Chapter 11
Biochemistry of the Host Pathogen Interaction

11.1 Introduction

Biochemical studies of the growth and survival of a pathogen and the changes it induces in its host can ultimately lead to a better understanding of the disease development, its epidemiology, and control. Ideal prerequisites for meaningful studies of the biochemistry of the host-parasite interaction are:

(a) A clear understanding of the genetic control of virulence and avirulence in the parasite and of susceptibility and resistance in the host
(b) Precise histological and cytological descriptions of spore germination, infection, and the establishment and development of the host-pathogen interaction
(c) The availability of methods for maintaining the pathogen alone, and in combination with its host, under controlled conditions. Unfortunately, these criteria have not yet been satisfactorily met for downy mildew of crucifers.

11.2 Metabolic Changes

Many marked shifts were observed in the metabolic processes of plant tissues following infection by biotrophic parasites. These included changes in respiration, photosynthesis, nucleic acid, and protein synthesis and phenol metabolism. There could also be changes in the translocation and accumulation of nutrients and in the levels of endogenous growth substances.

The respiration rate was raised sharply soon after *H. parasitica* infection of cabbage cotyledons reached a maximum, almost twice that of uninfected controls, at the time of the initiation of sporulation (Fig. 11.1). The chlorophyll content of infected and non-infected cotyledons did not differ significantly at any time (Fig. 11.2). The increased respiration rate of the infected tissues did not reflect any significant changes to the pentose phosphate pathway in this infection since no

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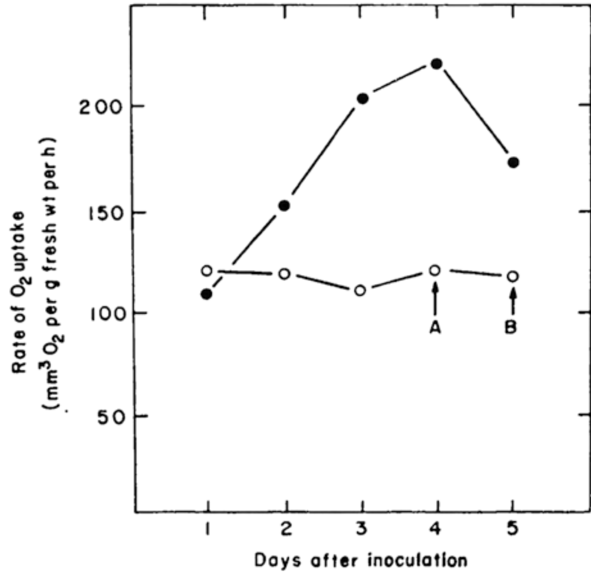

Fig. 11.1 Rates of O_2 uptake of infected and uninfected cotyledons at various times after inoculation: (●-●), infected; (○-○), uninfected; A, visible signs of sporulation (Thornton and Cooke 1974)

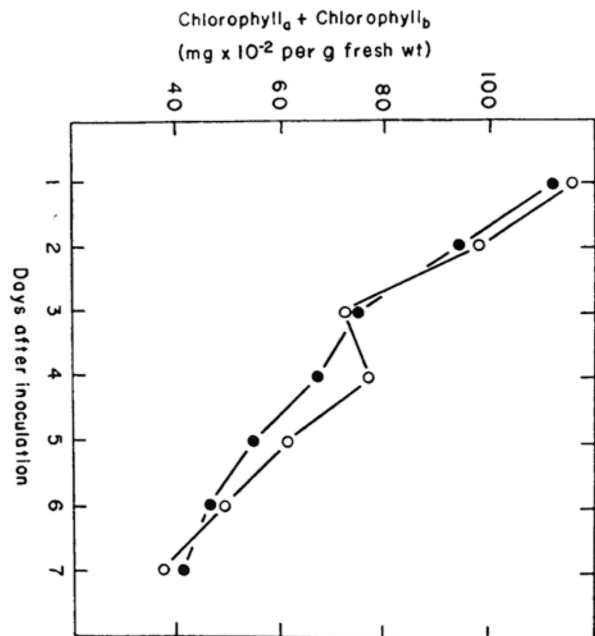

Fig. 11.2 Chlorophyll$_a$ plus chlorophyll$_b$, content of infected, and uninfected cotyledons at various times after inoculation: (●-●), infected; (○-○), uninfected (Thornton and Cooke 1974)

acyclic polyhydric alcohols were detected in soluble extracts of either infected leaves or fungal conidia (Thornton and Cooke 1974) (Figs. 11.3 and 11.4).

Pathogenesis in *Brassica*-*Hyaloperonospora* combinations was observed to be accompanied by large increases in electrolyte leakage (Fig. 11.5, 11.6, 11.7, and

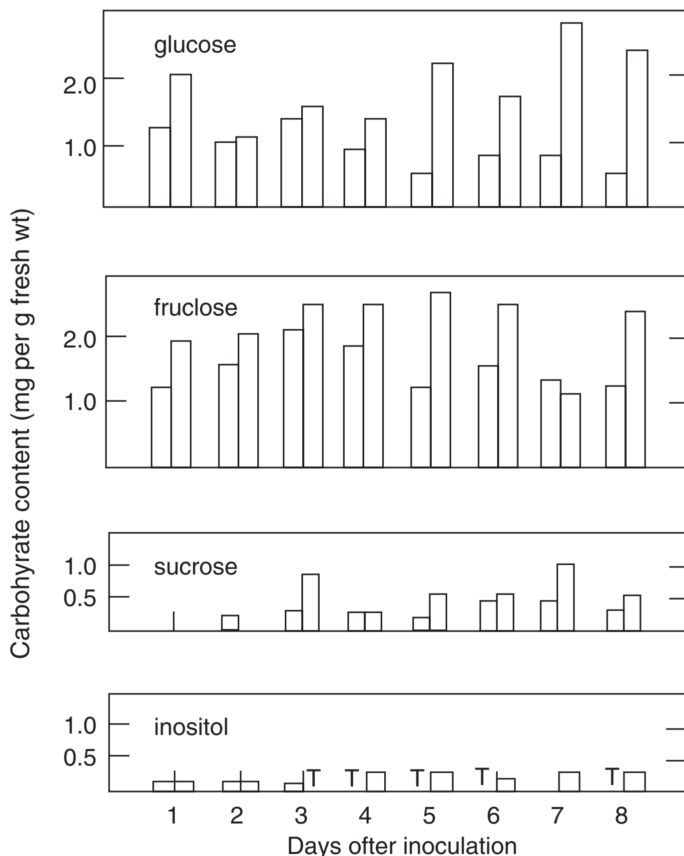

Fig. 11.3 Carbohydrate content of the alcohol-soluble fraction of infected and uninfected cotyledons at various times after inoculation with *Hyaloperonospora parasitica*: ■ = infected; □ = uninfected; *T* trace (indicating that the peak height of the TMS derivative was indeterminable at an attenuation of 20×10^3 (Thornton and Cooke 1974)

11.8) and increased activity of glucosidase (Fig. 11.6), ribonuclease (Fig. 11.7), and peroxidase (Fig. 11.8) (Kluczewski and Lucas 1982). The large increase in glucosidase was of pathogen origin, while enhanced ribonuclease activity was due to a new post-inflectional form of the enzyme. In vivo infected leaves of *B. juncea* produced cellulase, endo-PMG, and endo-PG (Singh et al. 1980).

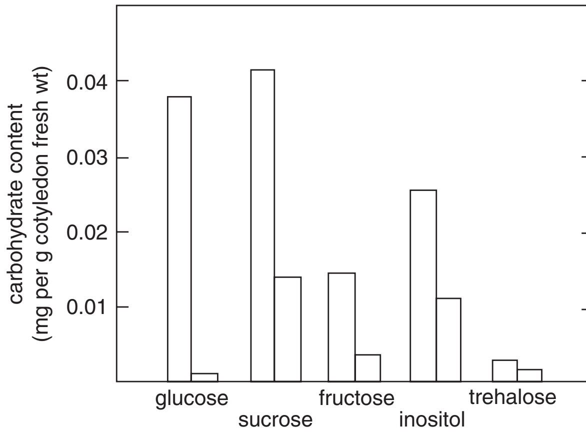

Fig. 11.4 Principal carbohydrates of the alcohol-soluble fraction of sporangia from infected cotyledons and control washings, 7 days after inoculation with *Hyaloperonospora parasitica.* ■ = infected; □ = uninfected (Thornton and Cooke 1974)

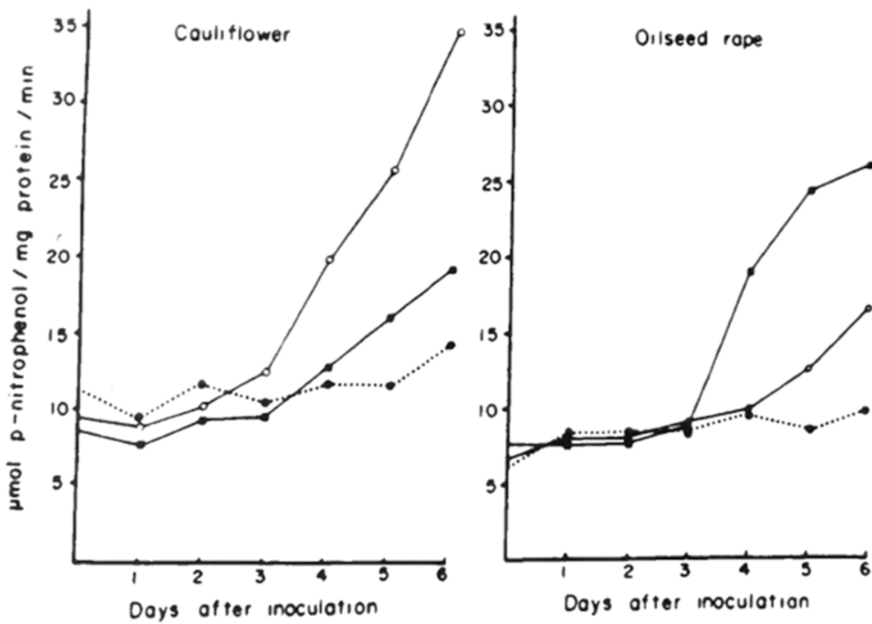

Fig. 11.5 Conductivity changes of de-ionized glass-distilled water containing samples of uninfected cotyledons (...) and cotyledons infected (−) by *Hyaloperonospora parasitica* isolate from cauliflower (○) and oilseed rape (–) (Kluczewski and Lucas 1982). Each point represents the mean of four replicates

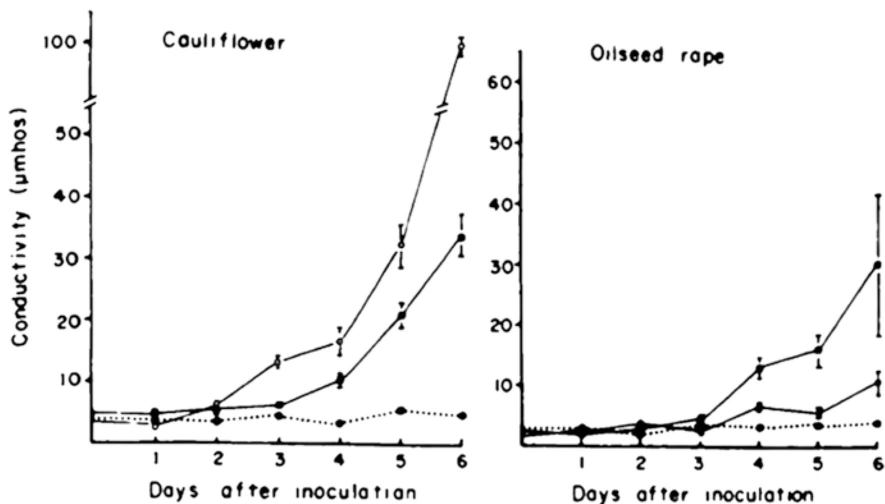

Fig. 11.6 β-glucosidase activity in extracts of control cotyledons (...) and cotyledons infected (−) by either cauliflower (◯) or oilseed rape (−) isolate of *Hyaloperonospora parasitica* (Kluczewski and Lucas 1982)

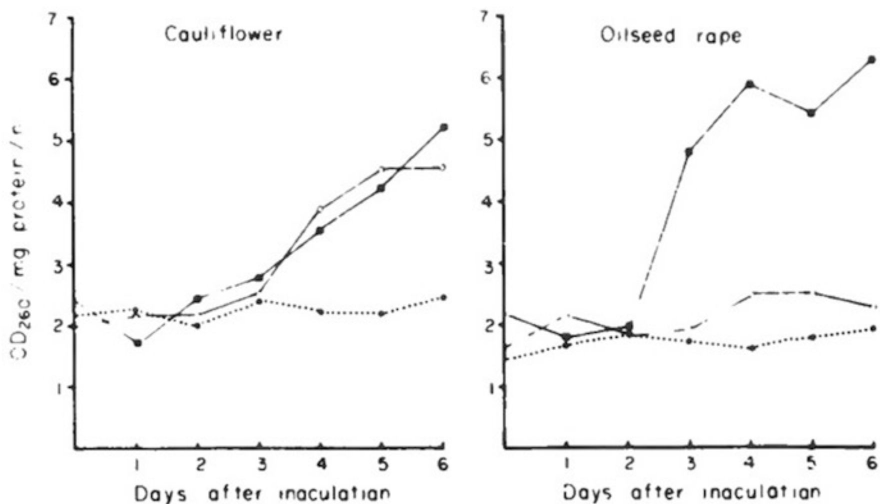

Fig. 11.7 Acid ribonuclease activity in extracts of control cotyledons (...) and cotyledons infected (−) by *Hyaloperonospora parasitica* isolate from cauliflower (◯) and oilseed rape (−) (Kluczewski and Lucas 1982)

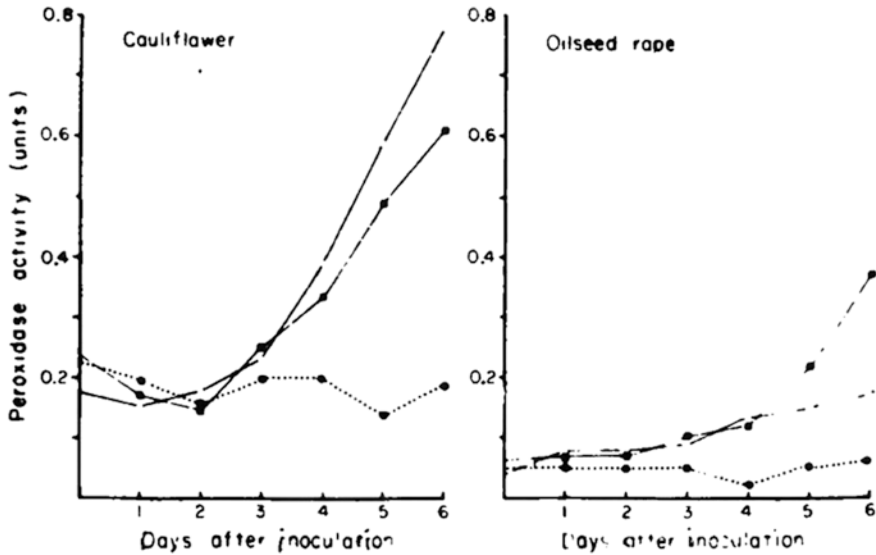

Fig. 11.8 Peroxidase activity in extracts of control cotyledons (...) and cotyledons infected by either cauliflower (○) or oilseed rape (−) isolate of *Hyaloperonospora parasitica* (−) (Kluczewski and Lucas 1982)

11.3 Role of Natural Biochemical Compounds

There are a number of natural biochemical compounds present in host tissues which may influence the defence mechanism of crucifers against downy mildew infection. The role of phenolic compounds, glucosinolates, phytoalexins, and flavour volatile compounds in providing resistance to crucifers against downy mildew infection has been explained in Sect. 12.6, 'Biochemical Basis of Resistance', of Chap. 12, 'Host Resistance'.

11.4 Biochemistry of Disease Resistance

Plants can employ chemical defence either as preformed antimicrobial substances (phytoanticipins) or as induced antimicrobial substances which accumulate after contact with the pathogen (phytoalexins) (Van Etten et al. 1994). In *Arabidopsis*, as in other *Brassica*, glucosinolates come into question as potential phytoanticipins. However, phytoanticipins are generally correlated with non-host resistance (Mansfield 2000), and there is no evidence that glucosinolates play a role in the interaction of *Arabidopsis* with *H. parasitica*. The accumulation of defence gene transcripts such as *PAL* and *CHS* and the increase in peroxidase activity in cell suspension cultures treated with elicitor were reported by Davis and Ausubel (1989)

and indicated that stimulation of phenylpropanoid metabolism in *Arabidopsis* could be expected to be associated with resistance, as observed in several other plant species (Dixon and Paiva 1995). In the *Arabidopsis*-*H. parasitica* interaction, an early oxidative burst of H_2O_2 production is observed on penetration of the epidermis by an avirulent race of the pathogen. Following the oxidative burst is the genetically programmed hypersensitive cell death response (HR). In an HR against an avirulent *Pseudomonad*, a shift from housekeeping to defence metabolism which affected an estimated 10% of the transcriptome was demonstrated (Scheideler et al. 2002), and this is presumably similar in the HR against *H. parasitica*. Since *H. parasitica* is an obligate biotroph, the rapid hypersensitive response (HR) which occurs in the epidermal cells adjacent to the penetration hyphae, and sometimes an additional few cells in the mesophyll (Koch and Slusarenko 1990), would be expected to be sufficient to effectively condition resistance by preventing the establishment of the highly co-evolved nutritional relationship between host cell and pathogen, which depends on haustoria. Nevertheless, the HR to *H. parasitica* is associated with the accumulation of at least one antimicrobial phytoalexin, camalexin (Slusarenko and Mauch-Mani 1991; Tsuji et al. 1992), and it was reported that the phytoalexin-deficient mutants (*pad1*-1, *pad2*-1, and *pad3*-1 and the double mutants *pad1*-1/*pad2*-1, *pad1*-1/*pad3*-1, and *pad2*-1/*pad3*-1) showed increased susceptibility in incompatible combinations with five races of *H. parasitica*, namely, EMOY, EMWA, CALA, HIKS, and HIND (Glazebrook et al. 1997).

Camalexin, the only *Arabidopsis* phytoalexin so far described, is an indole thiazole derivative, as are other phytoalexins so far reported from the Brassicaceae. Thus, camalexin is synthesized from tryptophan, and not from phenylalanine, which is a precursor for phenolics in plants. This metabolic distinction was made use to assess the relative contributions of phenolics and phenolic polymers to the resistance of *Arabidopsis* to *H. parasitica*, separately from phytoalexins (Mauch-Mani and Slusarenko 1996). Thus, in *Arabidopsis,* it is possible to inhibit phenolic metabolism without directly interfering with phytoalexin synthesis (Fig. 11.9). Using specific inhibitors of *PAL* (2-aminoindan-2-phosphonic acid, AIP) and cinnamyl alcohol dehydrogenase (2-hydroxyphenyl-aminosulphinyl acetic acid 1, 1-dimethyl ester, OH-PAS) to inhibit phenolic metabolism in general or lignifications in particular, it was shown that loss of lignification caused a mild shift towards susceptibility but that a more general inhibition of the phenolic metabolism resulted in complete susceptibility (Mauch-Mani and Slusarenko 1996). Feeding salicylic acid (SA) back into the system restored resistance in the presence of AIP and was interpreted as showing the dependence of resistance expression on SA. These results conform with the observations of Delaney et al. (1994) for transgenic *Arabidopsis* carrying the bacterial *nahG* gene and are therefore unable to accumulate SA. However, despite the overwhelming effect of SA, the contribution of lignification to resistance against *H. parasitica* was shown, and this highlights the multifactorial nature of the resistant response. It has been suggested that the SA that is required for *PR1* gene expression and SAR might be predominantly synthesized via isochorismate, whereas the SA which modulates cell death in the HR might arise predominantly from phenylalanine (Fig. 11.9) (Wildermuth et al. 2001).

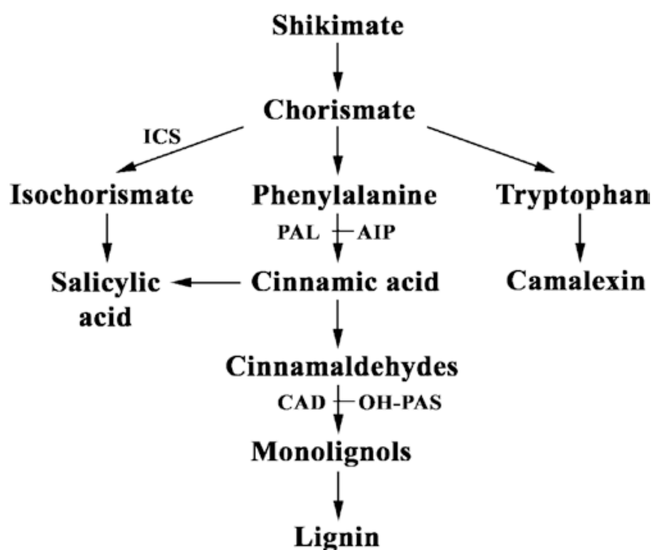

Fig. 11.9 Simplified scheme of the biosynthesis of the defence-related compounds camalexin, salicylic acid, and lignin in *Arabidopsis*. Chorismate is the first branch point, since camalexin arises via tryptophan, while salicylic acid is synthesized via isochorismate and phenylalanine, and lignins arise via phenylalanine. Chorismate is converted into isochorismate by isochorismate synthase (*ICS*). Phenylalanine ammonia-lyase (*PAL*) converts phenylalanine into cinnamic acid and is specifically inhibited by aminoindan phosphonic acid (*AIP*). From cinnamic acid, the pathway branch to produce salicylic acid or, via cinnamaldehydes and monolignols, lignin. The conversion from cinnamaldehydes to monolignols by cinnamyl alcohol dehydrogenase (*CAD*) is inhibited by hydroxyphenyl-aminosulphinyl acetic acid dimethyl ester (OH-PAS) (Slusarenko and Schlaich 2003)

The idea that plants might lignify not only their own walls to strengthen them as barriers to pathogen spread (Hijwegen 1963), but that the pathogens themselves might be inactivated by polymerization of monilignols to lignin in their walls as part of the peroxidase catalysed intercellular free radical condensation reaction, was proposed by Hammerschmidt and Kuc (1982) and Ride (1983). However, Slusarenko and Schlaich (2003) have found evidence for the active lignification of hyphae in intercellular spaces (Mauch-Mani and Slusarenko 1996). *Arabidopsis* host-pathosystem is not an easy subject for biochemical study, and comparative studies are complicated by leaf age- and ecotype-specific differences in basal enzyme activity levels (Mauch-Mani et al. 1993). After the inoculation of plants with virulent or avirulent isolates of *H. parasitica*, Slusarenko and Schlaich (2003) did not observe a significant pattern of change in the activities of superoxide dismutase, catalase, ascorbate peroxidase, lipolytic acyl-hydrolase, lipoxygenase, or linolenic acid 13-hydroperoxide decomposing activity. All these enzymes have been reported to be important in one or more pathosystems. It is possible that in *Arabidopsis*, these enzymes have no role in resistance against *H. parasitica* or, because only relatively few cells show a HR, any changes are diluted out in comparison with the bulk of

non-stimulated cells in the leaf. In contrast, transcripts for lipoxygenase were reported to be induced by treatment with *Pseudomonas* (Melan et al. 1993), which leads to a quantitatively greater leaf response than in the *H. parasitica* pathosystem where Slusarenko and Schlaich (2003) found no increased transcript levels using the same probes (Mauch-Mani et al. 1993; Slusarenko 1996). Thus, SA seems to be important for defence against most *H. parasitica* races, presumably because of its role as a signal amplifier, and it seems that lignification and camalexin might be needed for full resistance, since some resistance is lost when lignifications is blocked or in *pad4* or *pad1* + 2, 1 + 3, or 2 + 3 mutants.

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