22 • Photosynthetic responses to biotic stress

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22.1. INTRODUCTION TO PLANT BIOTIC STRESS

Agricultural and native plants are subject to a myriad of biotic stresses inflicted by other living organisms, from viruses to mammals, and many of these damaging agents affect photosynthesis, either by altering its underlying metabolism (primary photochemistry, electron transport, Calvin cycle) or gas diffusion, or by reducing photosynthetic leaf area. Pathogens (fungal, bacteria or viral agents) and animal pests causes on average, a 15% and 18% reduction in crop yield, respectively (Oerke and Dehne, 2004). Biotic stresses on plants have had enormous repercussions for humanity. For example, the potato blight (*Phytophthora infestans*) caused widespread famine in England, Ireland and Belgium, or the introduction of grape phylloxera (*Daktulosphaira vitifoliae* or *Phylloxera vastatrix*) from America in the mid 19th century nearly put an end to the French wine industry. In addition, it is predicted that plant-pathogen interactions will favour pathogens under the high CO_2 conditions expected for the next decades (Lake and Wade, 2009).

This chapter reviews the effects of parasitic plants, pathogens (virus, bacteria and fungi) and arthropods on photosynthesis. While competition among plants clearly is a 'biotic interaction', we have not included it in this discussion as competition often manifests itself through reduced availability of growth-limiting resources and does not affect photosynthesis *per se*. The underlying mechanisms by which biotic agents affect photosynthesis vary widely and it may be useful to classify these agents into different 'damage guilds' (Table 22.1), although it has been claimed that biotic stresses generally downregulate photosynthesis genes (Bilgin *et al.*, 2010). Chewing insects, for example, reduce carbon gain primarily by reducing leaf area, where virus infections rarely reduce leaf area but instead depress the rate of photosynthesis per unit leaf area. Among fungi, leaf-disease fungi (rust, mildew, etc.) behave like herbivores in their ability to reduce photosynthetic leaf area, where vascular-wilt fungi compromise plant water transport and reduce photosynthesis by inducing stomatal closure. In addition to exploring how different biotic agents affect photosynthesis, this chapter concludes with a brief discussion of different methods for detecting the effects of biotic stress on photosynthesis, sometimes in advance of the development of visual symptoms.

22.2. EFFECTS OF PARASITIC PLANTS ON THEIR HOSTS' PHOTOSYNTHESIS

Parasitic plants depend on their hosts for carbohydrates and other resources, and while the mechanisms by which they affect photosynthesis in their hosts are not well understood, they have enormous potential to manipulate source-sink balance. This taxonomically diverse group represents about 1% of all flowering plants (~4500 species); they invade host tissues, above- or belowground, and remove resources via a specialised structure known as the haustorium (Watling and Press, 2001). The degree of dependence on the host varies, from holoparasitic species that have no capacity for independent photosynthesis, to hemiparasitic species that retain some capacity to fix carbon. There is some evidence that hemiparasitic and homoparasitic species affect photosynthesis in their hosts differently.

Holoparasitic species lack chl. and are totally dependent on the host for photosynthate (Watling and Press, 2001; Bungard, 2004). The loss of photosynthetic capacity seems to depend on several evolutionary steps towards holoparasitism. For instance, some gene losses (such as those related to chlororespiration) occurred in the early stages in the evolution of parasitism in *Cuscuta reflexa*, below the loss of photosynthetic capacity (Bungard, 2004). Other species, like *Lathraea clandestina*, have lost photosynthetic function but

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Table 22.1. *A summary of the described effects of several biotic stresses on photosynthetic parameters. The relative importance of each factor is indicated (–, effect not described; X, effect described occasionally; XX, effect described several times; XXX, main effect). Notice that a factor not indicated as important may not necessarily mean that it is not, as the literature available is fragmentary and some of the effects have never been analysed under some of the biotic stresses. In the case of endophytic fungi, both positive (i.e., symbiotic) and negative stomatal and non-stomatal effects have been described, depending on the fungus–host combination and/or environmental conditions.*

Biotic stress	Defoliation, leaf destruction/ necrosis*	Stomatal limitations		Non-stomatal limitations			
	Importance	Importance	Importance	Structural effects*	Photosynthetic primary reactions	Carboxylation/ Calvin-cycle reactions	Carbohydrate metabolism/ transport
Virus	—	—	XXX	XXX	XXX	XX	XX
Bacteria	—	XX	XXX	X	XXX	X	X
Fungi – endophytic	—	X (+/–effects)	X (+/–effects)	—	X	—	X
Fungi – vascular wilt	X	XXX	X	X	X	X	—
Fungi – leaf disease	XXX	X	XXX	X	X	XX	XX
Nematodes	X	X	—	—	—	—	—

* Including leaf shape and size, leaf internal structure, and organelle form and distribution

produce functional Rubisco large subunits (RbcL), while *Epifagus virginiana* have lost all trace of the gene encoding the RbcL protein (*rbc*L gene) (Bungard, 2004). Because they depend on host plants for carbohydrates, it is likely that holoparasitic plants interact with host photosynthesis mostly through source-sink interactions.

The few studies of holoparasitic plants do not point to a common mechanism for reducing photosynthesis in their hosts. Biomass, particularly leaf mass of tomato plants parasitised by *Orobanche aegyptiaca*, is severely reduced (Barker *et al.*, 1996). In contrast, tobacco infected with *Orobanche cernua* maintained the same leaf area as uninfected plants and increased carbon allocation to roots by 77%, of which 73% was removed by the parasite (Hibberd *et al.*, 1998b). In addition, leaf senescence was delayed, which resulted in a 20% increase in canopy photosynthesis compared with uninfected plants (Hibberd *et al.*, 1998b). Sink stimulation of host photosynthesis was also observed in *Lupinus alba* infected with the stem holoparasite *Cuscuta reflexa* (Jeschke *et al.*, 1997). In this case and despite a reduction of host nitrogen, the effect was induced through increased leaf-area-based photosynthesis in the host, which was associated with increased chl. contents. Unlike other holoparastic plants, infection by the stem holoparasite *Pilostyles ingae* had no effect on photosynthesis of its host, *Mimosa naguirei* (Fernandes *et al.*, 1998).

Even thought hemiparasitic species contain chl. and can fix carbon autotrophically (Strong *et al.*, 2000), they are obligate parasites either because they pass through a holoparasitic stage during development or because they depend on the host for resources other than carbon, such as water and mineral nutrients (Watling and Press, 2001). Although some proportion of their carbon can be obtained from host plants, it is likely that the effect of hemiparasitic species on host photosynthesis does not occur through source-sink interactions. In fact, they often reduce rather than stimulate host photosynthesis.

Striga hermonthica, the most extensively studied hemiparasitic species, causes severe losses of yield in cereal crops such as maize and sorghum in the semi-arid tropics. The removal of resources (i.e., carbon and others) by the parasitic plant

is only responsible for 20% of the observed decrease in host growth, while the other 80% is attributed to the impact of the parasite on host photosynthesis (Graves *et al.*, 1989), reducing its leaf area and leaf photosynthesis (Seel and Press, 1996; Watling and Press, 2001). The reduction in photosynthesis is achieved by parasite-induced stomatal closure, associated with increased ABA synthesis in the host (Taylor *et al.*, 1996; Frost *et al.*, 1997). That photosynthesis is inhibited by stomatal closure is reinforced by the observations that infected and non-infected plants have the same A_N/C_i responses (Frost *et al.*, 1997; but see Cechin and Press, 1993); in addition, increasing nitrogen availability, which typically stimulates photosynthetic capacity, had no effect on photosynthesis in infected plants (Gurney *et al.*, 1995). There is, however, one example of non-stomatal reduction in photosynthesis by a hemiparasite. The reduction in photosynthesis of the grass *Phleum bertolinii* by the facultative hemiparasitic plant *Rhinanthus minor* was associated with substantial reduction in chl. and Rubisco content (Cameron *et al.*, 2007).

Summarising, it is evident that many parasitic plants manipulate the photosynthetic performance of their hosts, the magnitude of this effect, as well as the underlying mechanisms, being highly variable. Increasing the span of parasitic – host systems studied, both holo- and hemiparasitic, would be necessary for a better understanding of the mechanisms leading to photosynthesis regulation in these interactive plant systems.

22.3. EFFECT OF VIRAL INFECTION ON A HOST'S PHOTOSYNTHESIS

Goodman *et al.* (1986) summarised changes in the photosynthetic behaviour of the host plants induced by virus, bacteria and fungi, showing many points of similarity. Early works demonstrated that leaf photosynthesis rates are reduced in host plants infected by a number of viral families (Table 22.2).

Viruses cause only small reductions of A_N in host plants at early stages of infection (He *et al.*, 2004; Rowland *et al.*, 2005), but at later stages A_N is often depressed by as much as 50–85% compared with non-infected plants (Smith and Neales, 1977; Balachandran *et al.*, 1997; Sampol *et al.*, 2003; Bertamini *et al.*, 2004; Rowland *et al.*, 2005). Gas-exchange and Chl-F analysis have revealed that virus-induced depressions of photosynthesis are caused almost exclusively by non-stomatal limitations (Table 22.1). Further evidence for the non-stomatal inhibition of photosynthesis by viruses is provided by the strong interaction between virus infection and plant nitrogen availability: the effects of viruses are pronounced when superimposed with nitrogen deficiency (Balachandran *et al.*, 1997; Sampol *et al.*, 2003). The nature of these non-stomatal limitations can be variable, possibly depending on factors such as the virus sp. or strain, the resistance of the host plant and the environmental variations.

Reductions in photosynthesis by viral infection are typically accompanied by chlorosis and structural changes in photosynthetic organelles (Goodman *et al.*, 1986), decreased mesophyll conductance to CO_2 (Sampol *et al.*, 2003), altered photochemistry and primary photosynthetic reactions (Balachandran *et al.*, 1997; Rahoutei *et al.*, 1999, 2000; Bertamini *et al.*, 2004; Pérez Bueno *et al.*, 2006; Sajnani *et al.*, 2007), inhibition of Rubisco and other photosynthetic enzymes (Balachandran *et al.*, 1997; Sampol *et al.*, 2003; Bertamini *et al.*, 2004) as well as inhibition of carbohydrate export (Shalitin and Wolf, 2000). Indeed, gene-expression studies show that virus infection induces downregulation of numerous genes involved in photon capture and thylakoid processes, CO_2 uptake and photosynthesis metabolism (Golem and Culver, 2003; Whitham *et al.*, 2003; Kokkinos *et al.*, 2006; Espinoza *et al.*, 2007).

22.3.1. Viral-induced changes on chloroplast ultrastructure and starch accumulation

Viral pathogens can affect chloroplast number, size, morphology and content, as well as the size and number of chloroplast inclusions (plastoglobuli, starch grains, etc). Almási *et al.* (2001) summarised chloroplast aberrations in virus-infected plants; they differ both qualitatively and quantitatively among host-pathogen systems. Alterations in both starch accumulation and metabolism appear as a common feature of pathogen infection.

Goodman *et al.* (1986) cited a number of early studies that reported starch accumulation during viral pathogenesis (Carroll, 1970; Conti *et al.*, 1972; Favali *et al.*, 1975; Tomlinson and Webb, 1978). Starch lesions or rings spots on inoculated leaves could appear before the visible disease symptoms (Cohen and Loebenstein, 1975). In marrow inoculated with the *Cucumber mosaic virus* (CMV), starch-accumulating cells display increased photosynthetic capacity relative to uninfected cells, or cells in which virus replication is actively occurring (Técsi *et al.*, 1996). During infection of a starch-depleted mutant line of *Arabidopsis thaliana* with *Turnip vein-clearing virus* (TVCV), CMV or *Cauliflower mosaic virus* (CaMV), Handford and Carr (2007) demonstrated that starch accumulation during infection is not required for successful viral infection; however, carbohydrate metabolism does influence symptom development.

Table 22.2. *Summary of the most representative studies on the effect of different viral families on the photosynthetic process.*

Family/Virus	Host plant	Effects on photosynthesis-related parameters	Reference(s)
Tobamovirus			
Tobacco mosaic virus (TMV)	Tobacco	Chloroplast malformations Loss of Chl-protein complexes OEC alterations PSII alterations Decreased PSII quantum yield Increased NPQ Altered Calvin cycle Starch accumulation BGF increase during HR Leaf temperature increase	Zaitlin and Jagendorf, 1960; Esau, 1968; Carroll, 1970; Carroll and Kosuge, 1989; Hodgson *et al.*, 1989; Koiwa *et al.*, 1989; Montalbini and Lupattelli, 1989; Chaerle *et al.*, 1999; van Kooten *et al.*, 1990; Balachandran *et al.*, 1997; Abbink *et al.*, 2002; Hlavácková *et al.*, 2002; Lehto *et al.*, 2003; Wilhelmová *et al.*, 2005; Chaerle *et al.*, 2007
	Cucumber	Swollen and deformed plastids Accumulation of osmiophilic plastoglobuli Starch lesions or ringspots Large and irregular starch grains	Cohen and Loebenstein, 1975
	Tomato	Loss of Chl-protein complexes	Koiwa *et al.*, 1992
	Arabidopsis thaliana	Repression of plastid-associated genes	Golem and Culver, 2003
Turnip vein-clearing virus (TVCV)	*Arabidopsis thaliana*	Altered starch accumulation	Handford and Carr, 2007
Pepper mild mottle virus (PMMoV)	*Nicotiana benthamiana*	Swollen and deformed plastids Accumulation of osmiophilic plastoglobuli Large and irregular starch grains OEC alterations PSII alterations Decreased PSII quantum yield BGF increase NPQ increase Leaf temperature increase Changes of the assimilatory potential of infected leaves	Rahoutei *et al.*, 1999, 2000; Pérez-Bueno *et al.*, 2004, 2006; Chaerle *et al.*, 2006; Pineda *et al.*, 2008 a,b; Sajnani *et al.*, 2007.
Calimovirus			
Cauliflower mosaic virus (CaMV)	Cabbage	Enlargement and accumulation of starch grains	Conti *et al.*, 1972
	Arabidopsis thaliana	Altered starch accumulation	Handford and Carr, 2007

Luteovirus			
Beet western yellow virus (BWYV)	Lettuce	Starch accumulation	Tomlinson and Webb, 1978
Barley yellow dwarf virus (BYDV)	Barley	Starch accumulation	Jensen, 1972
Cucumovirus			
Cucumber mosaic virus (CMV)	Cucumber	Reduced carbohydrate export from leaves	Shalitin and Wolf, 2000
	Marrow	Inhibition of lamellar development Starch lesions or ringspots	Técsi *et al.*, 1996
	Tobacco	OEC alterations Inhibition of lamellar development	Ehara and Misawa, 1975; Takahashi and Ehara, 1992; Roberts and Wood, 1982
	Arabidopsis thaliana	Repression of plastid-associated genes Altered starch accumulation	Whitham *et al.*, 2003 Handford and Carr, 2007
Hordeovirus			
Barley stripe mosaic virus (BSMV)	Barley	Cytoplasmatic invaginations	Carroll, 1970; MacMullen *et al.*, 1978
Tymovirus			
Turnip yellow mosaic tymovirus (TYMV)	Chinese cabbage	Cytoplasmatic invaginations Inhibition of Hill reactions Changes in the ratios Chl-F/BGF	Goffeau and Bové, 1965; Matthews and Sarkar, 1976
Potyvirus			
Potato virus Y (PVY)	Potato	Abundance of plastoglobuli	Schnablová *et al.*, 2005
Peanut green mosaic virus (PGMV)	Peanut	Inhibition of PSII electron transport OEC alterations	Naidu *et al.*, 1986
Plum pox virus (PPV)	*Nicotiana benthamiana*	Repression of plastid-associated genes PSII alterations	Jiménez *et al.*, 2006; Dardick, 2007
Sweet potato feathery mottle virus (SPFMV)	Sweet potato	Repression of plastid-associated genes	Kokkinos *et al.*, 2006
Turnip mosaic (TuMV)	*Arabidopsis thaliana*	Repression of plastid-associated genes	Whitham *et al.*, 2003; Yang *et al.*, 2007
Tobacco etch virus (TEV)	Tobacco	Inhibition of photosynthesis	Owen, 1957
Maize dwarf mosaic virus (MDMV)	Corn	Inhibition of photosynthesis	Tu and Ford, 1968

Table 22.2 (*cont.*)

Family/Virus	Host plant	Effects on photosynthesis-related parameters	Reference(s)
Geminivirus			
Tobacco leaf curl virus (TLCV)	*Eupatorium makinoi*	Loss of Chl-protein complexes	Funayama *et al.*, 1997a,b
Abutilon Mosaic Virus (AbMV)	*Abutilon striatum*	Impaired NPQ reflecting the state of symptom development	Osmond *et al.*, 1998; Lohaus *et al.*, 2000
Closterovirus			
Grapevine leaf roll associated virus (GLRaV)	Grapevine	Decreased mesophyll conductance to CO_2 Decreased chlorophyll content OEC alterations Decreased PSII quantum yield Decreased activity of Rubisco and nitrate reductase Repression of plastid-associated genes	Sampol *et al.*, 2003; Bertamini *et al.*, 2004; Espinoza *et al.*, 2007
Nepovirus			
Tomato ringspot nepovirus (ToRSV)	Tobacco	Inhibition of photosynthesis	Roberts and Corbett, 1965
Tomato ringspot nepovirus (ToRSV)	*Nicotiana benthamiana*	Repression of plastid-associated genes	Dardick, 2007
Grape fanleaf yellow-mosaic virus (GFYM)	Grapevine	Inhibition of CO_2 fixation	Pozsar *et al.*, 1969
Carmovirus			
Turnip crinkle- and saguaro cactus carmovirus	*Nicotiana benthamiana*, *Brassica pekinensis*, *Chenopodium amaranticolour*	Abundance of plastoglobuli	Russo and Martelli, 1982
Crinivirus			
Sweet potato chlorotic stunt virus (SPCSV)	Sweet potato	Repression of plastid-associated genes	Kokkinos *et al.*, 2006

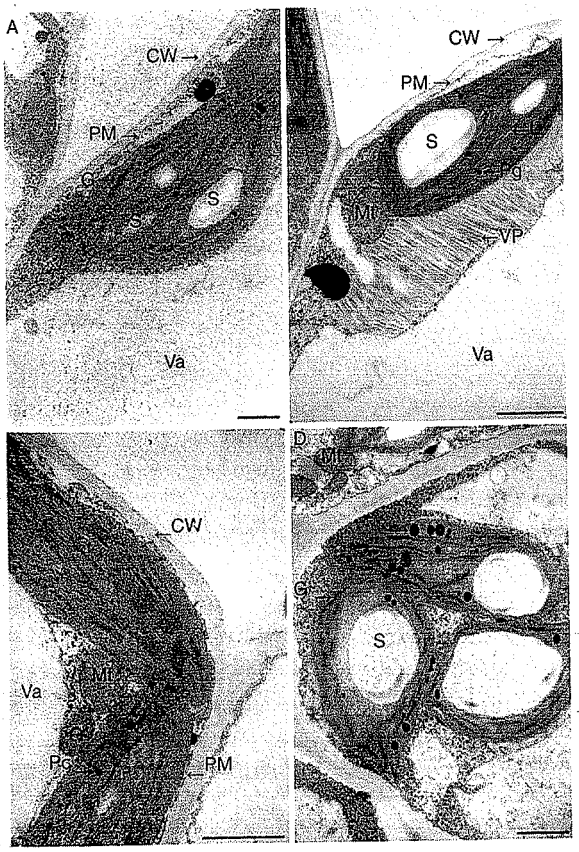

Fig. 22.1. Transmission electron micrographs of *N. benthamiana* chloroplasts. Control young leaves (**A**) and the corresponding symptomatic leaves of PMMoV-I infected plants at 7 dpi (**B**). Control old leaves (**C**) and the equivalent asymptomatic leaves of PMMoV-I infected plants at 17 dpi (**D**). C, chloroplast; CW, cell wall; L, stroma *lamellae* ; G, *grana*; M, mithochondrion; Pg, *plastoglobuli*; Pm, plasmatic membrane; S, starch grains; VP, virus particles; Va, vacuole. Scale, 1 μm. (Pictures by M.L. Pérez Bueno and N. Steffanie. R.Valcke´s lab. Hasselt University).

Starch accumulation also occurs in infected tissue in the absence of symptoms. Changes in *Pepper mild mottle virus* (PMMoV)-infected *N. benthamiana* cells from asymptomatic leaves (Pérez-Bueno *et al.*, 2006; Fig. 22.1), included the occurrence of large and irregular starch grains in swollen and deformed plastids, accumulation of osmiophilic plastoglobuli and disorganisation of chloroplast lamellar structures; these changes in chloroplast structure are similar to those detected in TMV-infected cucumber cotyledons (Cohen and Loebenstein, 1974), where the infection is restricted to the inoculated leaves forming starch lesions (Lindner *et al.*, 1959). Different metabolic processes, such as alterations in the Benson-Calvin cycle, modifications in the permeability of chloroplast membranes and disturbances of source-sink relationships were found to be correlated with starch accumulation (Wright *et al.*, 1995; Hull, 2002), but the precise responsible mechanisms remain unknown.

Chloroplasts of infected cells also show a wide range of alterations in their shape and inner structure, from swelling to complete rearrangement of the thylakoids (Esau, 1968; Koiwa *et al.*, 1992; Lehto *et al.*, 2003). The inhibition of lamellar development depends on when during development the leaf became infected (Ehara and Misawa, 1975; Roberts and Woods, 1982). The presence of vesicles within or attached to plastids was reported by various authors cited by Goodman *et al.* (1986) and Almási *et al.* (2001). In barley infected with *Barley stripe mosaic virus* (BSMV) (Carroll, 1970; McMullen *et al.*, 1978), chloroplasts became swollen, aggregated with parts of the cytoplasm trapped between adjacent organelles and contained cytoplasmatic invaginations. These changes resembled those observed in Chinese cabbage infected with *Turnip yellow mosaic tymovirus* (TYMV) (Matthews and Sarkar, 1976). In some cells, the vesicles were so large that they filled almost the entire space of the chloroplast, the membrane structure could become pushed to one side, producing a 'sickling effect'. It was proposed that these vesicles serve as sites of viral replication, but this is contested (Almási *et al.*, 2001).

Abundant plastoglobuli also occur in the swollen chloroplasts of infected leaves (Russo and Martelli, 1982; Schnablová *et al.*, 2005; Pérez-Bueno *et al.*, 2006). The formation of plastoglobuli is thought to be linked to the breakdown of thylakoids that accompanies senescence (del Río *et al.*, 1998). Thylakoid-membrane degradation by lipid peroxidation has been shown to take place at the final infection steps by PMMoV (Rahoutei *et al.*, 1999). Alternatively, the abundance of plastoglobules and disorganisation of chloroplast lamellar structures resemble those of chloroplasts from senescent leaves (del Río *et al.*, 1998; Almási *et al.*, 2001 and references therein). Recently, Bréhélin *et al.* (2007) suggested that plastoglobules participate in diverse secondary metabolism pathways and stress responses, and are not merely a 'passive storage' compartment. Lehto *et al.* (2003) assigned the chloroplast malformations in tobacco plants infected with the flavum strain of *Tobacco mosaic virus* (TMV) to deficient synthesis and assembly of thylakoid proteins; Koiwa *et al.* (1992) and Funayama *et al.* (1997b) suggested that the impact of infection on the chloroplast with tobamovirus or geminivirus, respectively, could be related to the loss of chl.-protein complexes, mainly from

the LHC. Disturbances of other photosynthetic complexes, such as the OEC of PSII, during early infection stages in PMMoV-infected *N. benthamiana* plants have also been demonstrated (Rahoutei *et al.*, 2000; Pérez-Bueno *et al.*, 2004).

22.3.2. Photosynthetic electron transport during viral pathogenesis

Studies of several plant–virus systems have demonstrated lower photosynthetic electron transport rates in infected plants, essentially at the PSII level (Table 22.2). The involvement of specific viral gene products in the inhibition of the photosynthetic $\mathcal{J}$ has been proposed frequently (Hodgson *et al.*, 1989; Reinero and Beachy, 1989).

That the decreased rate of photosynthesis in viral-infected plants is not simply caused by reduced chl. content was already evident in early studies (Spikes and Stout, 1955). Photosynthetic phosphorylation and the Hill reaction were decreased in chloroplasts isolated from TMV-infected plants (Zaitlin and Jagendorf, 1960; Montalbini and Lupattelli, 1989), as well as in Chinese cabbage leaves infected with TYMV (Goffeau and Bové, 1965).

Studies of *peanut green mosaic virus* (PGMV)-infected peanut revealed that reduced electron transport rates was caused primarily by direct inhibition of PSII (Naidu *et al.*, 1986 and references therein). For some authors (Takahashi and Ehara, 1992) disturbances of the OEC protein pattern are associated to the primary molecular processes of symptom expression during infection. The inactivation of PSII associated to a decrease in PsbO (a 33-kDa extrinsic protein) was also described in grapevine plants infected with the *grapevine leafroll virus* (Bertamini *et al.*, 2004).

Analysing the chloroplast proteome and the transcript profile of chloroplast proteins in *Nicotiana benthamiana* infected with two strains of PMMoV (Spanish and Italian strain, PMMoV-S and I, respectively), it was established that the OEC is the main target of the tobamovirus. PMMMoV-infected plants displayed a decrease of OEC proteins (PsbO, PsbP and PsbQ), whose synthesis could be transcriptionally regulated during pathogenesis. The OEC proteins were found to be products of three multigene families in *N. benthamiana*. The PsbP family was differentially regulated during the infection process (Rahoutei *et al.*, 2000; Pérez-Bueno *et al.*, 2004). Changes on the TL characteristics of chloroplasts isolated from PMMoV-infected plants were analysed, concluding that the formation of the higher S states of the OEC was inhibited during infection with both strains of the virus. The simultaneous appearance of high-temperature TL bands is indicative of lipid peroxidation in the photosynthetic membranes (Rahoutei *et al.*, 1999).

In addition to viral-induced changes affecting OEC, measurements of Chl-F revealed that viral infection caused a decrease in PSII photochemical efficiency and an increase in NPQ (van Kooten *et al.*, 1990; Balachandran *et al.*, 1997; Rahoutei *et al.*, 2000; Hlavácková *et al.*, 2002; Pérez-Bueno *et al.*, 2004; Wilhelmová *et al.*, 2005). PSII quantum yield and NPQ are affected to a different extent depending upon the virus and the developmental and growing condition of the plant, as well as leaf age. PSI electron transport was only slightly affected during pathogenesis.

Funayama *et al.* (1997a,b) found higher chl. *a/b* ratios and a preferential loss of LHCII in a *geminivirus*-infected *Eupatorium makinoi*. They suggested that decreased PSII quantum yield was mainly owing to the decreased energy allocation to PSII, altering the energy distribution balance between PSII and PSI. Subsequent growth experiments revealed that the performance of infected *E. makinoi* plants varied largely with growth light environment (Funayama *et al.*, 1997b).

Recently, Dardick (2007) has studied the gene expression profiles of *N. benthamiana* leaves systemically infected with three different fruit-tree viruses (*Plum pox potyvirus*, PPV, *Tomatoringspot nepovirus*, ToRSV and *Prunus necrotic ringspot ilarvirus*, PNRSV). Consistent with the severity of the symptoms, repression of plastid-associated genes (mainly nuclear-encoded proteins involved in electron transport, light harvesting and Benson-Calvin cycle) was observed for both PPV and ToRSV, but not for PNRSV. Yang *et al.* (2007) carried out a spatial analysis of *Arabidopsis thaliana* gene expression in response to *Turnip mosaic virus* (TuMV) infection tagged with the GFP. Downregulated genes are those associated with chloroplast functions, sulphate utilisation or cell-wall expansion. The extent to which TuMV-responsive genes were up- or downregulated primarily correlated with the amount of virus accumulation regardless of gene function.

Summarising, virus-induced depressions of photosynthesis are caused mainly by non-stomatal limitations of different nature. Structural changes in photosynthetic organelles, altered photochemistry, inhibition of the activity from Rubisco and other photosynthetic enzymes, as well as inhibition of carbohydrate export and changes in source-sink relationships are evident in plants infected with different viral families.

22.4. IMPACT OF FUNGAL INFECTION ON A HOST'S PHOTOSYNTHESIS

In contrast to viruses, the effects of fungi on host photosynthesis are considerably more varied. To characterise common patterns and modes of action, we have divided fungi into three damage guilds (i.e., such division does not have any taxonomic or systematic basis, but rather it is based on the effects on host plants): endophytic fungi, vascular-wilt fungi and leaf-disease fungi (Table 22.3). Their effects on photosynthesis-related parameters are summarised in Table 22.3.

Endophytic fungi are often considered symbiotic as infected plants perform better than non-infected plants. Infection induces increases in host plant A_N, although this is not always the case. Vascular-wilt fungi include a number of fungi that grow mostly on xylem tissues of host plants, causing a decrease in hydraulic conductance that leads to severe wilting of the foliage. Reductions of A_N induced by wilt-disease fungi can be as large as 30–90% and typically caused by reduction in stomatal conductance (g_s). As in the case of limited soil-water availability (see Chapter 20), non-stomatal limitations to photosynthesis (Table 22.1) appear only secondarily, when the infection is severe and long-lasting. Leaf-disease fungi live in mesophyll cells and their effects on photosynthesis are well characterised. Leaf diseases largely reduce CO_2 assimilation of host plants mostly by reducing the photosynthesising leaf area (Table 22.1), but they also induce a decrease in A_N in the remaining green-leaf areas that in some cases can be as high as 30–50% (see references for the three types of fungi in Table 22.3).

22.4.1 Fungal-induced changes on chloroplast ultrastructure

Chloroplast degeneration is also associated with decreased photosynthetic rates in fungal-infected plants. Goodman *et al.* (1986) describes a general damage or ageing of the photosynthetic machinery in fungal-infected plants. However, infected leaves are heterogeneous, consisting of regions of cells directly invaded by the pathogen and regions remote from the fungal colony; consequently, alterations in photosynthesis are often spatially and temporally complex and depend upon the particular host/pathogen interaction (Scholes and Rolfe, 1996; Chou *et al.*, 2000; Swarbrick *et al.*, 2006). Rust and powdery mildew induce the formation of *green islands* at the infection site, which are photosynthetically active with a higher chl. concentration and starch accumulation than in the uninfected cells, while the rest of the leaf remains chlorotic. Chloroplasts in the green island of rust-infected bean leaves are functional but contain abundant peripheral reticula (Sziráki *et al.*, 1984). In rust diseases starch content varies along the infection, increasing during the sporulation time in the chloroplast of the host cells adjacent to the fungal hyphae. Donald and Strobel (1970) found that the activity of the ADP-glucose pyrophosphorylase was similar to the pattern of starch accumulation, and was almost the inverse of the variation observed in inorganic phosphate in diseased leaves during the infection process.

22.4.2. Fungal-induced alterations on photochemistry and related processes

Fungal infections have been shown to induce alterations of leaf photochemistry. For instance, the decline in the rate of photosynthesis and the corresponding increase in NPQ during infection of *Arabidopsis thaliana* leaves with *Albugo candida* (Chou *et al.*, 2000) was restricted closely to invaded regions of the leaf. Changes in NPQ were interpreted as evidence of a greater reduction in the activity of the Calvin cycle relative to other components of the photosynthetic apparatus, and were associated with a lower amount of mRNA encoding the small Rubisco subunit (RbcS). The expression of *cab* (chl. *a*/*b*-binding protein) genes was also repressed during fungal pathogenesis. Soluble carbohydrates accumulated in the infected region, whereas the amount of starch declined. The reverse was seen in uninfected regions of the infected leaf. Aldea *et al.* (2006b) has summarised the effect on actual PSII efficiency (ϕ_{PSII}) of different biotic agents, including fungal infection (*Phyllosticta*, *Cercospora*, *Gymnosporangium*), on leaf tissues adjacent to the site of direct damage.

Phaeomoniella chlamydospora, *Phaeoacremonium aleophilum* and *Fomitiporia mediterranea* cause *esca* disease, a grapevine trunk disease (Petit *et al.*, 2006). Christen *et al.* (2007) compared the photosynthetic responses to *esca* and to drought stress; although both stresses modified the PSII performance, there was a differentiated functional pattern of PSII for the two stress types. Furthermore, infection of *Solanum tuberosum* with *Phytophora infestans* was shown to induce the reduction of the standard Chl-F parameters (F_v/F_m, F_v, F_m: maximum PSII efficiency, variable chl. fluorescence, maximum chl. fluorescence), as well as increase of qP (photochemical quenching of Chl-F) at an early stage of

Table 22.3. *Summary of the most representative studies on the effect of fungi on host photosynthesis, considering different damage guilds.*

Fungi	Host plant	Effects on photosynthesis-related parameters	References(s)
Leaf-disease fungi			
Uncinula necator, Erysiphe graminis, Plasmopara viticola	Grapevine	Reduction of CO_2 assimilation	Shtienberg, 1992; Scholes *et al.*, 1994; Moriondo *et al.*, 2005; Petit *et al.*, 2006; Agati *et al.*, 2008
Puccinia triticina	Wheat		Shtienberg, 1992; Robert *et al.*, 2005
Alternaria spp., *Blumeriella* spp., *Septoria* spp.	Several species		Niederleitner and Knoppik, 1997; Robert *et al.*, 2005; Roloff *et al.*, 2004
Phaeocryptopus gaeumannii	Douglas fir		Manter and Kavanagh, 2003
M. nubilosa, M. cryptica	Eucalyptus		Pinkard and Mohammed, 2006
Puccinia coronata	Oat	Alteration in several photosynthetic parameters in both infected and non-infected tissues	Scholes and Rolfe, 1996
Albugo candida	*Arabidopsis thaliana*	Alterations in phochemistry (NPQ), down-regulation of cab and *Calvin*-cycle genes	Chou *et al.*, 2000
Phytophthora ramorum	Tobacco SR1, *Rhododendron macrophyllum, Lithocarpus densiflorus, Umbellularia californica*	Disturbances on PSII function	Manter *et al.*, 2007
Phytophora infestans	*Solanum tuberosum*		Koch *et al.*, 1994
Blumeria graminis (during HR)	Barley	Alteration in source-sink relationships and carbon utilisation, changes on invertase activity, down-regulation of Calvin-cycle genes	Swarbrick *et al.*, 2006
Phytophtora nicotianae (during HR)	Tobacco	Stomatal closure, PSI inhibition, inactivation of Calvin cycle	Scharte *et al.*, 2005
Endophytic fungi			
Acremonium coenophialum,	*Festuca arundinacea*	*Changes* on A_N (light-saturated net photosynthesis)	Marks and Clay, 1996

Table 22.3. (*cont.*)

Fungi	Host plant	Effects on photosynthesis-related parameters	References(s)
Vascular-wilt fungi			
Fusarium oxysporum f. Sp. lycopersici	Tomato	A_N reduction by decreased stomatal conductance	Duniway and Slatyer, 1971; Lorenzini *et al.*, 1997; Nogués *et al.*, 2002
Verticillium dahliae	Potato		Bowden *et al.*, 1990; Haverkort *et al.*, 1990; Bowden and Rouse, 1991; Gent *et al.*, 1995, 1999; Saeed *et al.*, 1999; Goicoechea *et al.*, 2001; Rotenberg *et al.*, 2004
Phytophtora capsici	Pepper		Aguirreolea *et al.*, 1995
Phaeomoniella chlamydospora	Grapevine		Edwards *et al.*, 2007a,b

the disease, indicating a disturbance of PSII function (Koch *et al.*, 1994).

Comparatively little is known about the consequences for photosynthetic metabolism of activating resistance responses in plants challenged with pathogenic fungi. Swarbrick *et al.* (2006) studied barley leaves infected with *Blumeria graminis*. During resistance, photosynthesis was most severely inhibited in cells directly associated with attempted penetration of the fungus but also in surrounding cells as a result of cell death but also to an alteration in source-sink relationships and carbon utilisation. Invertase activity increased more rapidly and to a much greater extent than in infected susceptible leaves and was accompanied by an accumulation of hexoses and downregulation in the expression of *rbcS* and *cab* genes (to a lesser extent than in a compatible interaction).

Scharte *et al.* (2005) found in source leaves of *Nicotiana tabacum* infected with *Phytophthora nicotianae* that hypersensitive cell death (HR) did not appear until photosynthesis completely declined. The decline in assimilation occurs in two steps: first by stomatal closure and later by inhibition of electron donation to PSI, which prevents H_2O_2 release at PSI (Mehler reaction), and kept the stroma in an oxidised state inactivating the Calvin cycle. Moderate photoinhibition of PSII at the infection site could be a consequence, rather than a primary cause for restricted electron transport.

Beech seedlings infected with the root-rot pathogen *Phytophthora citricola* showed a decrease in the rate of A_N in the very early infection steps, indicating the involvement of a mobile signal from the root; later in infection, PSII electron quantum yield, leaf-water potential and total water consumption were slightly impaired and wilt symptoms occurred (Fleischmann *et al.*, 2005).

Giving the main points of the varied effects of fungi on host photosynthesis, it is evident that leaf-disease fungi (rust, mildew, etc.) reduce the photosynthetic leaf area and consequently the CO_2 assimilation. Although these fungi induce photosynthetic alterations spatially and temporally and depending upon the particular host/pathogen interaction, repression of photosynthetic genes, changes in chloroplast structure and starch metabolism as well as altered J were common to different host plants. In contrast, wilt fungi compromise plant water transport and reduce photosynthesis by inducing stomatal closure, and endophytic fungi, often considered symbiotic, could increase the photosynthetic performance of the host plant during infection.

22.5. IMPACT OF BACTERIAL CHALLENGE ON PHOTOSYNTHESIS

22.5.1. The effect of pathogenic bacteria on gas exchange and CO_2 assimilation

The nature of photosynthetic limitations imposed by bacteria are variable (Table 22.1, Table 22.4), although probably non-stomatal effects, mostly associated with leaf chlorosis,

Table 22.4. *Summary of the most representative studies on the effect of different pathogenic bacteria on the photosynthetic process.*

Pathogenic bacteria	Host plant	Effects on photosynthesis-related parameters	References(s)
Xanthomonas			
Xanthomonas campestris pv. *phaseoli*	Bean	Decreased in chl. content	Berova *et al.*, 2007
Xanthomonas campestris pv. *pelargonii*	Geranium	Decreased in CO_2 export from source leaves	Jiao *et al.*, 1999
Xanthomonas axonopodis pv. *manihotis*	Cassava	Downregulation of photosynthesis genes	López *et al.*, 2005
Pseudomonas			
Pseudomonas syringae pv. *tomato*	Tomato	Downregulation of photosynthetic genes	Berger *et al.*, 2004
Pseudomonas syringae pv. *glycinea*	Soybean		Zou *et al.*, 2005
Pseudomonas syringae pv. *tomato*	*Arabidopsis thaliana*	Decreased F_v/F_m, ϕ_{PSII} and NPQ	Bonfig *et al.*, 2006; Berger *et al.*, 2007
Pseudomonas syringae pv. *phaseolicola* and pv. tomato	Bean	Alterations in NPQ pattern	Rodriguez-Moreno *et al.*, 2008
Pseudomonas syringae pv. *syringae*	Tobacco	Change of ferredoxin levels	Huang *et al.*, 2007
Xylella			
Xylella fastidiosa	Sweet orange	Lower leaf-water potential, carboxylation efficiency and stomatal conductance, impairment of the biochemical reactions of photosynthesis, downregulation of genes encoding LHCII, RuBisco activase and PsaO	Habermann *et al.*, 2003a,b; Ribeiro *et al.*, 2003a,b; de Souza *et al.*, 2007
Xylella fastidiosa + temperature stress	*Parthenocissus quinquefolia*	Lower CO_2 assimilation rates and stomatal conductance	Hopkins, 1989; Ribeiro *et al.*, 2004
Xylella fastidiosa + water stress		Reduced photosynthesis by stomatal and not stomatal limitations (depending on the disease and drought severity)	McElrone *et al.*, 2003; McElrone and Forseth, 2004
Erwinia			
Erwinia carotovora ssp. *carotovora*	Potato	Decreased mRNA levels of PsaD, accumulation of hydrogen peroxide in chloroplasts	Montesano *et al.*, 2004
	Tobacco	Change of ferredoxin levels	Huang *et al.*, 2007

predominate (Goodman *et al.*, 1986). Different strains of *Xanthomonas campestris* produce almost exclusively non-stomatal limitations on photosynthesis. Nevertheless, the specific limitations may be different: *X. campestris pv. phaseoli* caused strong reductions in chl. content in beans, whereas *X. campestris pv. pelargonii* did not affect chl. concentration, but largely decreased carbon export from leaves. *X. axonopodis pv. manihotis* was shown to induce downregulation of photosynthetic genes in cassava, while genes against oxidative stress were upregulated (López *et al.*, 2005).

In the case of *Xylella fastidiosa*, causing Pierce's disease of grape and leaf scorch of different plants, little differences

in hydraulic conductivity and g_s were observed between healthy and infected plants in the absence of additional stress (Table 22.4).

Another disease induced by *Xylella fastidiosa* is the citrus variegated chlorosis (CVC). Diseased sweet orange (*Citrus sinensis* cv. Pera) plants showed stomatal disfunction as well as lower carboxylation efficiency (Habermann *et al.*, 2003b), related with impairment of the biochemical reactions of photosynthesis (Ribeiro *et al.*, 2004). The pathogenicity of *X. fastidiosa* was aggravated by the occurrence of additional stresses (Hopkins, 1989; Ribeiro *et al.*, 2004).

Bacterial infection has been shown to reduce plant CO_2 assimilation. The magnitude of the effect depends on the severity and timing of infection, but also on the particular type of bacteria and on genotype-associated resistance of the host plant (Jiao *et al.*, 1999; McElrone and Forseth, 2004; Berova *et al.*, 2007).

In addition, a potentially synergistic effect of CO_2 with bacterial infection was investigated in geranium (*Pelargonium* × *domesticum*, *'ScarletOrbit Improved'*) plants infected with *Xanthomonas campestris pv. pelargonii*. High CO_2 lowered the bacterial number in infected leaves, the reductions in photosynthesis and export of photoassimilates from 'source leaves' being greater than at ambient CO_2 (Jiao *et al.*, 1999).

Despite the lack of proper gas-exchange analysis in plants infected with *Pseudomonas syringae*, it can be deduced that it affects photosynthesis by both stomatal and non-stomatal limitations. Stomatal closure is part of a plant innate immune response to restrict bacterial invasion. To circumvent this innate immune response, plant pathogenic bacteria have evolved specific virulence factors to induce stomatal reopening (Melotto *et al.*, 2006). In view of the interaction between bacterial infection and high CO_2, as well as the importance of stomatal regulation on the plant innate immune response to the pathogen, it is likely that in a future secenario of climatic change the impact of bacterial infection on plant photosynthesis will be lower. Nevertheless, this interaction needs further studies.

22.5.2. Bacterial-induced alterations of the PET in the host plant

Bacterial infection strongly affects the photochemical steps of photosynthesis. In fact, downregulation of genes encoding photosynthetic functions (Tao *et al.*, 2003; Zou *et al.*, 2005; Truman *et al.*, 2006) as well as changes in PSII proteins (Jones *et al.*, 2006) have been reported in *P. syringae*-infected plants. Bacterial effects on fluorescence parameters have been associated with a decrease in ϕ_{PSII}, as well as an increase in NPQ in leaf areas infiltrated with an avirulent *P. syringae pv. glycinea* inducing the HR, although little effect was observed during a compatible interaction (Zou *et al.*, 2005). In contrast, Bonfig and collaborators (2006) found that maximum F_v/F_m, ϕ_{PSII} and NPQ decreased in *Arabidopsis* plants infected with either virulent or avirulent *P. syringae pv. tomato*. A novel combination of Chl-F imaging (Chl-FI) and statistical analysis (Matouš *et al.*, 2006) used to study *P. syringae* infection of *Arabidopsis* leaves resolved very early and late phases of the plant response to infection, allowing for pathogen detection before the appearance of visual symptoms (Berger *et al.*, 2007). Rodríguez-Moreno *et al.* (2008) could differentiate a compatible from an incompatible plant-bacteria interaction in asymptomatic leaf tissues from *Phaseolus vulgaris* plants inoculated with either *P. syringae* pv. *phaseolicola* or *P. syringae* pv. *tomato* by Chl-FI analysis. A decrease in NPQ, evident in both infiltrated and non-infiltrated leaf areas, was observed in Pph-infected plants as compared with corresponding values from controls and Pto-infected plants.

De Souza *et al.* (2007) found that transcripts of LHCII, RuBisco activase (Rca) and PsaO were downregulated in sweet orange plants exhibiting CVC symptoms; in contrast, the phosphoribulokinase of the Calvin cycle was upregulated. Queiroz-Voltan and Paradela-Filho (1999) found chloroplasts totally damaged in chlorotic regions present in CVC-symptomatic leaves. Therefore the downregulation of these photosynthesis-associated genes is perhaps a consequence of disorders that occur in the photosynthetic apparatus.

A downregulation of PSI has been demonstrated in *Solanum tuberosum* treated with *Erwinia carotovora* ssp. *carotovora*. The expression of the *psaD*, a nuclear gene encoding the PsaD subunit of PSI, was downregulated and this correlated with an accumulation of H_2O_2 in chloroplasts (Montesano *et al.*, 2004). The levels of another PSI protein, ferredoxin (Fd), seem to play an important role in plant defence against bacterial infection (Huang *et al.*, 2007).

Summarising, while the changes caused by infection with biotrophic fungi and viruses on the photosynthetic machinery are the best understood, more research is needed to elucidate the interaction with virulent as well as avirulent bacterial strains. The nature of photosynthetic limitations imposed by bacteria is variable, although probably non-stomatal effects predominate. Studies on the differential gene expression during HR versus susceptible interaction

show that bacterial challenge has a strong impact on photosynthesis through the downregulation of genes encoding photosynthetic functions. Bacterial effects on Chl-F parameters of the host plant have been associated with an inhibition of the photosynthetic J and changes on the non-photochemical processes of energy dissipation. Regarding bacterial-induced stomatal limitations of the photosynthetic process, the importance of stomatal regulation on the plant innate immune response to a pathogen is a promising research field for the future.

22.6. THE INFLUENCE OF NEMATODE INFESTATION ON THE HOST PHOTOSYNTHETIC METABOLISM

Infection with parasitic nematodes causes decreased transpiration and photosynthesis (Fatemy *et al.*, 1985; Postuka *et al.*, 1986; Melakeberhan *et al.*, 1990; Schans and Arntzen, 1991; Asmus and Ferraz, 2002), and associated decreases in leaf chl. content (Siddiqui and Mahmood, 1999) and plant growth (Decker, 1969; Melakeberhan *et al.*, 1990; Schans and Arntzen, 1991). The effect of the nematodes on physiology and morphology of the host increases with the duration and level of infection.

Soybean plants inoculated with the soybean cyst nematode *Heterodera glycines* showed a marked reduction in photosynthetic rate and chl. content, evident as leaf yellowing (Asmus and Ferraz, 2002). The reduced photosynthetic activity was primarily related to a lesser amount of nutrients, particularly nitrogen, either absorbed or translocated by the infected roots (Koenning and Barker, 1995). These results agree with those found for other plant-nematode interactions, such as *Meloidogyne incognita* Chitwood on vine (*Vitis vinifera* L.) varieties (Melakeberhan *et al.*, 1990) and *Globodera pallida* on potato (*Solanum tuberosum* L.) varieties (Schans and Arntzen, 1991). Alternatively, Bird (1974) hypothesised that the reduced photosynthetic rate could be related to partial closure of the stomata caused by water stress owing to the nematode-damaged roots.

Schmitz *et al.* (2006) examined the effect of infection of sugar beet leaves by *Heterodera schachtii* using laser-induced and pulse-amplitude-modulated (PAM) Chl-F. Sugar beet plants initially responded to *H. schachtii* infestation with a decrease in photosynthetic rate and later with a reduction in nitrogen uptake and chl. concentration.

Mazzafera *et al.* (2004) studied CO_2 fixation and photoassimilate partition in coffee (*Coffea arabica*) seedlings infested with the lesion nematode *Pratylenchus coffeae*, exposing the plants to $^{14}CO_2$. At the highest level of infestation, the carbon fixation in the leaves and partitioning to the roots were decreased.

Potato early dying (PED) is a vascular-wilt disease caused primarily by the fungus *Verticillium dahlia* (Rotenberg *et al.*, 2004). The lesion nematode *Pratylenchus penetrans* interacts synergistically with the fungus to enhance the development of visual PED symptoms and reduce photosynthesis and CO_2 exchange rates in plants co-infected with both pathogens. At early stages of infection, only a small decrease in photosynthetic rate was observed in diseased leaves, with no evidence of non-stomatal limitation to photosynthesis. Later, the joint infection seems to affect the Rubisco activity (Saeed *et al.*, 1999).

In contrast to the number of studies on pathogens and herbivores, there is a lack of studies on the impact of nematodes on photosynthesis. This makes it difficult to define their general mechanism of action on the chloroplast of the host plant. The effects of lesion nematodes on the carbon assimilation and partition are quite distinct from those with root-knot nematodes. In contrast to these nematodes, where the feeding sites are regarded as strong metabolic sinks, even leading to an increase of photosynthesis in the beginning of infestation, the root-lesion nematodes caused a rapid detrimental effect on carbon fixation and photoassimilate distribution in the plant owing to direct damage of the roots.

22.7. EFFECTS OF ARTHROPOD HERBIVORY ON PHOTOSYNTHESIS

Any herbivorous animal will obviously affect plant photosynthesis by leaf removal. Insect herbivory, in addition to inducing a general defoliation or feeding on specific tissues (e.g., phloem or xylem), triggers a complex and interacting array of molecular and physiological responses in plants (Fig. 22.2). This particular aspect, how insect herbivory affects plant photosynthesis in the remaing leaf, will be the scope of this subchapter. These responses potentially reduce the photosynthetic capacity in remaining leaf tissues to a greater extent than the direct removal of photosynthetic surface area. For example, the removal of only 5% of the area of an individual wild parsnip leaf by caterpillars reduced photosynthesis by 20% in the remaining foliage (Zangerl *et al.*, 2002; Nabity *et al.*, 2009). The mechanisms reducing photosynthesis in remaining leaf tissues are multifaceted, ranging from disruptions in fluid or nutrient transport to self-inflicted reductions in metabolic processes.

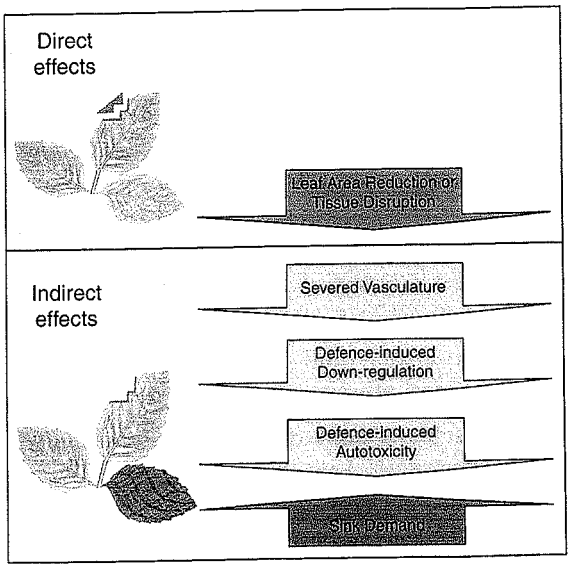

Fig. 22.2. Conceptual model of the direct effect of herbivory (removal of leaf area) and the indirect effects of herbivore damage to foliage on photosynthesis in the remaining leaf tissues.

Plant responses to arthropod herbivory traditionally have been assessed from the guild perspective, where different insect guilds are defined by their feeding mechanisms (Welter, 1989; Peterson, 2001). These guilds (e.g., chewing damage, piercing damage, etc.) were established in an effort to recognise 'homogeneity in physiological response' between different attacking agents (arthropods) that alter plant physiological processes in a similar manner. Welter (1989) examined an extensive body of literature across multiple guilds and found over 50% of all plant–insect interactions resulted in a loss of photosynthetic capacity. Defoliation generally increases photosynthesis of the remaining leaves, whereas specialised cell-content feeding decreases photosynthesis. Since then, several studies have examined plant responses to different insect-feeding guilds and even to different insects within guilds in an effort to develop models for predicting plant response to different feeding mechanisms. A brief review of the recent literature is not entirely consistent with the conclusions stated by Welter (1989). Feeding on specialised tissues typically reduces photosynthesis, regardless of whether the attacked component is the phloem or xylem (Heng-Moss *et al.*, 2006), the stem (Macedo *et al.*, 2007) or general leaf fluids (Haile and Higley, 2003). In contrast, defoliation injury often does not alter photosynthetic capacity, within plant families (e.g., legumes) or between hardwoods and crops (Peterson *et al.*, 2004); however, there are examples where defoliation decreased (Delaney and Higley, 2006) or increased photosynthesis.

The removal of leaf tissue by herbivores represents a 'direct' reduction of photosynthetic capacity. We define the suppression of photosynthesis in remaining leaf tissue by any one of a number of processes, including damage to the vasculature supplying that tissue, as an 'indirect' effect of herbivory. Arthropods damage xylem or phloem (Welter, 1989), which may alter water transport, stomatal aperture and sucrose transport and loading, thereby reducing photosynthesis in the remaining leaf tissue. Tissue disruption by severing vasculature alters leaf hydraulics and, subsequently, nutrient or osmotica transport (Sack and Holbrook, 2006). If insect feeding is subtle enough to avoid outright cell rupture, modulation of nutrients sequestered by feeding will alter plant osmotica or sink-source relationships (Dorchin *et al.*, 2006). These effects also may be mediated by the plant's response. Insect attack, or even the perception of attack, can induce a myriad of defence-related responses, while concomitantly reducing the expression of photosynthesis-related genes (Kessler and Baldwin, 2002). In instances where plant defences are constituently expressed, the release of biocidal compounds against attackers may damage photosynthetic or homeostatic mechanisms vital for plant function (e.g., Zangerl *et al.*, 2002). We assigned indirect effects of herbivory into four classes: severed vasculature; altered sink demand; defence-related autotoxicity; and defence-induced downregulation of photosynthesis (Fig. 22.2).

22.7.1. Insect herbivores can affect photosynthesis by severing leaf vasculature

Damage to leaf venation alters leaf hydraulic conductance, reducing g_s and photosynthesis. In the absence of alternative pathways for water transport, the consequences of damage to venation can persist for weeks after the initial injury and lead to desiccation (Sack and Holbrook, 2006). Defoliation injury that severs venation indiscriminately or by feeding on specific tissues, may physically obstruct fluid flow with insect mouthparts (stylets) or cell fragments and alter photosynthesis and water balance in the remaining leaf tissue (Delaney and Higley, 2006). In *Glycine max* (soybean) a form of defoliation (skeletonisation) that removes patches of tissue reduced photosynthesis in the remaining tissue on damaged leaves and on adjacent undamaged leaflets (Peterson *et al.*, 1998). Interestingly, soybean increased carbon-uptake rates and transpiration in the remaining leaf tissue when

one or two leaflets were completely lost (Suwignyo *et al.*, 1995), but when leaf-area removal (no patches) occurred to only part of a leaflet, CO_2 uptake did not decrease in the remaining leaflet tissue (Peterson *et al.*, 2004).

Aldea *et al.* (2005) confirmed that skeletonising soybean leaves by Japanese beetles substantially increased water loss from the cut edges. Damaging the interveinal tissue had no effect on CO_2 exchange, but increased transpiration by 150% for up to four days post injury. In contrast to the response to interveinal damage, severed vasculature caused a decrease in CO_2 exchange and an increase in ϕ_{PSII}, suggesting that insect damage transiently decoupled photosynthetic J from carbon assimilation (Aldea *et al.*, 2005). Damage to *Arabidopsis* by cabbage looper (*Trichoplusia ni*) larvae also increased water loss from the cut edges and caused a localised reduction in ϕ_{PSII} (Tang *et al.*, 2006). That the reduction in ϕ_{PSII} could be reversed by exposing the leaf to higher concentrations of CO_2, suggests that profligate water loss near cut edges reduced ϕ_{PSII} and increased NPQ by causing localised stomatal closure in the remaining undamaged leaf tissue.

22.7.2. Changes in sink demand by insect herbivory can affect photosynthesis

In instances where plants respond to herbivory with increased CO_2 uptake, the mechanism typically is linked to compensation or an increase in the sink demand within the leaf (Trumble *et al.*, 1993). For some gall-forming insects, gall tissue itself increases photosynthesis relative to uninjured tissue. In *Ilex aquifolium* (holly), increased ϕ_{PSII} and J enhanced carbon assimilation (Retuerto *et al.*, 2004), whereas a reduction in respiration in *Acacia pycnantha* galls contributed to an increase in A_N (Dorchin *et al.*, 2006).

In other galls of hardwoods, feeding damage reduced photosynthesis and altered water balance. Gall formation in red maple, pignut hickory and black oak reduced ϕ_{PSII} and increased NPQ, indicating a downregulation of the PSII reaction centres in the area around galls (Aldea *et al.*, 2006b). A sharp reduction in leaf temperature near galls suggests that transpiration was greater and fluid and nutrient transport increased near the point of damage. In contrast to gall-forming insects, a leaf-mining moth that lives enclosed within leaf tissue of apple trees, reduced carbon assimilation rates by decreasing transpiration (Pincebourd *et al.*, 2006).

Defoliation of plants may also increase photosynthesis by altering sink demand, but concerns over how remaining tissues were measured have been noted (Welter, 1989). By enclosing severed edges within gas-exchange cuvettes or measuring treatment effects on leaves where adjacent leaves were removed (within-plant controls), the data may not accurately describe plant responses specific to the herbivory treatment. Despite these challenges and potential limitations, data suggest defoliation may improve photosynthesis in the remaining leaf tissue (Thomson *et al.*, 2003) as a result of increased carboxylation efficiency and RuBP regeneration (Turnbull *et al.*, 2007a).

22.7.3. Autotoxicity following herbivory may reduce photosynthesis

Plants invest heavily in chemical defences (Berenbaum and Zangerl, 2008) and they run the risk of autotoxicity because of the biocidal properties of many secondary compounds employed in defence. Although in-vivo studies of autotoxicity are limited, photosynthesis may be severely reduced for some species. For example, wild parsnip (*Pastinaca sativa*) contains an arsenal of defence compounds including furanocoumarins, which are photoactivated and biocidal against a variety of organisms. Furanocoumarins are contained in oil tubes under positive pressure and bleed profusely from the wounding site (Gog *et al.*, 2005). When herbivores sever these tubes, the release of furanocoumarins reduces ϕ_{PSII} and gas exchange at considerable distances from the actual point of insect damage (Zangerl *et al.*, 2002; Gog *et al.*, 2005).

The autotoxic effect of defensive compounds on photosynthesis is highly species specific. Essential oils derived from parsley (*Petroselinum crispum*), wild parsnip and rough lemon (*Citrus jambhiri*) reduce ϕ_{PSII} when applied to leaves of conspecifics; however, oils from parsley affected a two-fold greater area than the other species (Gog *et al.*, 2005). Baldwin and Callahan (1993) fed nicotine to two species of tobacco (*Nicotiana sylvestris, N. glauca*) that naturally synthesised this alkaloid as a defence, and to two other solanaceous species lacking nicotine (*Datura stramonium, Lycopersicon esculentum*). Photosynthetic rates declined in both species that synthesised nicotine but only in one that did not (*L. esculentum*). Reduced photosynthesis contributed to reduced growth and fitness.

22.7.4. Herbivory and the defence response cause downregulation of photosynthesis-related genes

Jasmonates play a central role in regulating plant-defence responses to herbivores. The mechanism by which herbivore-induced jasmonate synthesis promotes global

reprogramming of defence gene expression, as well as the regulation of this response, have been reviewed recently (Howe and Jander, 2008). Although jasmonates induce defences, they also inhibit growth and photosynthesis (Giri *et al.*, 2006).

Transcription analysis of plant–herbivore interactions revealed that photosynthesis-related genes are downregulated after attack; however, few studies have demonstrated the effects of herbivore attack on photosynthesis at the proteome and physiological levels. Attack by herbivores reduces Rubisco transcription (Hui *et al.*, 2003). Using two-dimensional electrophoresis, Giri *et al.* (2006) observed that herbivory reduced the abundance of the gene coding for Rubisco activase (*rca*) in *N. attenuata*.

Partial defoliation of individual leaves by herbivores largely increases evapotranspiration via enhanced water loss from cut edges and produces leaf dehydration (Aldea *et al.*, 2005), which not only reduces photosynthesis by causing stomata to close, but also by initiating senescence signalling (Lim *et al.*, 2007). A number of genes are induced by endogenous ABA in response to dehydration through the synthesis of the regulating transcription factors MYC and MYB (Yamaguchi-Shinozaki and Shinozaki, 2006). Both MYC and MYB function as *cis*-acting elements that regulate transcription of dehydration-related genes. Transgenic plants overproducing MYC and MYB had higher osmotic stress tolerance, and microarray analysis indicated the presence of ABA- and JA-inducible genes (Abe *et al.*, 2003). It has been suggested that cross talk occurs on AtMYC2 between ABA- and JA-responsive gene expression at the MYC recognition sites in the promoters, and that AtMYC2 is a common transcription factor of ABA and JA pathways in *Arabidopsis* (Yamaguchi-Shinozaki and Shinozaki, 2006).

The lipoxygenase pathway leading to the production of JA is differentially induced depending on the attacking agent (Kempema *et al.*, 2007), and the initiation of jasmonate signalling reduces photosynthesis and vegetative growth.

Plants treated with methyl jasmonate develop shorter petioles than control plants (Cipollini, 2005), and *Arabidopsis* mutants that accumulate higher JA concentrations have shorter petioles than wildtype (Bonaventure *et al.*, 2007); these effects of JA on plant growth are modulated by the gene JASMONATE-ASSOCIATED1 (*JAS1*; Yan *et al.*, 2007). It has been suggested that the slower growth and downregulation of photosynthetic-related genes by herbivore elicitation may be required to free up resources for defence-related processes (Baldwin, 2001). It is not clear whether the change in carbon allocation affects photosynthetic rate *per se*, but growth reduction would affect leaf expansion and total plant photosynthesis.

22.8. IMAGING METHODS FOR BIOTIC-STRESS DETECTION

22.8.1. Chl-F imaging

Pathogens induce in their hosts a wide range of foliar visual symptoms with a heterogeneous distribution (mottle, mosaic, chlorosis, necrosis, etc.) and different Chl-FI prototypes have provided valuable tools to follow the infection and investigate the spatial and temporal heterogeneity of the foliar photosynthetic efficiency during pathogenesis (see reviews Nilsson, 1995; Nedbal and Whitmarsh, 2004). Measurements have been carried out in both inoculated and systemically infected leaves during pathogen challenge, showing photosynthesis impairment in symptomatic and asymptomatic areas during pathogenesis (Esfeld *et al.*, 1995; Scholes and Rolfe, 1996; Osmond *et al.*, 1998; Chou *et al.*, 2000; Lohaus *et al.*, 2000). In some cases, the rate of photosynthetic inhibition is associated to the severity of the symptoms; however, changes in some fluorescence parameters could precede the symptom development and allow a presymptomatic diagnosis of the disease (Chaerle *et al.*, 2007b; Fig. 22.3). A correlation between the pattern of Chl-F quenchings and virus distribution in leaves was found during the infection of *Nicotiana benthamiana* with PMMoV (Fig. 22.4; Pérez-Bueno *et al.*, 2006; Pineda *et al.*, 2008b), as well as in *Abutilon* mosaic virus-infected *Abutilon striatum* leaves (Osmond *et al.*, 1998; Lohaus *et al.*, 2000).

Alterations in photosynthesis of fungi-infected plants mapped by Chl-FI are also spatially and temporally complex. Infected leaves consist of regions of cells directly invaded by the pathogen and regions remote from the fungal colony (e.g., Scholes and Rolfe 1996; Osmond *et al.*, 1998; Chou *et al.*, 2000; Meyer *et al.*, 2001).

Imaging analysis of the changes on photosynthesis parameters during bacterial challenge in compatible and incompatible interactions has also deserved special attention (Zou *et al.*, 2005; Bonfig *et al.*, 2006; Rodriguez-Moreno *et al.*, 2008).

The fluorescent parameter best suited for either evaluating damage or carrying out presymptomatic diagnosis depends on the type of infection. Invasion of bean leaves by rust fungi (*Uromyces appendiculatus*) was revealed by changes in the fluorescence-induction kinetics (Peterson and Aylor, 1995). Cedar needles (*Torreya taxifolia*) infected by the fungus *Pestaliopsis* spp. were identified by an empirical estimate

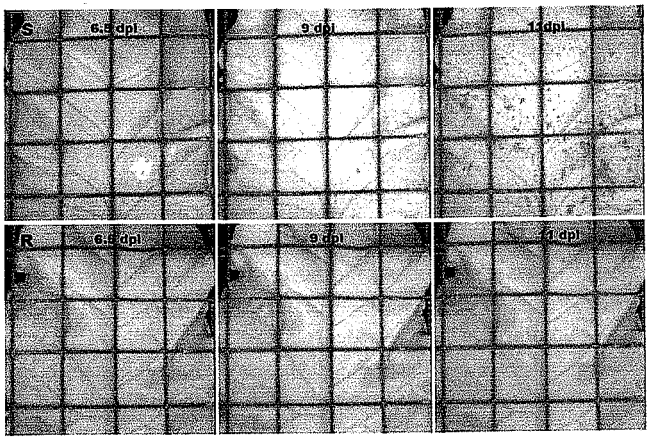

Fig. 22.3. Presymptomatic chlorophyll-fluorescence (Chl-F) increase upon *Cercospora* infection of attached sugar beet leaves. Chl-F images of infected leaves are captured at different days post-infection (dpi) in susceptible (S) and resistant (R) plants. The first Chl-F symptoms appear at 6 dpi on the S leaf. At 8 dpi, a general increase of Chl-F intensity was apparent. At 11 dpi widespread cell death (as indicated by low intensity spots) was visualised in the S leaf. Modified with permission from Chaerle *et al.* (2007a).

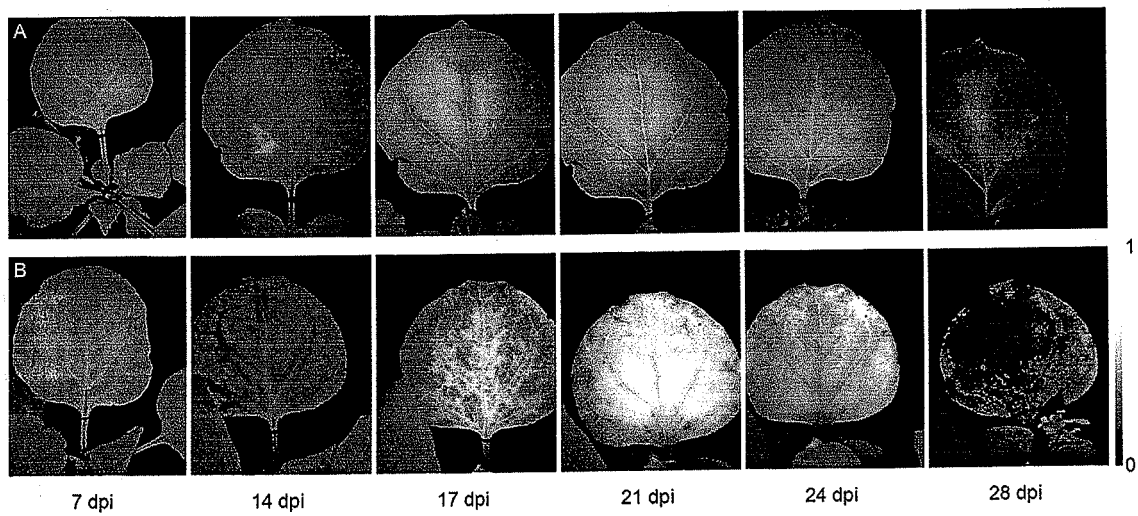

Fig. 22.4. Images of NPQ_{300} (at 300 s during the fluorescence-induction kinetics) from healthy (A) and asymptomatic (B) leaves of PMMoV-I infected *Nicotiana benthamiana* plants during pathogenesis. Modified, with permission from Pérez –Bueno *et al.* (2006).

of quantum yield (Ning *et al.*, 1995), which also was used to visualise the impact of fungal phytotoxins in hibiscus leaves (*Hibiscus sabdariffa*) (Bowyer *et al.*, 1998). Soukupová *et al.* (2003) proposed an experimental algorithm to identify the combination of fluorescence parameters providing the highest contrast between affected and unaffected plants in canola (*Brassica napus*) and white mustard (*Sinapis alba*) leaves exposed to phytotoxins of *Alternaria brassicae*. Imaging of ϕ_{PSII} of chickpea leaves was used to assess the impact of a fungal pathogen from *Ascochyta rabiei* that altered source-sink distribution (Esfeld *et al.*, 1995; Weis *et al.*, 1998). Changes of photosynthetic parameters were also visualised during host resistance (Repka, 2002; Swarbrick *et al.*, 2006).

22.8.2. Multicolour fluorescence imaging

There is a lack of multicolour fluorescence (MCF) studies, including the analysis of BGF signals in pathogen-infected

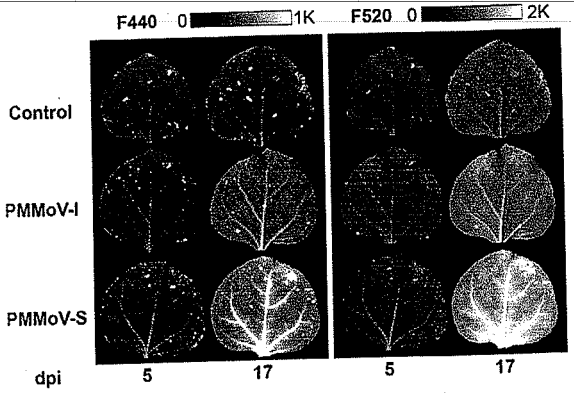

Fig. 22.5. Images of blue (F440) and green (F520) fluorescence emission from the abaxial surface of asymptomatic leaves from *Nicotiana benthamiana* control and PMMoV-infected plants. Images obtained before (5 dpi) and after (17 dpi) fluorescence changes occurred are displayed. Modified, with permission from Pineda *et al.* (2008a).

plants. Fungal infection has been reported to increase F440 either by the fungus autofluorescence (Lüdeker *et al.*, 1996) or by the production of plant phytoalexins (Niemann *et al.*, 1991). Buschmann and Lichtenthaler (1998) reported an early visualisation by MCFI of small punctures made by tobacco flies on leaves and mite attack on bean. Pineda *et al.* (2008a) monitored a systemic viral infection in PMMoV-infected *Nicotiana benthamiana* plants using a compact flash-lamp MCF system (Fig. 22.5). BGF increase linked to the accumulation of different phenolic compounds was monitored during the HR in tobacco plants challenged with TMV (Chaerle *et al.*, 2007c), *Phytophtora megasperma*, (Dorey *et al.*, 1997) and *Phytophthora nicotianae* (Scharte *et al.*, 2005).

22.8.3. Other imaging techniques for visualisation of the plant-pathogen interaction and herbivore attack

Each representative of the wide range of plant-pathogen and plant-insect interactions likely affects different plant physiological processes to a varying extent. Using several imaging techniques in parallel could reveal *disease signatures*, allowing diagnosis in the absence of symptoms in the visible spectrum (Nedbal and Whitmarsh, 2004; Aldea *et al.*, 2006a; Chaerle *et al.*, 2007c). Parallel measurements of thermal and Chl-F imaging permit to study the spatial and temporal heterogeneity of leaf transpiration and photosynthesis under biotic stress. Downy mildew infection in cucumber was visualised at an early stage by thermal imaging (Lindenthal *et al.*, 2005; Oerke *et al.*, 2006). In PMMoV-infected plants, virus immunolocalisation on tissue prints matched well with the concomitant pattern of thermal and Chl-F increase (Chaerle *et al.*, 2006). Infection with the fungus *Phyllosticta* of two conifer species induces a temperature increase in areas surrounding the inoculation point (Aldea *et al.*, 2006b); the corresponding stomata closure is associated to a decrease of ϕ_{PSII} and NPQ increase.

Development of cell death was visualised by Chl-F and thermal imaging during the HR of potato to *Phytophthora* (Scharte *et al.*, 2005), as well as in tobacco challenged with TMV (Chaerle *et al.*, 1999) and *Nicotiana sylvestris* inoculated with *Erwinia amylovora* (Boccara *et al.*, 2001). Chaerle *et al.* (2004) observed opposite effects on leaf temperature in a necrotrophic fungal infection (*Cercospora*-bean) versus a viral-induced HR (resistant tobacco-TMV).

A number of new imaging techniques have been used for following the interaction of the host plant with either virulent of avirulent pathogens: autoluminescence or biophoton imaging, associated with oxidative stress reactions (Mansfield, 2005; Havaux *et al.*, 2006 and references therein; Kobayashi *et al.*, 2007); NIR imaging for field applications (Zandonadi *et al.*, 2005; Pethybridge *et al.*, 2008); and hyperspectral imagery (Franke and Menz, 2007; Huang *et al.*, 2007). Obtaining an overview of spatial in-field variability would thus be a pre-requisite for site-specific disease management. This will be important in the context of precision agriculture, where different imaging techniques could be combined into a multispectral visualisation approach.

23.9. CONCLUSIONS

The effects of pathogens and insects on photosynthesis are as varied as their life forms and feeding behaviours, but some intriguing commonalities are emerging. Although virtually all forms os biotic damage applied directly to foliage cause some form of direct reduction of photosynthesis by removing or killing portions of the leaf, the reduction of photosynthesis in the remain tissue represents a 'hidden' and potentially considerable consequence of biotic stress. Although there are exceptions, many forms of biotic damage downregulate genes coding for the component processes of photosynthesis. The effects of biotic damage on

photosynthesis are notoriously heterogeneous. Imaging the patterns of Chl-F and leaf temperature across damaged leaves has greatly increased our understanding of the plant responses to biotic attack. Understanding the underlying molecular and biochemical mechanisms governing the response of photosynthesis has not kept pace because of a more limited ability to measure the spatial patterns of these processes. Future advances in the ability to map transcriptional and proteomic responses will shed new light on how photosynthesis is regulated following biotic damage.