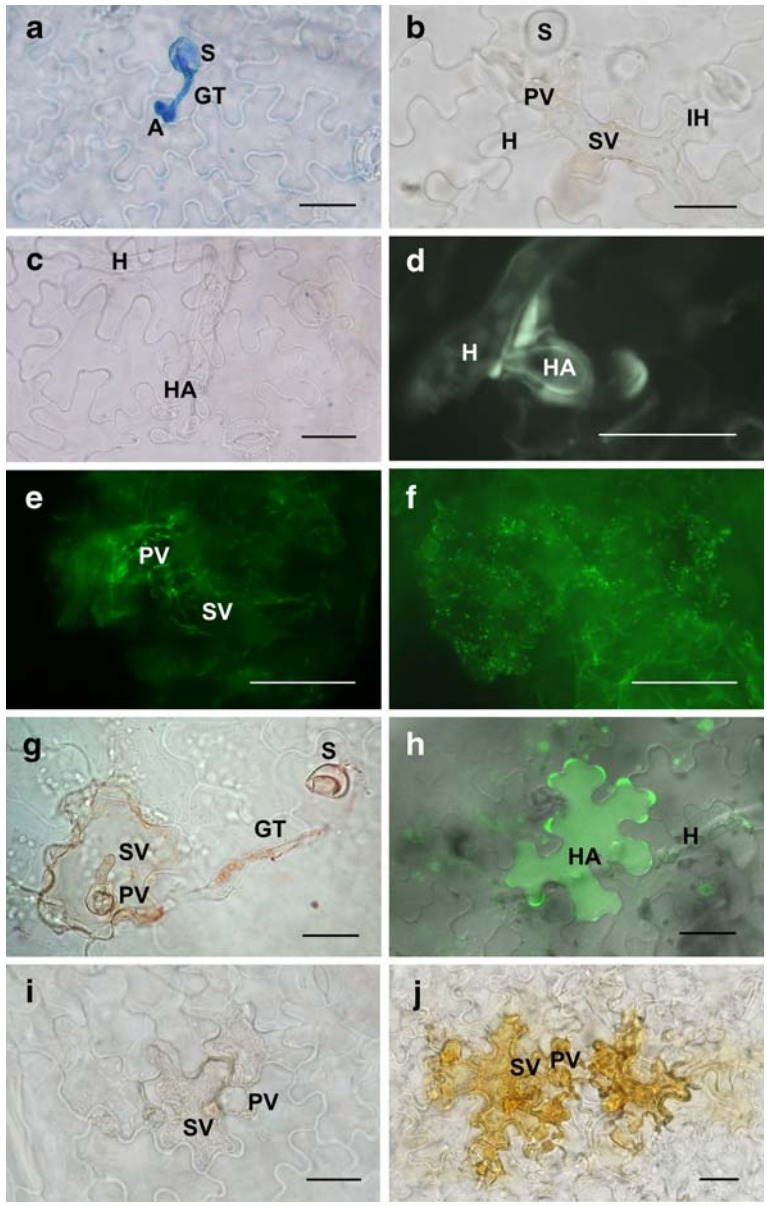

Fig. 1 Development of *Bremia lactucae* (race BL16) and the response of *Lactuca* spp. cells. **a**–**c**. Pathogen growth within tissues of susceptible *L. sativa* (Cobham Green); **a**, germination and appressorial formation (12 hai); **b**, colonisation of host tissues (48 hai); **c**, formation of numerous haustoria (120 hai). **d**. A detail of haustorium with callose deposited around its neck (168 hai), susceptible *L. sativa* (British Hilde). **e**, **f**. Realignment of host MTs due to infection (immunolocalisation of α-tubulin); **e**, microtubular 'basket' formed with host plasma membrane invagination (48 hai), susceptible *L. sativa* (UCDM2); **f**, depolymerization of cortical microtubules induced by initiation of HR (48 hai), resistant *L. serriola* (PIVT 1309). **g**. Peroxidase activity localised both in pathogen infection structures and in the cell of susceptible *L. sativa* (UCDM2; 24 hai). **h**. Signal for NO in epidermal cell of susceptible *L. sativa* (Cobham Green) penetrated by haustoria beneath the growing hypha (192 hai). **i**, **j**. Hypersensitive reaction (HR); **i**, initial stages of HR with granulation of cytoplasm (48 hai), *L. virosa* (NVRS 10.001602); **j**, necrosis, a visible outcome of HR is caused by oxidation of phenolics (336 hai), resistant *L. serriola* (PIVT 1309). Infection structures: spore (*S*), germ tube (*GT*), appressorium (*A*), primary (*PV*) and secondary vesicle (*SV*), intracellular hypha (*IH*), intercellular hypha (*H*), haustorium (*HA*). The bar corresponds to 20 μm. The micrographs were obtained by conventional light microscopy (**a**–**c**, **g**, **i**, **j**), fluorescence microscopy (**d**–**f**) and confocal laser scanning microscopy (**h**). Photo courtesy by M. Sedlářová

L. saligna, L. virosa), whereas no significant differences were found among the wild *Lactuca* spp. (Sedlářová et al. 2001b).

Penetration and development of primary and secondary vesicles

Penetration of plant surfaces by oomycetes is performed by a combination of mechanical force and secreted chemicals, as with other fungal pathogens (Latijnhouwers et al. 2003; Lebeda et al. 2001a). Appressoria of *B. lactucae* are a prerequisite for penetration, and exert high pressure on cell walls allowing penetration pegs to pierce the periclinal cell wall, and to colonize the underlying epidermal cell by the formation of primary and secondary vesicles (Sargent et al. 1973). To date, the turgor pressure exerted by appressoria has not been quantified in any oomycete species. The cuticular penetration predominates in *B. lactucae*, with penetration via stomata incident in about 1–5% of germ tubes (Lebeda and Reinink 1991).

Environmental factors are crucial for the *B. lactucae* penetration process. The effect of temperature is especially important, as seen from an optimum of 15–20°C for germination (Sargent 1976) but 12–15°C for penetration (MacLean and Tommerup 1979). Some details related to the timing of this process were summarized by Lebeda et al. (2001b). The penetration rate is a frequently studied parameter in screening studies (e.g. Lebeda and Pink 1998; Lebeda et al. 2006; Sedlářová et al. 2001b).

Chemical degradation of the cuticle and cell wall is the second necessity for successful penetration of plant cells. Secretion of a wide range of degradative enzymes has been described for fungi and oomycetes (Lebeda et al. 2001a), including hemibiotrophic *Phytophthora* and *Pythium* spp. (Hardham 2007). Extensive genomic studies have initiated the characterization of genes encoding these enzymes. However, direct demonstration of the action of cell wall-degrading enzymes is a perspective for future work (Hardham 2007).

Data on *B. lactucae*-derived enzymes are quite limited, and only polygalacturonase, esterase and protease activities have been reported (Van Pelt-Heerschap and Smit-Bakker 1993). Pathogen lipolytic enzymes, mentioned above in the context of germination (Characteristics of leaf surface: influence on conidial germination and appressorium formation), enable lipid degradation in the cuticle and enable subsequent pathogenesis (Sargent et al. 1973). Lipase activity is also elevated during penetration and the formation of primary vesicles (Duddridge and Sargent 1978). At this stage, the lipolytic activity is localized in lomasomes which occur mostly at the periphery of the expanding vesicle, as illustrated by transmission electron microscopy (Zinkernagel 1985; Zinkernagel and Bartscherer 1978).

Once the pathogen overcomes the barriers of the cuticle and cell wall by the activity of cell-wall degrading enzymes (Lebeda et al. 2001a; Sargent et al. 1973), it gains access to the cell lumen. *Bremia lactucae* forms primary infection structures within the host epidermal cell by invagination of the host plasma membrane. Formation of the primary vesicle (PV), secondary vesicle (SV), and intracellular hypha (IH; Fig. 1b) are not initially followed by destruction of the host cell plasma membrane (Ingram et al. 1976). Only subsequently is the plasma membrane perforated, with colonization of sub-epidermal tissues by intercellular hyphae (H) and haustoria (HA) ensuing (Fig. 1c). Various aspects of this process were reviewed by Lebeda et al. (2001b).

Early stages after inoculation are very important for recognition and initiation of defence responses. In lettuce–*Bremia* interactions, both pre-haustorial and post-haustorial recognition occurs, based on specific *Dm*/Avr gene combinations (Mansfield et al. 1997). Incidence of PVs and SVs, as well as the timing of their formation, may differ in interactions with an identical phenotype (Lebeda and Pink 1998; Lebeda et al. 2006; Sedlářová et al. 2001b). Formation of PVs and SVs also can be found in non-host plants, though at significantly lower frequencies than in host plants (summarized in Lebeda et al. 2002). In some *L. saligna* interactions with *B. lactucae*, the SV represents the final stage of oomycete development which is considered to distinguish non-host resistance (Lebeda and Reinink 1994; Lebeda et al. 2002, 2006; Sedlářová et al. 2001b). Quite intriguing is *B. lactucae* development in interspecific hybrids of lettuce (*L. sativa*) with wild *Lactuca* spp., where an 'heterosis' effect was recorded (increased rate of *B. lactucae* infection structures in F1 hybrids compared to both parents). Details can be found elsewhere (Lebeda and Reinink 1994; Lebeda et al. 2001b, 2006).

Development of hyphae and haustoria

The most important period for hyphal development of *B. lactucae* is 24–48 hai, but the extent and speed of formation of intra- (IH) and inter-cellular (H) hyphae and haustoria (HA) is extremely variable among non-host and host genotypes (Table 1; Lebeda et al. 2001b, 2002). The growth of intercellular hyphae of *B. lactucae* in mesophyll tissue even starts at 12 hai in some compatible interactions (Sedlářová et al. 2001b). Although delayed, the development of IH and H occur in most incompatible (non-host and host) interactions (Lebeda and Schwinn 1994).

An extreme variability of incompatible reactions in *Lactuca* spp. has been described (Lebeda et al. 2006). In *L. saligna* (LSA/6), which is considered as a non-host genotype (Lebeda et al. 2001b), *B. lactucae* did not form any intra- and intercellular hyphae (Lebeda et al. 2006). However, in many other incompatible interactions both types of hyphae were formed with significant differences in frequency (Lebeda and Pink 1998; Lebeda and Reinink 1994; Lebeda et al. 2006). Formation of IH and H was suggested as a crucial developmental stage for *B. lactucae*, and as a limiting factor in host–parasite communication as well as expression of various resistance mechanisms (Lebeda et al. 2001b, 2006). Quantitative comparative studies showed significant variation in size (length and width) of hyphae (Lebeda and Pink 1998; Lebeda and Reinink 1994), thus supporting the assumption that these features relate to differences in the physiology of resistance (Lebeda and Reinink 1991, 1994; Lebeda and Pink 1998; Lebeda et al. 2006; Sedlářová et al. 2001b).

Pyriform haustoria, characteristic for *B. lactucae* (Voglmayr et al. 2004), originate as hyphal side branches in penetrated cells to accomplish parasitic feeding. The haustorium remains outside the plant protoplast and an altered interface is developed that probably assists uptake of nutrients and the exchange of signals between both partners (Spencer-Phillips 1997). Composition of the extrahaustorial membrane (EHM), separating the haustorium from the host cytoplasm, differs from the semi-permeable plasma membrane as described in detail for *Hyaloperonospora arabidopsidis* (O'Connell and Panstruga 2006). In this respect, the EHM in both oomycete and fungal infections (e.g. in powdery mildews; Koh et al. 2005) are similar. Callose deposits may be formed around haustoria in lettuce–*Bremia* interactions (for detail, see Plasma membrane homeostasis and deposition of callose).

The frequency and timing of haustorial formation and the final size of haustoria are very specific features of host–parasite interactions (Lebeda et al. 2001b). In non-host resistance of *L. saligna*, haustoria form neither on IH nor directly on SV (Lebeda et al. 2006). Frequency of haustorial formation varies specifically among *Lactuca* spp. genotypes carrying different *Dm* genes and/or R-factors for host resistance. In compatible host–parasite interactions, the frequency and size of haustoria is significantly higher than in incompatible interactions (Lebeda et al. 2002, 2006; Sedlářová et al. 2001b). The 'heterosis' effect mentioned for PV and SV also was also reported for haustoria (Lebeda and Reinink 1994; Lebeda and Pink 1998).

Components of host resistance and variability of defence

An integrated approach is being adopted in plant science to understand intercellular signalling, i.e. how plants perceive and respond to external and internal stimuli. Combination of molecular, chemical and electrical components is essential (Birch et al. 2006; Mansfield 2005; O'Connell and Panstruga 2006; Robatzek 2007; Takemoto and Hardham 2004; Walters and McRoberts 2006). Several chemical and physical factors that condition shifts in plant metabolism and architecture induced by oomycete pathogenesis, as well as their importance for resistance of *Lactuca* spp. to *B. lactucae*, are considered below.

Reactive oxygen species (ROS), antioxidants and ROS-scavenging enzymes

Release of reactive oxygen (ROS), nitrogen (RNS) and sulphur (RSS) species intermediates, combined with transport of phytohormones, are amongst the early chemical signals during plant–pathogen interactions. ROS affect establishment of infection, enable redox signal transduction (e.g. hydrogen peroxide together with NO and SA amplifies resistance responses; Delledonne et al. 2003) and trigger programmed cell death (Kamoun et al. 1999). Therefore, generation of

ROS may serve as a marker of pathogenesis and/or plant defence initiation and progress.

Hydrogen peroxide (H_2O_2), a secondary messenger molecule, was accumulated in *Lactuca* spp. tissues challenged by *B. lactucae,* whereas superoxide (O_2^-) was not detected (Sedlářová et al. 2007a). Dramatic changes of H_2O_2 correlate with race-specific resistance, especially in *L. virosa* where it is characterized by early HR onset. In contrast, the supposed non-host resistance in *L. saligna* (CGN 05271) is accompanied by only minor changes in the level of H_2O_2, the content of which is generally lower compared to the other species (Sedlářová et al. 2007a).

High antioxidant status in plants was reported to hinder transportation of ROS across the cell (Neill et al. 2002); therefore our experiments have included the use of antioxidant enzymes and non-enzymatic ROS scavengers. Changes in peroxidase (POX), catalase and polyphenoloxidase activities in lettuce tissue, in relation to the infection process of *B. lactucae*, were demonstrated by Zinkernagel (1986). Our study focused on the dynamics and isozyme spectrum of three ROS-scavenging enzymes, catalase, peroxidase and superoxide dismutase, and unveiled the importance of peroxidase (POX). However, POX activity (Fig. 1g) was found only in the cytosolic fraction, with a higher basic level in wild *Lactuca* spp. compared to cultivated lettuce. Increase of POX activity was linked to expression of race-specific resistance in prickly lettuce (*L. serriola*) and great lettuce (*L. virosa*), with a two-peak timing (6–12 hai, the recognition phase, and from 24 hai at induction of HR; Sedlářová et al. 2007a). The relationship between the increase of pre-infection POX activity and level of field resistance to *B. lactucae* was demonstrated in lettuce (*L. sativa*) cultivars and accessions of *L. serriola* (Reuveni et al. 1991). Thus POX was proposed to serve as a marker in the selection for field resistance to different downy mildew pathogens (Lebeda and Schwinn 1994). From a large group of molecules with an antioxidative action, contents of quercetin and rutin were studied in the leaf extracts (for details see Flavonoids, phenolic acids and PAL).

Nevertheless, the complexity of leaf phytochemistry raises the possibility that many other antioxidants may be involved in the interplay between *Lactuca* spp. and *B. lactucae*. This merits investigation as it would provide a better understanding of host–parasite interactions.

Nitric oxide, NO synthase and NO modulators

Nitric oxide (NO) performs a variety of phytochemical roles during pathogenesis. NO and its metabolites mediate transcription of specific genes during pathogenesis (Neill et al. 2002); synchronized formation of NO and H_2O_2 co-regulates the cell death programme and the phenylpropanoid pathway (Dellendone et al. 2003).

In lettuce–*Bremia* interactions, the formation of NO was localized in cells penetrated by either primary infection structures or haustoria (Fig. 1h). NO synthase (NOS) activity was followed by the oxyhemoglobin method to detect NO production in lettuce and wild *Lactuca* spp. leaf extracts up to 216 hai. A significant increase of NOS activity was found in *L. virosa* early after inoculation (4–8 hai), with a second lower peak at 168 hai. Non-host resistance of *L. saligna* (CGN 05271) correlated with low amounts of NO production and relatively small-scale increase of NOS activity (Petřivalský, unpubl.).

Modulators of NO metabolism were applied to *L. sativa* tissues to follow their influence on *B. lactucae* development up to 48 hai (Petřivalský et al. 2007). Sodium nitroprusside, a model NO donor, decreased conidial germination rate at 4 hai and strongly inhibited further pathogen growth. On the contrary, PTIO as a specific NO scavenger, showed a strong stimulatory effect on pathogen development at 24–48 hai. However, no significant effect of either L-NAME (competitive inhibitor of animal nitric oxide synthases) or sodium tungstate (specific inhibitor of plant nitrate reductase) was found. This may be explained by either the possible contribution of another NO-generating system in *Lactuca* spp., or the lower bioavailability and chemical stability of these substances during leaf tissue treatment (for more details, see Petřivalský et al. 2007).

Flavonoids, phenolic acids and PAL

Phenolic compounds are abundant in plants of the Asteraceae family (Bohm and Stuessy 2001). Screening was conducted in the early 1980s in order to utilize flavoids and flavonols of the genus *Lactuca* in chemotaxonomy (Rees and Harborne 1984). Recently, the phenolic compounds in lettuce have been studied in relation to: leafy vegetable processing to avoid browning due to mechanical injury (Saltveita et al. 2005) and

human medications for anti-inflammatory, anti-bacterial, anti-diabetic and anti-proliferative effects (Chen et al. 2007).

Quercetin, rutin, caffeic acid and chlorogenic acid and several other phenolic compounds are known as major components of lettuce extracts (e.g. Chen et al. 2007). Quercetin, one of the aglycones, was reported from *L. sativa, L. serriola* and *L. virosa*, whilst only traces were found in *L. saligna* and (Rees and Harborne 1984). Quercetin and rutin molecules operate as strong antioxidants. A study was conducted to measure their amount in all four of these *Lactuca* species during the course of *B. lactucae* pathogenesis, determined by the accumulation of autofluorescent phenolics near the plasma membrane of penetrated cells (Bennett et al. 1996; Sedlářová and Lebeda 2001). Quantitative analysis of leaf extracts led to the finding that *L. sativa* genotypes with non-effective race-specific resistance significantly differ in quercetin content (approx. 10 μmol g^{-1} FW) from *L. sativa* with effective race-specific resistance and three wild lettuce species (less than 1 μmol g^{-1} FW). This may help in the balancing of oxidative processes induced by *B. lactucae*. Content of rutin varied slightly from 0.28 μmol g^{-1} FW in *L. virosa* to 0.87 μmol g^{-1} FW in *L. sativa* (Petřivalský, unpubl.). Whilst no striking linkage between rutin level and genotype susceptibility/resistance was found, the external application of rutin solution to lettuce tissues delayed *B. lactucae* germination and penetration (Petřivalský et al. 2007).

The content of phenolic acids (PAs) changes during ontogenesis of *Lactuca* spp.; in cotyledons, chlorogenic acid prevails, whereas amounts of other PAs increase with plant development (Grúz, unpubl.). A time-course study of PAs in adult *L. sativa* (cv. Mariska) showed significant changes in caffeic acid and minor changes in chlorogenic acids after inoculation with incompatible *B. lactucae* race BL16. A two-peak (6–24 and 72 hai) decrease in their level (Grúz, unpubl.) corresponds with induction of oxidative processes (see also previously).

Preliminary studies of phenylalanine ammonium lyase (PAL), a key enzyme of the phenylpropanoid pathway, have not disclosed a relationship between PAL activity and *B. lactucae* colonization (Sedlářová, unpubl.). The phenylpropanoid pathway is also known to be connected with the formation of structural barriers to pathogen ingress by the deposition of lignin (Mauch-Mani and Slusarenko 1996). Although large pools of phenolic compounds that might serve as a source of precursors for incorporation in the lettuce cell wall were found due to *B. lactucae* challenge (Bennett et al. 1996), lignification has not been proved (Sedlářová and Lebeda 2001).

Reorganisation of the cytoskeleton

Host cells challenged by oomycetes undergo drastic changes similar to cells targeted by fungal pathogens (Latijnhouwers et al. 2003; Takemoto and Hardham 2004). Rapid rearrangements of cytoskeletal components (microtubules and F-actin) begin even prior to penetration of the cell wall (during maturation of the appressorium), and link to the relocation of cytoplasm, nuclei and other organelles within epidermal cells in contact with the pathogen (Koh et al. 2005; Takemoto et al. 2003). Pathogens that continue colonisation beyond the epidermis via intercellular hyphae induce similar alterations of architecture in mesophyll cells penetrated by haustoria (Spencer-Phillips 1997).

The multitude of binding proteins associated with the cytoskeleton and its extraordinary dynamics facilitate trafficking of many pathogen-derived signals. The vital role of the host cytoskeleton in non-pathogen and pathogen recognition (Takemoto et al. 2003), the binding of effector molecules (Binet et al. 2001), gene expression (Hamada 2007) and in relation to defects in cell wall microfibril orientation (Wasteneys 2004) are well documented. In lettuce–*Bremia* interactions, actin filaments were not detected in epidermal cells after contact with the pathogen, whereas cortical microtubules (MTs) supported invagination of the plasma membrane and formation of primary and secondary vesicles (Sedlářová et al. 2001a). In compatible interactions, such a unique layout resembles a 'microtubular basket' (Fig. 1e), and is characterised by a high density of MTs at the necks of vesicles (Sedlářová, unpubl.). This suggests a role in the deposition of callose at these locations (Sedlářová and Lebeda 2001). In resistant plants, the timing and extent of the destruction of MTs (Fig. 1f) is correlated with a hypersensitive reaction and typically affects one cell per infection site in *L. sativa*, and 2–3 cells in *L. virosa* (Lebeda and Pink 1998; Lebeda et al. 2006; Sedlářová et al. 2001b).

Construction of *Arabidopsis thaliana* mutants with GFP-tagged cytoskeleton or organelles made it

possible to follow subcellular changes *in vivo* (Hardham 2007; Koh et al. 2005; Takemoto et al. 2003). Rapid and continuing intracellular realignment during *Hyaloperonospora arabidopsidis* challenge was shown elegantly by Takemoto et al. (2003), including 'focusing-to-pathogen' of F-actin below the penetration site and in neighbouring cells. Secretion of plant materials around the infection site, indicated by an aggregation of ER and Golgi bodies, did not stop penetration by an avirulent isolate of *H. arabidopsidis* and even the non-pathogen *Phytophthora sojae*.

A number of detailed experimental data raise the question: what facilitates the extreme plasticity of the plant cytoskeleton in reaction to oomycete and fungal pathogens? As well as the high degree of conservation of tubulin and actin throughout a variety of genomes, a wide array of associated molecules (proteins, RNA) was found in the cytoskeletal complexes. The rapid rearrangement of MTs in reaction to external/internal stimuli occurs because the nucleation sites of MTs are based on γ-tubulin anchors which can be relocated easily within the plant cell (Hamada 2007).

Plasma membrane homeostasis and deposition of callose

Similar adaptations, including haustorial development, have evolved in oomycetes and fungi to enhance a parasitic life strategy (Latijnhouwers et al. 2003). As the integrity of the plasma membrane is a crucial prerequisite for plant cell functionality, as well as the homestasis of cellular processes, the biotrophic pathogens deploy mechanisms to minimise disruption of the host cell (Glazebrook 2005; Grenville-Briggs and van West 2005; Koh et al. 2005; O'Connell and Panstruga 2006; Walters and McRoberts 2006). The plasma membrane regulates osmotic processes (Bennett et al. 1996). Host transmembrane proteins are engaged in the perception of pathogen-associated molecular patterns (O'Connell and Panstruga 2006; Robatzek 2007) and with the aid of the cytoskeleton, facilitate vesicle trafficking (Robatzek 2007; Takemoto and Hardham 2004).

After penetration of the host cell wall, primary and secondary vesicles of *B. lactucae* are formed in the first epidermal cell by invagination of the host plasma membrane (Fig. 1g), as described above. Initiation of intercellular growth is usually linked to membrane damage (Woods et al. 1988) and accumulation of autofluorescent phenolics (Bennett et al. 1996). Pathogen recognition in resistant cells results in irreversible loss of membrane integrity and initiation of the HR. In compatible interactions, the hyphae growing between mesophyll cells penetrate adjacent cell walls (Fig. 1c) to form haustoria that invaginate host plasma membranes. A new interface, the extrahaustorial membrane (EHM), arises from the secretion of proteinaceous and carbohydrate compounds by both partners (Koh et al. 2005; O'Connell and Panstruga 2006).

Formation of *Bremia* infection structures is associated with the deposition of callose, especially in the necks between PVs and SVs, and between SVs and haustoria, forming sheath-like structures around haustoria (Fig. 1d; Sargent et al. 1973). The callose is of host origin and the strongest deposition was reported in compatible interactions (Sedlářová and Lebeda 2001). The chemical composition of callose is very similar to components of extracellular matrices released by *H. arabidopsidis* spores upon germination, namely β-1,3-glucans which has a protective action (Carzaniga et al. 2001).

Hypersensitive response: extent and timing

The hypersensitive reaction (HR), a form of programmed cell death (Kamoun et al. 1999), is one of the most important features in race-specific resistance of lettuce to *B. lactucae* (Lebeda et al. 2001b). On a small scale, it has been reported to also occur in compatible or non-host interactions (Lebeda et al. 2002). Necrosis of affected plant cells and tissues (Fig. 1i,j) is used for phenotypic evaluation (Lebeda and Petrželová 2008). Contemporary methods are able to detect the onset of cell death before visible symptoms occur, either by measuring natural bioluminiscence as the emission of biophotons (Mansfield 2005) or by the application of osmotic stress to test the plasma membrane functionality (Bennett et al. 1996). It was concluded that lettuce cells undergoing the HR experience a prolonged oxidative stress (Bestwick et al. 2001).

In a wide range of lettuce–*Bremia* interactions, the substantial differences found in timing and rate of formation of infection structures correspond with detailed histological investigations of HR (Lebeda and Pink 1998; Lebeda and Reinink 1994; Lebeda et al. 2001b, 2002, 2006; Sedlářová et al. 2001b). Post-haustorial resistance in *Lactuca* spp. with race

specificity includes an intensive HR. Although the extent of the HR is specific for a genotype–race interaction, the number of cells involved in the HR is generally higher than one in wild lettuce species (Fig. 1i,j), especially *L. virosa*, where cells of underlying mesophyll tissue often also show the HR (Lebeda et al. 2006; Sedlářová et al. 2001b). Conversely, the non-host resistance in *L. saligna* (CGN 05271) is expressed before haustorial formation (Sedlářová et al. 2001b), and is characterized by a lack of the HR which might relate to the previously mentioned adjustment of oxidative processes (see Reactive oxygen species (ROS), antioxidants and ROS-scavenging enzymes and nitric oxide, NO synthase and NO modulators; details in Sedlářová et al., 2007a).

Plant energetics

The life strategy of parasites is based on the need to derive nutrients from host tissues, thus affecting plant energetics. Economically damaging infections of crops by powdery mildews and rusts has led to intensive research in this area, with the aim of reducing losses in yield. Although the chlorotic symptoms typical of downy mildew infections are well known (Lebeda and Schwinn 1994), relatively little research has attempted to elucidate the consequent changes of host photosynthetic processes. Interactions with hemibiotrophic *Phytophthora* species, *P. capsici* (Aguirreolea et al. 1995), *P. citricola* and *P. cambivora* (Fleischmann et al. 2002, 2005), *P. infestans* (Restrepo et al. 2005; Schnabel et al. 1998) and *P. nicotianae* (Scharte et al. 2005), and the necrotroph *Pythium aphanidermatum* (Johnstone et al. 2005) have been investigated. As for biotrophs, the effect of white blister rust (*Albugo candida*) on the photosynthetic and carbohydrate metabolism of *Arabidopsis thaliana* was studied by Tang et al. (1996). They showed that infection caused a decrease in chlorophyll and Rubisco content, as well as an inhibition of photosynthetic rates which might result from accumulation of soluble carbohydrates and starch in infected leaves. Moriondo et al. (2005) indicated that *Plasmopara viticola* reduced functional green leaf area of grapevine (*Vitis vinifera*), with decreased chlorophyll content, and affected stomatal closure and transpiration in lesions and adjacent tissues. The reduced assimilation rate was not limited by changes in electron transport capacity and generation of ATP and NADPH.

Prior to Restrepo et al. (2005) reporting the suppression of a large group of photosynthesis-related genes in susceptible potato following *Phytophthora infestans* infection, photosynthesis was supposed to be affected indirectly. Loss of photosynthetic activity has been attributed to the reduction of photosynthetically-active leaf area and lower pigment content (Moriondo et al. 2005; Tang et al. 1996), and to changes in stomatal aperture and transpiration (Aguirreolea et al. 1995). Impairment of the photosynthetic apparatus also was reported by other authors (Koch et al. 1994; Fleischmann et al. 2005).

Recently, we investigated the impact of *B. lactucae* (race BL16) on photosynthetic parameters of *Lactuca* spp. plants. Analyses of chlorophyll fluorescence induction curves and content of photosynthetic pigments (chlorophylls and carotenoids) revealed a linkage between deterioration of the photosynthetic apparatus and compatibility by 13 dai. In susceptible genotypes of *L. sativa* (Cobham Green and UCMD2), an impairment of photosystem II (PSII) photochemistry and decreased content of photosynthetic pigments were noticed due to profuse growth of *B. lactucae*. In resistant *L. virosa* (NVRS 10.001602), characterised by rapid pathogen elimination via the HR (Sedlářová et al. 2001b), no significant influence of inoculation was observed (Prokopová, unpubl.). Our data are in agreement with results of other authors such as Schnabel et al. (1998), who showed a correlation between degree of resistance and changes in PSII photochemistry. Transmission electron microscopy (TEM) studies of susceptible *L. sativa* (Cobham Green) cells revealed a decrease of chloroplast area in sections of cells following challenge by *B. lactucae* (race BL16). On the other hand, the number and area of starch granules within host cells did not change within 13 dai (Novotný and Sedlářová, unpubl.).

Further experiments addressed the effect of treating host tissues with cytokinins (*meta*-topolin, BAP and 3-MeOBAPR) before inoculation. These compounds significantly reduced *B. lactucae* sporulation on susceptible lettuce tissues, and influenced optical parametres of the leaves (Prokopová et al. 2007; Sedlářová et al. 2007b). All cytokinins (CKs) applied retained the maximal quantum yield of PSII photochemistry and content of photosynthetic pigments in infected leaf discs, whereas they slightly reduced these parameters in non-infected controls (Prokopová, unpubl.). CKs also increased the area and number of

chloroplasts and starch granules within infected tissues alone (Novotný and Sedlářová, unpubl.). These results relate to several physiological aspects. In plant–parasite interactions the parasitic partner operates as sink to which carbohydrates are relocated. CKs are also known to enhance sinks for the transport of solutes, e.g. from older to younger parts of a plant. Some biotrophs themselves produce CKs to modulate nutrient transport, tissue senescence and even host morphology (Walters and McRoberts 2006). Either infection or application of CKs alone disturbs the normal functioning of processes linked to photosynthesis. In the concurrent presence of both factors, CKs might suppress the effect of pathogenesis. Increasing concentration of CKs in leaf tissues by external application preceding inoculation thus represents a competitor with pathogen-driven relocation of photosynthates. Although the feedback mechanisms of assimilates on the enzymes of photosynthesis are quite complex and still not completely understood, the increase of invertase leading to carbohydrate accumulation seems to be a principal mechanism (Walters and McRoberts 2006).

Future perspectives

From the data summarized in this review it is evident that relationships between plants and oomycetes are heterogeneous and complex. During the last two decades, the understanding of the biology of these associations has advanced significantly. However, much basic information is still needed before the very complicated mosaic of components, processes, interactions and feedbacks can be assembled to obtain a more complete view about host–oomycete specificity. In particular, the *Lactuca* spp. and *B. lactucae* interactions have been recognized as reflecting a very diverse and complicated system (Lebeda et al. 2001b, 2002), and provide a suitable model for studies of host-parasite specificity and variability of plant defence mechanisms (Lebeda et al. 2001b).

Several investigations worldwide on the lettuce–*Bremia* pathosystem are expected to contribute substantially to complement our present fragmentary knowledge. These include: (1) molecular and ultrastructural studies on pathogen taxonomy and phylogeny (Choi et al. 2007; Göker et al. 2007; Voglmayr, 2008; Voglmayr et al. 2004); (2) characterization of *B. lactucae* population structure, virulence evolution (Lebeda and Zinkernagel 2003a) and gene-flow between natural and cultivated plant pathosystems (Lebeda et al. 2008); (3) collecting and characterization of *Lactuca* spp. germplasm variation (Lebeda et al. 2007), screening of lettuce germplasm for resistance to *B. lactucae* (Lebeda and Petrželová 2008) and detection of new sources of resistance (Beharav et al. 2006; Lebeda and Zinkernagel 2003b; Petrželová et al. 2007), their genetic characterization (Bonnier et al. 1994) and utilization in lettuce breeding (Lebeda et al. 2007); (4) detailed studies of resistance mechanisms within lettuce genotypes and *Lactuca* spp. (Bestwick et al. 2001; Grube and Ochoa 2005; Jeuken and Lindhout 2002; Lebeda et al. 2002, 2006; Sedlářová et al. 2007a); (5.) molecular mapping of genes responsible for resistance/susceptibility and virulence/avirulence both in host plants and the pathogen (Kuang et al. 2004, 2006; Michelmore and Wong 2008); (6.) development of new control methods based on screening of various ‘natural’ compounds for their activity to suppress lettuce downy mildew (Portz et al. 2008; Sedlářová et al. 2007b), utilization of new generations of fungicides (Cohen et al. 2008; Gisi and Sierotzki 2008) and induced resistance.

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References

Aguirreolea, J., Irigoyen, J., Sanchez-Diaz, M., & Salaverri, J. (1995). Physiological alterations in pepper during wilt induced by *Phytophthora capsici* and soil water deficit. *Plant Pathology*, *44*, 587–596.

Andrews, J. H. (1975). Distribution of label from ^{3}H-glucose and ^{3}H-leucine in lettuce cotyledons during the early stages of infection with *Bremia lactucae*. *Canadian Journal of Botany*, *53*, 1103–1115.

Beharav, A., Lewinsohn, D., Lebeda, A., & Nevo, E. (2006). New wild *Lactuca* genetic resources with resistance against *Bremia lactucae*. *Genetic Resources and Crop Evolution*, *53*, 467–474.

Bennett, M., Gallagher, M., Fagg, J., Bestwick, C., Paul, T., Beale, M., et al. (1996). The hypersensitive reaction,

membrane damage and accumulation of autofluorescence phenolics in lettuce cells challenged by *Bremia lactucae*. *The Plant Journal*, *9*, 851–865.

Bestwick, C. S., Adam, A. L., Puri, N., & Mansfield, J. W. (2001). Characterisation of changes to pro- and anti-oxidant enzyme activities during the hypersensitive reaction in lettuce (*Lactuca sativa* L.). *Plant Science*, *161*, 497–506.

Binet, M. N., Humbert, C., Lecourieux, D., Vantard, M., & Pugin, A. (2001). Disruption of microtubular cytoskeleton induced by cryptogein, an elicitor of hypersensitive response in tobacco cells. *Plant Physiology*, *125*, 564–572.

Birch, P. R. J., Rehmany, A. P., Pritchard, L., Kamoun, S., & Beynon, J. L. (2006). Trafficking arms: Oomycete effectors enter host plant cells. *Trends in Microbiology*, *14*, 8–11.

Bohm, B. A., & Stuessy, T. F. (2001). *Flavonoids of the sunflower family (Asteraceae)*. Wien: Springer.

Bonnier, F. J. M., Reinink, K., & Groenwold, R. (1994). Genetic analysis of *Lactuca* accessions with new major gene resistance to lettuce downy mildew. *Phytopathology*, *84*, 462–468.

Carzaniga, R., Bowyer, P., & O'Connell, R. J. (2001). Production of extracellular matrices during development of infection structures by the downy mildew *Peronospora parasitica*. *New Phytologist*, *149*, 83–93.

Chen, Y. H., Chen, H. Y., Hsu, C. L., & Yen, G. C. (2007). Induction of apoptosis by *Lactuca indica* L. in human leukemia cell line and its active components. *Journal of Agricultural and Food Chemistry*, *55*, 1743–1749.

Choi, Y. J., Hong, S. B., & Shin, H. D. (2007). Extreme size and sequence variation in the ITS rDNA of *Bremia lactucae*. *Mycopathologia*, *163*, 91–95.

Clark, J. S. C., & Spencer-Phillips, P. T. N. (2004). The compatible interaction in downy mildew infections. In P. T. N. Spencer-Phillips, & M. Jeger (Eds.) *Advances in downy mildew research, vol. 2* (pp. 1–34). Dordrecht: Kluwer.

Cohen, Y., Rubin, E., & Gotlieb, D. (2008). Activity of carboxylic acid amide (CAA) fungicides against *Bremia lactucae*. *European Journal of Plant Pathology* (this issue).

Crute, I. R. (1992a). Downy mildew of lettuce. In H. S. Chaube, J. Kumar, A. N. Mukhopadhyay, & U. S. Singh (Eds.) *Plant diseases of international importance, vol. II. Diseases of vegetable and oil seed crops* (pp. 165–185). New Jersey: Prentice Hall.

Crute, I. R. (1992b). From breeding to cloning (and back again?): A case study with lettuce downy mildew. *Annual Review of Phytopathology*, *30*, 485–506.

Crute, I. R., & Dickinson, C. R. (1976). The behaviour of *Bremia lactucae* on cultivars of *Lactuca sativa* and on other Composites. *Annals of Applied Biology*, *82*, 433–450.

Crute, I. R., & Dixon, G. R. (1981). Downy mildew diseases caused by the genus *Bremia* Regel. In D. M. Spencer (Ed.) *The downy mildews* (pp. 421–460). London: Academic.

Crute, I. R., & Johnson, A. G. (1976). The genetic relationship between races of *Bremia lactucae* and cultivars of *Lactuca sativa*. *Annals of Applied Biology*, *83*, 125–137.

Crute, I. R., & Norwood, J. M. (1981). The identification and characteristics of field resistance to lettuce downy mildew (*Bremia lactucae* Regel). *Euphytica*, *30*, 707–717.

Davis, R. M., Subbarao, K. V., Raid, R. N., & Kurtz, E. A. (1997). *Compendium of lettuce diseases*. St. Paul, MN. USA: APS.

Delledonne, M., Polverari, A., & Murgia, I. (2003). The functions of nitric oxide-mediated signalling and changes in gene expression during the hypersensitive response. *Antioxidants and Redox Signaling*, *5*, 33–41.

Duddridge, J. A., & Sargent, J. A. (1978). A cytochemical study of lipolytic activity in *Bremia lactucae* Regel during germination of the conidium and penetration of the host. *Physiological Plant Pathology*, *12*, 289–296.

Fleischmann, F., Koehl, J., Portz, R., Beltrame, A. B., & Oßwald, W. F. (2005). Physiological changes of *Fagus silvatica* seedlings infected with *Phytophthora citricola* and the contribution of its elicitin "Citricolin" to pathogenesis. *Plant Biology*, *7*, 650–658.

Fleischmann, F., Schneider, D., Matyssek, R., & Oßwald, W. F. (2002). Investigation on net CO_2 assimilation, transpiration and root growth of *Fagus sylvatica* infected with four different *Phytophthora* species. *Plant Biology*, *4*, 144–152.

Gisi, U., & Sierotzki, H. (2008). Fungicides modes of action and resistance in downy mildews. *European Journal of Plant Pathology* DOI 10.1007/s10658-008-9290-5.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology*, *43*, 205–227.

Göker, M., Voglmayr, H., Riethmüller, A., & Oberwinkler, F. (2007). How do obligate parasites evolve? A multi-gene phylogenetic analysis of downy mildews. *Fungal Genetics and Biology*, *44*, 105–122.

Grenville-Briggs, L. J., & van West, P. (2005). The biotrophic stages of oomycete–plant interactions. *Advances in Applied Microbiology*, *57*, 217–243.

Grube, R. C., & Ochoa, O. E. (2005). Comparative genetic analysis of field resistance to downy mildew in the lettuce cultivars 'Grand Rapids' and 'Iceberg'. *Euphytica*, *142*, 205–215.

Hamada, T. (2007). Microtubule-associated proteins in higher plants. *Journal of Plant Research*, *120*, 79–98.

Hardham, A. R. (2007). Cell biology of plant–oomycete interactions. *Cellular Microbiology*, *9*, 31–39.

Holub, E. B., & Cooper, A. (2004). Matrix, reinvention in plants: How genetics is unveiling secrets of non-host disease resistance. *Trends in Plant Science*, *9*, 211–214.

Hooftman, D. A. P., Nieuwenhuis, B. P. S., Posthuma, K. I., Oostermeijer, J. G. B., & den Nijs, H. J. C. M. (2007). Introgression potential of downy mildew resistance from lettuce to *Lactuca serriola* and its relevance for plant fitness. *Basic and Applied Ecology*, *8*, 135–146.

Hulbert, S. H., Webb, C. A., Smith, S. M., & Sun, Q. (2001). Resistance gene complexes: Evolution and utilization. *Annual Review of Phytopathology*, *39*, 285–312.

Ingram, D. S., Sargent, J. A., & Tommerup, I. C. (1976). Structural aspects of infection by biotrophic fungi. In J. Friend & D. R. Threlfall (Eds.), *Biochemical aspects of plant–parasitic relationships* (pp. 43–78). *Annual Proceedings of Phytochemical Society, 13.*

Jeuken, M., & Lindhout, P. (2002). *Lactuca saligna*, a non-host for lettuce downy mildew (*Bremia lactucae*), harbors a new race-specific *Dm* gene and three QTLs for resistance. *Theoretical and Applied Genetics*, *105*, 384–391.

Johnstone, M., Chatterton, S., Sutton, J. C., & Grodzinski, B. (2005). Net carbon gain and growth of bell peppers, *Capsicum annuum* 'Cubico', following root infection by *Pythium aphanidermatum*. *Phytopathology*, *95*, 354–361.

Judelson, H. S., & Michelmore, R. W. (1992). Temperature and genotype interactions in the expression of host resistance in lettuce downy mildew. *Physiological and Molecular Plant Pathology*, *40*, 233–245.

Kamoun, S. (2006). A catalogue of the effector secretome of plant pathogenic oomycetes. *Annual Review of Phytopathology*, *44*, 41–60.

Kamoun, S., Huitema, E., & Vleeshouwers, V. G. A. A. (1999). Resistance to oomycetes: A general role for the hypersensitive response. *Trends in Plant Science*, *4*, 196–200.

Kitner, M., Lebeda, A., Doležalová, I., Maras, M., Křístková, E., Nevo, E., Pavlícek, T., Meglic, V., & Beharav, A. (2008). AFLP analysis of *Lactuca saligna* gemplasm collections from four European and three Middle East Countries. *Israel Journal of Plant Sciences* (in press)

Koch, C., Noga, G., & Strittmatter, G. (1994). Photosynthetic electron transport is differentially affected during early stages of cultivar/race-specific interactions between potato and *Phytophthora infestans*. *Planta*, *193*, 551–557.

Koh, S., André, A., Edwards, H., Ehrhardt, D., & Somerville, S. (2005). *Arabidopsis thaliana* subcellular responses to compatible *Erysiphe cichoracearum* infections. *The Plant Journal*, *44*, 516–529.

Koike, S. T., & Ochoa, O. E. (2007). Downy mildew caused by *Bremia lactucae* on strawflower (*Helichrysum bracteatum*) in California. *Plant Disease*, *91*, 326.

Kuang, H., Ochoa, O. E., Nevo, E., & Michelmore, R. W. (2006). The disease resistance gene *Dm3* is infrequent in natural populations of *Lactuca serriola* due to deletions and frequent gene conversions at the RGC2 locus. *The Plant Journal*, *47*, 38–48.

Kuang, H., Woo, S. S., Meyers, B. C., Nevo, E., & Michelmore, R. W. (2004). Multiple genetic processes result in heterogeneous rates of evolution within the major cluster disease resistance genes in lettuce. *The Plant Cell*, *16*, 2870–2894.

Latijnhouwers, M., de Wit, P. J., & Govers, F. (2003). Oomycetes and fungi: Similar weaponry to attack plants. *Trends in Microbiology*, *11*, 462–469.

Lebeda, A. (1986). Specificity of interactions between wild *Lactuca* species and *Bremia lactucae* isolates from *Lactuca serriola*. *Journal of Phytopathology*, *117*, 54–64.

Lebeda, A. (2002). Occurrence and variation in virulence of *Bremia lactucae* in natural populations of *Lactuca serriola*. In P. T. N. Spencer-Phillips, U. Gisi, & A. Lebeda (Eds.) *Advances in downy mildew research* (pp. 179–183). Dordrecht: Kluwer.

Lebeda, A., & Boukema, I. W. (1991). Further investigation of the specificity of interactions between wild *Lactuca* spp. and *Bremia lactucae* isolates from *Lactuca serriola*. *Journal of Phytopathology*, *133*, 57–64.

Lebeda, A., Doležalová, I., Feráková, V., & Astley, D. (2004). Geographical distribution of wild *Lactuca* species (Asteraceae, Lactuceae). *The Botanical Review*, *70*, 328–356.

Lebeda, A., Doležalová, I., Křístková, E., Vinter, V., Vránová, O., Doležal, K., et al. (1999). Complex research of taxonomy and ecobiology of wild *Lactuca* spp. genetic resources. In A. Lebeda, & E. Křístková (Eds.) *Eucarpia leafy vegetables '99* (pp. 117–131). Olomouc (Czech Republic): Palacký University in Olomouc.

Lebeda, A., Luhová, L., Sedlářová, M., & Jančová, D. (2001a). The role of enzymes in plant–fungal pathogens interactions. *Journal of Plant Diseases and Protection*, *108*, 89–111.

Lebeda, A., & Petrželová, I. (2004). Occurrence of race-specific resistance to *Bremia lactucae* in *Lactuca serriola* germplasm originating from four European countries. In J. Vollmann, H. Grausgruber, & P. Ruckenbauer (Eds.) *Genetic variation for plant breeding* (pp. (pp. 113–116)). Vienna, Austria: EUCARPIA & BOKU-University of Natural Resources and Applied Life Sciences.

Lebeda, A., & Petrželová, I. (2008). Screening for resistance to lettuce downy mildew (*Bremia lactucae*). In M. Miranda & A. Lebeda (Eds.), Disease resistance mass screening techniques for banana and other crops. IAEA, Vienna and Springer, Dordrecht, the Netherlands (in press).

Lebeda, A., Petrželová, I., & Maryška, Z. (2008). Structure and variation in the wild-plant pathosystem: *Lactuca serriola–Bremia lactucae. European Journal of Plant Pathology,* DOI 10.1007/s10658-008-9291-4.

Lebeda, A., & Pink, D. A. C. (1998). Histological aspects of the response of wild *Lactuca* spp. and their hybrids, with *L. sativa* to lettuce downy mildew (*Bremia lactucae*). *Plant Pathology*, *47*, 723–736.

Lebeda, A., Pink, D. A. C., & Astley, D. (2002). Aspects of the interactions between wild *Lactuca* spp. and related genera and lettuce downy mildew (*Bremia lactucae*). In P. T. N. Spencer-Phillips, U. Gisi, & A. Lebeda (Eds.) *Advances in downy mildew research* (pp. 85–117). Dordrecht: Kluwer.

Lebeda, A., Pink, D. A. C., & Mieslerová, B. (2001b). Host–parasite specificity and defense variability in the *Lactuca* spp.–*Bremia lactucae* pathosystem. *Journal of Plant Pathology*, *83*, 25–35.

Lebeda, A., & Reinink, K. (1991). Variation in the early development of *Bremia lactucae* on lettuce cultivars with different levels of field resistance. *Plant Pathology*, *40*, 232–237.

Lebeda, A., & Reinink, K. (1994). Histological characterization of resistance in *Lactuca saligna* to lettuce downy mildew (*Bremia lactucae*). *Physiological and Molecular Plant Pathology*, *44*, 125–139.

Lebeda, A., Ryder, E. J., Grube, R., Doležalová, I., & Křístková, E. (2007). Lettuce (Asteraceae; *Lactuca* spp.), Chapter 9. In R. Singh (Ed.) *Genetic resources, chromosome engineering, and crop improvement series, volume 3—vegetable crops* (pp. 377–472). Boca Raton, FL, USA: CRC.

Lebeda, A., & Schwinn, F. J. (1994). The downy mildews—an overview of recent research progress. *Journal of Plant Diseases and Protection*, *101*, 225–254.

Lebeda, A., Sedlářová, M., Lynn, J., & Pink, D. A. C. (2006). Phenotypic and histological expression of different genetic backgrounds in interactions between lettuce, wild *Lactuca* spp., *L. sativa* × *L. serriola* hybrids and *Bremia lactucae*. *European Journal of Plant Pathology*, *115*, 431–441.

Lebeda, A., & Syrovátko, P. (1988). Specificity of *Bremia lactucae* isolates from *Lactuca sativa* and some *Asteraceae* plants. *Acta Phytopathologica et Entomologica Hungarica*, *23*, 39–48.

Lebeda, A., & Zinkernagel, V. (2003a). Evolution and distribution of virulence in the German population of *Bremia lactucae*. *Plant Pathology*, *52*, 41–51.

Lebeda, A., & Zinkernagel, V. (2003b). Characterization of new highly virulent German isolates of *Bremia lactucae* and efficiency of resistance in wild *Lactuca* spp. germplasm. *Journal of Phytopathology*, *151*, 274–282.

Lipka, V., Dittgen, J., Bednarek, P., Bhat, R., Wiermer, M., Stein, M., et al. (2005). Pre- and postinvasion defenses both contribute to nonhost resistance in *Arabidopsis*. *Science*, *310*, 1180–1183.

Lucas, J. A., Hayter, J. B. R., & Crute, I. R. (1995). The downy mildews: Host specificity and pathogenesis. In K. Kohmoto, U. S. Singh, & R. P. Singh (Eds.) *Pathogenesis and host specificity in plant diseases* (pp. 217–238). Oxford: Pergamon.

MacLean, D. J., & Tommerup, I. C. (1979). Histology and physiology of compatibility between lettuce and the downy mildew fungus, *Bremia lactucae* Regel. *Physiological Plant Pathology*, *14*, 291–312.

Mahajan, M., & Dhillon, M. (2003). Relation of leaf epidermal characteristics with susceptibility and resistance of potato (*Solanum tuberosum*) to late blight disease (*Phytophthora infestans*). *Indian Journal of Agricultural Sciences*, *73*, 656–660.

Mansfield, J. W. (2005). Biophoton distress flares signal the onset of the hypersensitive reaction. *Trends in Plant Science*, *10*, 307–309.

Mansfield, J. W., Bennett, M., Bestwick, C., & Woods-Tör, A. (1997). Phenotypic expression of gene-for-gene interaction involving fungal and bacterial pathogens: Variation from recognition to response. In I. R. Crute, E. B. Holub, & J. J. Burdon (Eds.) *The gene-for-gene relationship in plant parasite interactions* (pp. 265–291). Wallingford, UK: CABI.

Mauch-Mani, B. (2002). Host resistance to downy mildew diseases. In P. T. N. Spencer-Phillips, U. Gisi, & A. Lebeda (Eds.) *Advances in downy mildew research* (pp. 59–83). Dordrecht: Kluwer.

Mauch-Mani, B., & Slusarenko, A. J. (1996). Production of salicylic acid precursors is a major function of phenylalanine ammonia-lyase in the resistance of *Arabidopsis* to *Peronospora parasitica*. *Plant Cell*, *8*, 203–212.

Michelmore, R. W., & Wong, J. W. (2008). Classical and molecular genetics of *Bremia lactucae*, lettuce downy mildew. *European Journal of Plant Pathology* DOI 10.1007/s10658-008-9305-2.

Michelmore, R. W., Ochoa, O. E., Truco, M. J., Grube, R., & Hayes, R. (2005). Breeding crisphead lettuce. In *California Lettuce Research Board Annual Report (April 1, 2004 through March 31, 2005)* (pp. 68–78). Salinas, CA, USA: California Lettuce Research Board.

Moriondo, M., Orlandini, S., Giuntoli, A., & Bindi, M. (2005). The effect of downy and powdery mildew on grapevine (*Vitis vinifera* L.) leaf gas exchange. *Journal of Phytopathology*, *153*, 350–357.

Neill, S. J., Desikan, R., Clarke, A., Hurst, R. D., & Hancock, J. T. (2002). Hydrogen peroxide and nitric oxide as signalling molecules in plants. *Journal of Experimental Botany*, *53*, 1237–1247.

Nordskog, B., Gadoury, D. M., Seem, R. C., & Hermansen, A. (2007). Impact of diurnal periodicity, temperature and light on sporulation of *Bremia lactucae*. *Phytopathology*, *97*, 979–986.

Norwood, J. M., Crute, I. R., & Lebeda, A. (1981). The utilization of novel sources of resistance to *Bremia lactucae* from wild *Lactuca* species. *Euphytica*, *30*, 659–668.

O'Connell, R. J., & Panstruga, R. (2006). Tête à tête inside a plant cell: Establishing compatibility between plants and biotrophic fungi and oomycetes. *New Phytologist*, *171*, 699–718.

Pesacreta, T. C., & Hasenstein, K. H. (1999). The internal cuticle of *Cirsium horridulum* (Asteraceae) leaves. *American Journal of Botany*, *86*, 923–928.

Petrželová, I., & Lebeda, A. (2004). Occurrence of *Bremia lactucae* in natural populations of *Lactuca serriola*. *Journal of Phytopathology*, *152*, 391–398.

Petrželová, I., Lebeda, A., Nevo, E., & Beharav, A. (2007). Variation of response against *Bremia lactucae* in natural populations of *Lactuca saligna*. In A. Lebeda, & P. T. N. Spencer-Phillips (Eds.) *Advances in downy mildew research, vol. 3. Proceedings of the 2nd international downy mildews symposium* (pp. 169–173). Olomouc and Kostelec na Hané, Czech Republic: Palacký University in Olomouc and JOLA.

Petřivalský, M., Kočířová, J., Sedlářová, M., Piterková, J., Luhová, L., & Lebeda, A. (2007). On the role of nitric oxide in *Bremia lactucae* pathogenesis on *Lactuca sativa*. In A. Lebeda, & P. T. N. Spencer-Phillips (Eds.) *Advances in downy mildew research, vol. 3. Proceedings of the 2nd international downy mildews symposium* (pp. 175–184). Olomouc and Kostelec na Hané, Czech Republic: Palacký University in Olomouc and JOLA.

Pink, D. A. C. (2002). Strategies using genes for non-durable disease resistance. *Euphytica*, *124*, 227–236.

Portz, D., Koch, E., & Slusarenko, A. (2008). Effects of garlic (*Allium sativum*) juice containing allicin on *Phytophthora infestans* and on downy mildew of cucumber caused by *Pseudoperonospora cubensis*. *European Journal of Plant Pathology* (this issue).

Prokopová, J., Nauš, J., Špundová, M., & Sedlářová, M. (2007). The effect of *meta*-topolin on optical parameters of lettuce leaf discs infected by *Bremia lactucae*. In A. Lebeda, & P. T. N. Spencer-Phillips (Eds.) *Advances in downy mildew research, vol. 3. Proceedings of the 2nd international downy mildews symposium* (pp. 73–78). Olomouc and Kostelec na Hané, Czech Republic: Palacký University in Olomouc and JOLA.

Rees, S., & Harborne, J. (1984). Flavonoids and other phenolics of *Cichorium* and related members of the Lactuceae (Compositae). *Botanical Journal of the Linnean Society*, *89*, 313–319.

Restrepo, S., Myers, K. L., del Pozo, O., Martin, G. B., Hart, A. L., Buell, C. R., et al. (2005). Gene profiling of a compatible interaction between *Phytophthora infestans* and *Solanum tuberosum* suggests a role for carbonic anhydrase. *Molecular Plant–Microbe Interactions*, *18*, 913–922.

Reuveni, R., Shimoni, M., & Crute, I. R. (1991). An association between high peroxidase activity in lettuce (*Lactuca sativa*) and field resistance do downy mildew (*Bremia lactucae*). *Journal of Phytopathology*, *132*, 312–318.

Robatzek, S. (2007). Vesicle trafficking in plant immune responses. *Cellular Microbiology*, *9*, 1–8.

Saltveita, M. E., Choia, Y. J., & Tomás-Barberán, F. A. (2005). Mono-carboxylic acids and their salts inhibit wound-induced

phenolic accumulation in excised lettuce (*Lactuca sativa*) leaf tissue. *Physiologia Plantarum*, *125*, 454–463.

Sargent, J. A. (1976). Germination of spores of *Bremia lactucae*. *Annals of Applied Biology*, *84*, 290–294.

Sargent, J. A., & Payne, H. L. (1974). Effect of temperature on germination, viability and fine structure of conidia of *Bremia lactucae*. *Transactions of British Mycological Society*, *63*, 509–518.

Sargent, J. A., Tommerup, I. C., & Ingram, D. S. (1973). The penetration of a susceptible lettuce variety by the downy mildew fungus, *Bremia lactucae* Regel. *Physiological Plant Pathology*, *3*, 231–239.

Scharte, J., Schön, H., & Weis, E. (2005). Photosynthesis and carbohydrate metabolism in tobacco leaves during an incompatible interaction with *Phytophthora nicotianae*. *Plant, Cell and Environment*, *28*, 1421–1435.

Schnabel, G., Strittmatter, G., & Noga, G. (1998). Changes in photosynthetic electron transport in potato cultivars with different field resistance after infection with *Phytophthora infestans*. *Journal of Phytopathology*, *146*, 205–210.

Sedlářová, M., Binarová, P., & Lebeda, A. (2001a). Changes in microtubular alignment in *Lactuca* spp. (*Asteraceae*) epidermal cells during early stages of infection by *Bremia lactucae* (*Peronosporaceae*). *Phyton*, *41*, 21–34.

Sedlářová, M., & Lebeda, A. (2001). Histochemical detection and role of phenolic compounds in defence response of *Lactuca* spp. to lettuce downy mildew (*Bremia lactucae*). *Journal of Phytopathology*, *149*, 1–5.

Sedlářová, M., Lebeda, A., & Pink, D. A. C. (2001b). The early stages of interaction between effective and non-effective race-specific genes in *Lactuca sativa*, wild *Lactuca* spp. and *Bremia lactucae* (race NL16). *Journal of Plant Diseases and Protection*, *108*, 477–489.

Sedlářová, M., Luhová, L., Petřivalský, M., & Lebeda, A. (2007a). Localisation and metabolism of reactive oxygen species during *Bremia lactucae* pathogenesis in *Lactuca sativa* and wild *Lactuca* spp. *Plant Physiology and Biochemistry*, *45*, 607–616.

Sedlářová, M., Výtisková, M., Doležal, K., & Lebeda, A. (2007b). Pre-incubation with cytokinins delays chlorophyll degradation in *Lactuca* spp. tissues and reduces *Bremia lactucae* sporulation. In A. Lebeda, & P. T. N. Spencer-Phillips (Eds.) *Advances in downy mildew research, vol. 3. Proceedings of the 2nd international downy mildews symposium* (pp. 185–194). Olomouc and Kostelec na Hané, Czech Republic: Palacký University in Olomouc and JOLA.

Skidmore, D. I., & Ingram, D. S. (1985). Conidial morphology and the specialization of *Bremia lactucae* Regel (Peronosporaceae) on hosts in the family Compositae. *Botanical Journal of the Linnean Society*, *91*, 503–522.

Spencer-Phillips, P. T. N. (1997). Function of fungal haustoria in epiphytic and endophytic infections. *Advances in Botanical Research*, *24*, 309–333.

Takemoto, D., & Hardham, A. R. (2004). The cytoskeleton as a regulator and target of biotic interactions in plants. *Plant Physiology*, *136*, 3864–3876.

Takemoto, D., Jones, D. A., & Hardham, A. R. (2003). GFP-tagging of cell components reveals the dynamics of subcellular re-organization in response to infection of *Arabidopsis* by oomycete pathogens. *The Plant Journal*, *33*, 775–792.

Tang, X., Rolfe, S. A., & Scholes, J. D. (1996). The effect of *Albugo candida* (white blister rust) on the photosynthetic and carbohydrate metabolism of leaves of *Arabidopsis thaliana*. *Plant, Cell and Environment*, *19*, 967–975.

Thines, M. (2007). Use of scanning electron microscopy in downy mildew systematics. In A. Lebeda, & P. T. N. Spencer-Phillips (Eds.) *Advances in downy mildew research, vol. 3. Proceedings of the 2nd international downy mildews symposium* (pp. 17–23). Olomouc and Kostelec na Hané, Czech Republic: Palacký University in Olomouc and JOLA.

Thines, M., Göker, M., Spring, O., & Oberwinkler, F. (2006). A revision of *Bremia graminicola*. *Mycological Research*, *110*, 646–656.

Van Pelt-Heerschap, H., & Smit-Bakker, O. (1993). Cell-wall degrading enzymes synthesized by the obligate pathogen *Bremia lactucae*. In B. Fritig, & M. Legrand (Eds.) *Mechanisms of plant defense responses* (p. 82). Dordrecht: Kluwer.

Vieira, B. S., & Barreto, R. W. (2006). First record of *Bremia lactucae* infecting *Sonchus oleraceus* and *Sonchus asper* in Brazil and its infectivity to lettuce. *Journal of Phytopathology*, *154*, 84–87.

Voglmayr, H. (2008). Progress and challenge in systematics of downy mildews and white blister rusts: New insights from genes and morphology. *European Journal of Plant Pathology* (this issue).

Voglmayr, H., Riethmuller, A., Göker, M., Weiss, M., & Oberwinkler, F. (2004). Phylogenetic relationships of *Plasmopara, Bremia* and other genera of downy mildew pathogens with pyriform haustoria based on Bayesian analysis of partial LSU rDNA sequence data. *Mycological Research*, *108*, 1011–1024.

Walters, D., & McRoberts, N. (2006). Plants and biotrophs: A pivotal role for cytokinins. *Trends in Plant Science*, *11*, 581–586.

Wasteneys, G. O. (2004). Progress in understanding the role of microtubules in plant cells. *Current Opinion in Plant Biology*, *7*, 651–660.

Witsenboer, H., Vogel, J., & Michelmore, R. W. (1997). Identification, genetic localization, and allelic diversity of selectively amplified microsatellite polymorphic loci in lettuce and wild relatives (*Lactuca* spp.). *Genome*, *40*, 923–936.

Woods, A. M., Fagg, J., & Mansfield, J. W. (1988). Fungal development and irreversible membrane damage in cells of *Lactuca sativa* undergoing the hypersensitive reaction to the downy mildew fungus *Bremia lactucae*. *Physiological and Molecular Plant Pathology*, *32*, 483–498.

Zinkernagel, V. (1985). Investigations on the susceptibility and resistance of head lettuce (*Lactuca sativa*) to downy mildew (*Bremia lactucae*). 2. Light and electron-microscopic examinations of the host–parasite interface. *Journal of Phytopathology*, *112*, 238–258.

Zinkernagel, V. (1986). Investigations on the susceptibility of head lettuce (*Lactuca sativa*) to downy mildew (*Bremia lactucae*). 3. Activities of peroxidase, catalase and polyphenoloxidase. *Journal of Phytopathology*, *115*, 257–266.

Zinkernagel, V., & Bartscherer, H. C. (1978). The host–parasite interface in *Bremia lactucae* with *Lactuca sativa*. In *Book of Abstracts, 3rd International Congress of Plant Pathology* (p. 112). München, Germany.