Chapter A14

Plant Metabolism Associated with Resistance and Susceptibility

Michael G. Handford[1] and John P. Carr[2]
1. Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Las Palmeras 3425, Ñuñoa, Casilla 653, Santiago, Chile. 2. Department of Plant Sciences, University of Cambridge, Downing St., Cambridge CB2 3EA, United Kingdom. Email: jpc1005@hermes.cam.ac.uk

Introduction

Viruses are subcellular parasites that replicate within a host cell with no intervening membrane to insulate host and viral gene products from each other (Hull, 2002). The highly intimate nature of this relationship suggests that the biochemical and physiological processes occurring in the various host cell types through which a virus must propagate will significantly affect the outcome of the infection. In plants, drastic alterations in, and re-direction of, host metabolism have been observed in many studies of both incompatible and compatible host-virus interactions. However, is it safe to suggest that these changes in plant metabolism influence whether a plant is resistant or susceptible to the virus infection? The answer to this question is important for a number of reasons. Firstly, it will lead to a better general understanding of the plant-virus interaction. Secondly, it may reveal mechanisms underlying induced resistance phenomena. Finally, it may allow us to identify targets for novel, artificial methods of inducing resistance to plant viruses.

In this review we will examine how certain aspects of plant photosynthetic and respiratory metabolism are altered by infection by viruses while others may play role(s) in counteracting it.

G. Loebenstein and J. P. Carr (eds.), Natural Resistance Mechanisms of Plants to Viruses, 315-340.

Virus-induced changes in carbon metabolism in susceptible plants

The effects of virus infection on the metabolism of a susceptible plant are frequently profound, influencing multiple pathways such as respiration, carbohydrate partitioning and photosynthesis in both directly- inoculated and systemically -infected tissue (Hull, 2002). Some of these effects are due to virus-induced changes in gene expression controlled at the transcriptional level (Wang and Maule, 1995). Others may be influenced post-transcriptionally through perturbation of levels of microRNAs (miRNAs) by certain viral gene products such as the potyviral HC-Pro (reviewed by Bartel, 2004). Other virus-induced effects on metabolism can occur through a more direct physical perturbation of subcellular structures, for example the disruption of photosynthetic activity by the interaction of viral gene products with components of the photosynthetic apparatus (see **Photosynthesis** below), or the deregulation of carbohydrate partitioning by viral movement proteins (Herbers et al. 1996a;b). Metabolic disturbances are also observed in plants undergoing an incompatible (resistance) reaction to virus infection and some of these are discussed in the section 2 of this review as well as in the accompanying chapter by Loebenstein and Akad.

Virus-infected plants do not respond uniformly because not all of the host cells become infected and those that are may not have become infected at the same time. Even with the most concentrated of virus inocula, only about 0.1% of the cells in directly inoculated leaves actually become infected (Matthews, 1991). Therefore, the results of the many studies that have examined the physiological responses of a mixed population of virus-infected and non-infected cells, which for example, occurs in systemically, infected tissue, need to be interpreted cautiously. Thus, whenever possible, virus-induced metabolic alterations are best studied in the earliest phases of infection of directly inoculated tissues since these show the greatest degree of synchrony with respect to the changes caused by the infection. However, even in directly inoculated tissue virus-induced metabolic changes in plants are not uniform (Doke and Hirai, 1970; Técsi et al. 1994a, 1994b, 1996; Wang and Maule, 1995; also discussed in Hull, 2002).

Starch. Starch is the major carbohydrate store in most plants and, compared to most other plant metabolites, it is relatively easy to detect and assay using iodine staining. Thus, starch accumulation has been, historically, one of the most commonly used indicators for virus-induced alterations in plant metabolism during both compatible and incompatible interactions. Abnormal accumulation or disappearance of starch is diagnostic for net alterations in the balance between those processes

responsible for creation and utilization of carbohydrate namely, photosynthesis and respiration.

It has been known for many decades that changes in the accumulation of starch frequently precede the appearance of virus symptoms (Bolas and Bewley, 1930; Holmes, 1931). For example, Holmes (1931) noted discrete regions of the tobacco leaf retained starch at the end of the night after inoculation with *Tobacco mosaic virus* (TMV), whilst the rest of the leaf was starch-less. At four days post-inoculation, the starch-rich region had expanded into a ring, surrounding a zone of starch-less cells termed a starch ringspot. Holmes (1931) also found that lesions contained less starch than the surrounding, uninfected tissue if staining was carried out at the end of the day. This important elementary work demonstrated that early in the infection process, even before symptoms are discernible, virus infection alters both the starch production during the day, and its degradation and/or mobilization at night. Starch ringspots have also been seen in other plant-virus systems (eg Técsi et al. 1994a; Roberts and Wood, 1982) including *Arabidopsis thaliana* leaves (Fig.1). Subsequently, chloroplasts containing enlarged starch grains were observed using TEM (e.g. Zechmann et al. 2003). For example, two zones containing chloroplasts with altered structure were discernible in tobacco leaves inoculated with *Cucumber mosaic virus* (CMV) (Cohen and Loebenstein, 1975). In cells at the center of the lesion, CMV particles were visible and all chloroplasts contained enlarged starch grains. In contrast, cells at the outer edge of the lesion contained fewer virus particles and only about half the cells contained chloroplasts with large starch grains. The development of starch ringspots in inoculated tissue has been examined in greatest detail in marrow (Técsi et al. 1994a, 1994b, 1996, see below).

Changes in starch level are even more apparent once the virus starts to spread from the initial inoculation site. In point-inoculated leaves stained at the end of the night, the path taken by the virus through veins towards the midrib was seen as a zone of starch accumulation (Holmes, 1931). Conversely, regions of starch-less cells reveal the path of virus movement in leaves stained during the day (Bolas and Bewley, 1930; Samuel, 1934).

Partitioning of carbohydrate. Many studies carried out over the last 40 years, on a variety of host-virus systems, have shown that partitioning of newly fixed carbon between soluble sugars (sucrose, fructose and glucose) and organic and amino acids is perturbed (reviewed by Porter, 1959; Goodman et al. 1986). For example, decreased soluble sugar content in infected tissue has been seen in Chinese cabbage infected with *Turnip yellow mosaic virus* (TYMV; Bedbrook and Matthews, 1973) and *Squash mosaic*

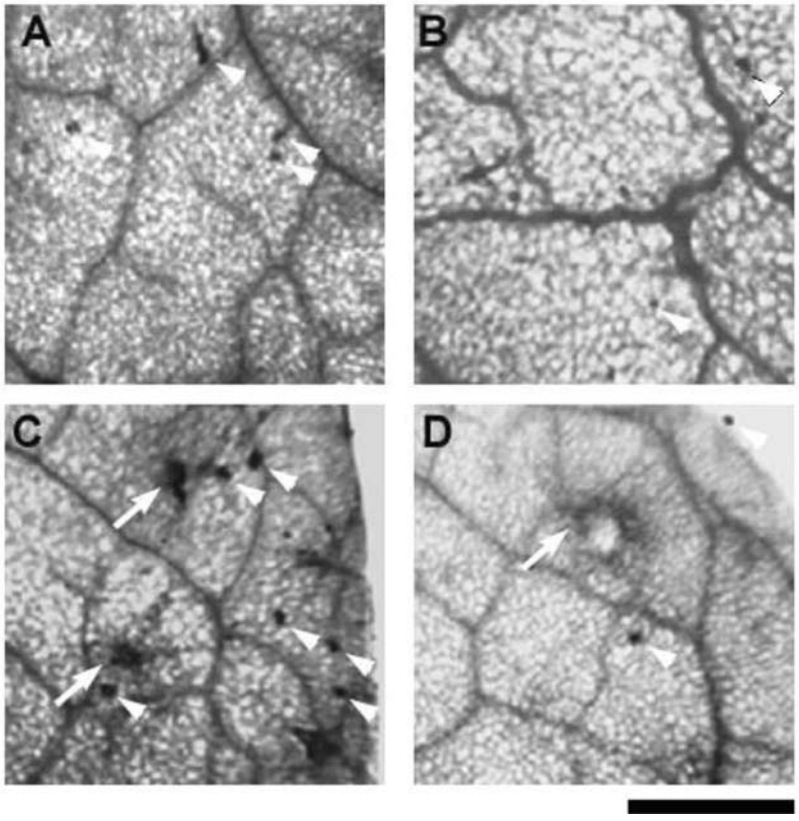

Fig. 1. Virus-induced starch lesions in TMV UI-inoculated *Arabidopsis* leaves *Arabidopsis thaliana* (Col-0) plants (17 days post-seeding) were dusted with carborundum and mock- (panels A and B) or TMV U1-inoculated (10 µg/ml: panels C and D). Two (panels A and C) and three (B and D) days post-inoculation (dpi), the inoculated leaves were removed two hours into the light period, decolorized in boiling ethanol, stained for starch in I_2/KI solution and imaged. Starch lesions (arrows) and carborundum particles (arrowheads) are marked. At 1 dpi, both mock- and virus-inoculated leaves had no zones of altered starch content (data not shown). Discrete regions of elevated starch became visible 2 dpi for leaves inoculated with TMV, expanding into a ring surrounding a region of starch-less cells by 3 dpi. Size bar: 500 µm.

virus (SqMV)-infected squash (Magyarosy et al. 1973). In contrast, sucrose levels in *Zucchini yellow mosaic virus* (ZYMV)-infected marrow plants increased relative to levels in healthy controls (Blua et al. 1994). Organic acid levels rose in CMV-infected tobacco (Porter and Weinstein, 1957) while amino acid content rose in ZYMV-infected marrow (Blua et al. 1994) and *Tomato spotted wilt virus* (TSWV)-infected tomato (Selman et al. 1961). Increases in both fractions were found in TYMV-infected Chinese cabbage (Bedbrook and Matthews, 1973), SqMV-infected squash (Magyarosy et al. 1973) and CMV-infected cowpea (Welkie et al. 1967).

Due to the limitations of biochemical analytical methods available at the time most of these early studies focused on a small number of metabolic changes. One of us (Handford, 2000) carried out a 'metabolomic' study of how virus infection alters carbohydrate partitioning in the model plant *A. thaliana*. Plants (ecotype Col-0) were fed with $^{13}CO_2$ 17 days after inoculation with two strains of TMV known to cause mild or severe symptoms in tobacco (TMV strains U1 and YSI/1, respectively; Banerjee et al. 1995) and which accumulate to significant levels in Arabidopsis (Handford, 2000). The methanol-extractable components from these plants were analyzed by 2-dimensional ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectroscopy. As shown in Table 1, incorporation of ^{13}C-label into carbohydrates, amino acids and organic acids was 2-4 times higher in virus-inoculated plants compared to the mock-inoculated control, and was proportionally greater after inoculation with the U1 strain of TMV. Such changes could reflect a greater pool size of the metabolites in TMV-infected plants, similar to the findings of, for example, Blua et al. (1994) and Bedbrook and Matthews (1973). Alternatively, the rise could reflect a decrease in turnover of the metabolites, a phenomenon seen by Técsi et al. (1994b) where photosynthetically fixed ^{14}C was lost at a lower rate in CMV-infected marrow cotyledons compared to healthy controls. To conclude, in a wide variety of plant-virus interactions there is a shift in carbon partitioning away from soluble sugars towards organic and amino acids, although this is not necessarily the case in every system analyzed.

Respiration. In by far the majority of cases, the respiration rate (broadly defined as net O_2 uptake) is increased in virus-infected plants. Pennazio (1996) reviewed reports of respiration rates in virus-infected and healthy plants and documented a rise in 17 cases, a fall in one and no change in three compatible interactions. For example, the respiration rate of tomato tissue systemically infected with *Tomato yellow mosaic virus* (ToYMV) was 80-100% higher than in healthy plants (Leal and Lastra, 1984). Similarly, in barley leaves inoculated with *Barley yellow dwarf virus* (BYDV), respiration rate per gram of fresh tissue increased, whilst that of the healthy leaf decreased over the course of the experiment (Jensen, 1967).

Table 1. Distribution of ^{13}C in aerial tissues of mock- and TMV-inoculated Arabidopsis

Fraction	n	^{13}C per fraction as % of ^{13}C fixed by mock			P		
		Mock	U1	YSI/1	Mock v U1	Mock v YSI/1	U1 v YSI/1
Carbohydrates	10	100	363 ± 9	181 ± 8	0.000	0.000	0.000
Amino acids	8	100	242 ± 18	177 ± 19	0.001	0.005	0.009
Organic acids	6	100	470 ± 129	256 ± 53	0.035	0.032	0.041

Arabidopsis plants were mock-, TMV U1- or TMV YSI/1-inoculated and after 17 days were incubated in a $^{13}CO_2$-rich atmosphere for 24 h. Following incubation, shoots were excised, weighed and extracted twice in methanol. The methanol-soluble components were analysed by 2D ^{1}H- and ^{13}C-NMR, and peaks were identified by comparing to standards. The labelling intensity of compounds in each fraction from mock-inoculated plants was assigned as 100%. The labelling intensity of compounds in each fraction from TMV-inoculated samples is expressed relative to mock-inoculated plants of the same line and corrected for shoot biomass. Each value is the mean labelling intensity per fraction ± SEM (n). Values were compared by a paired two sample, two-tailed Student's t-test and the probability (P) of the two samples being from the same population is shown.

Photosynthesis. Many reports have suggested that photosynthetic rate, determined by $^{14}CO_2$ incorporation, is decreased by virus infection (reviewed by Diener, 1963; Zaitlin and Hull, 1987). For example, Leal and Lastra (1984), Jensen (1967) and Naidu et al. (1984) observed falls in photosynthetic rate and chlorophyll amount in ToYMV-infected tomato, BYDV-infected barley and peanut infected with *Peanut green mosaic virus* (genus Potyvirus), respectively. Changes in chloroplast ultrastructure are also associated with virus infection in systemically infected tissue. These chloroplasts typically have disrupted integrity of the grana and stromal lamellae as well as a reduced number of thylakoids (Hršel, 1962; Honda and Matsui, 1974; Ehara and Misawa, 1975; Izaguirre-Mayoral et al. 1990; Zechmann et al. 2003).

In plants infected with TMV, damage to chloroplast structure and function is induced by uptake of viral coat protein (CP) into chloroplasts *via* an apparently unique mechanism involving aggregated forms of the CP (Gunasinghe and Berger, 1991; Naderi and Berger, 1997; Banerjee and Zaitlin, 1992; Banerjee et al. 1995; Carr, 2004). In tobacco plants infected with TMV strains causing severe chlorotic symptoms (e.g. YSI/1, *flavum* or PV230) there is a greater proportion of CP inside the chloroplasts than in plants infected with strains causing milder symptoms, e.g. TMV-U1 and TMV-PV42 (Reinero and Beachy, 1986; Banerjee et al. 1995; Lehto et al. 2003). In addition, the ratio of CP associated with thylakoids to that associated with the stromal fraction is greater in infections by severe strains than for mild strains (Reinero and Beachy, 1989; Banerjee et al. 1995). TMV CP is not loosely attached to the thylakoid membrane surface but rather it is embedded within it (Reinero and Beachy, 1986).

TMV CP embedded in the thylakoids hinders the rate of photosynthetic electron transfer through photosystem (PS) II, but not PSI (Reinero and Beachy, 1989; Hodgson et al. 1989; Naidu et al. 1984; Seaton *et al.* 1996; Funayama et al. 1997). Moreover, the decrease in PSII activity seen in chloroplasts isolated from plants infected with a severe TMV strain, but not a mild strain, and correlates with the proportion of CP taken up into the thylakoids (Reinero and Beachy, 1989; Barón et al. 1995; Banerjee et al. 1995). But how could CP inhibit electron transfer through PSII? Recent results from Lehto et al. (2003) suggest that the CP promotes an increase in the breakdown of specific proteins within the PSII core complexes leading to increased light-induced free radical accumulation and consequent break-down of the chloroplasts' protective pigments. Eventually this results in damage to chloroplast structure and the visible symptom of chlorosis.

Early metabolic events in the inoculated tissue appear to be coordinated and highly organized

Doke and Hirai (1970) analyzed changes in host physiology in space and time around the virus entry site by autoradiography of mock- and TMV-inoculated tobacco leaves after a brief exposure to $^{14}CO_2$. At one day post-inoculation, small, heavily labeled areas of tissue were visible only in virus-inoculated samples. By two days post-inoculation the lesions had expanded, forming a ring of heavily labeled tissue surrounding a region without labeling. This heavily labeled ring enlarged further, and by about 5 days post-inoculation the labeled areas were diffuse. Doke and Hirai (1970) interpreted these discrete, heavily labeled regions as infected tissue with an elevated photosynthesis rate. The idea that virus-induced changes in metabolism are highly localized and coordinated was later explored using

CMV-inoculated marrow cotyledons by Maule, Leegood, Técsi and colleagues. In a series of important papers these workers used a variety of immunohistochemical and cytological techniques, to explore the highly dynamic nature of the viral infection site (Técsi et al. 1994a, 1994b, 1995, 1996).

Localized changes in starch levels and photosynthetic rate in CMV-inoculated marrow cotyledons. At two days post-inoculation, iodine staining of CMV-inoculated cotyledons revealed localized accumulation of starch. By four days post-inoculation, the infection zone had expanded producing a ring of starch-rich cells, surrounding a zone of starch-less cells with a small group of starch-rich cells at the centre. By 6 days post-inoculation, the lesion was diffuse, due to adjacent infection sites coalescing. Analysis of serially sectioned lesions alongside iodine staining and immunocytochemistry revealed that at four days post-inoculation, the starch ring lay just inside the expanding infection front. Incorporation of $^{14}CO_2$ demonstrated that maximal photosynthesis roughly coincided with the starch-rich cells in the central dot and peripheral ring, and that in the zone of starchless cells, $^{14}CO_2$ fixation was below levels in the surrounding uninfected cotyledon (Técsi et al. 1994a). These findings parallel those of Doke and Hirai (1970) and it was concluded that the outer starch ring was a consequence of raised photosynthetic rates induced in some fashion by CMV replication.

Chlorophyll *a* fluorescence quenching measurements for photosynthetic activity were consistent with these conclusions. These studies showed that the rate of fluorescence quenching was elevated in a ring-dot pattern like that observed for starch accumulation. However, superimposition of the starch and fluorescence quenching data revealed that the cells affected were not coincident. There was roughly a 24-h time delay with cells showing enhanced chlorophyll *a* fluorescence quenching preceding those with enhanced starch content (Técsi et al. 1994a).

Local changes in other biochemical processes in CMV-inoculated marrow cotyledons. Subsequently, Técsi et al. (1996) examined the metabolic processes responsible for the fluctuations in starch, photosynthetic $^{14}CO_2$ fixation and chlorophyll *a* quenching. Their hypothesis was that in infected cells, carbohydrate reserves might be diverted into the synthesis of CMV RNA and proteins. Using *in situ* assays, activities of specific enzymes involved in carbohydrate metabolism and CMV replication were analyzed across the viral lesion. Enzyme activities were also correlated with starch accumulation.

Experiments were carried out at three days post-inoculation, when the starch-rich ring and starchless central zone had developed but before the appearance of the central starch-rich dot. Although all cells in the lesion contained virions, replication only occurred at the leading edge of the expanding infection front, a phenomenon also seen in pea cotyledons point-inoculated with a potyvirus *Pea seed-borne mosaic vir*us (PSbMV: Wang and Maule, 1995; Aranda et al. 1996). Behind the virus replication zone was the band of cells with raised photosynthetic capacity. Protein synthesis in general, monitored by ^{35}S-methionine incorporation, and viral CP synthesis in particular, was raised in this zone. In the region of starchless cells, several metabolic changes were noted. Firstly, the activities of enzymes of the oxidative pentose phosphate pathway, and of glycolysis were raised. Secondly, no reductive pentose phosphate pathway (Calvin cycle) activity was detected in regions coinciding with the decreased rates of photosynthetic $^{14}CO_2$ fixation and lower chlorophyll *a* fluorescence. Finally, total starch hydrolase activity was raised. It was concluded that these complex changes in photosynthetic capacity and starch catabolic activities across a viral lesion could account for the development of starch rings (Técsi et al. 1996).

Localized changes in metabolism are probably controlled through changes in host gene expression. The precise cause(s) of these biochemical alterations in virus-infected tissues is unclear. However, virus infection does alter host gene expression in a complex fashion in and around the infected cells. This is best exemplified by work on the PSbMV-pea embryo interaction (Wang and Maule, 1995; Aranda et al. 1996).

In situ hybridization for minus-strand viral RNA showed that PSbMV replication in the pea seed was limited to a narrow band of cells, as in the CMV-marrow cotyledon system (Técsi et al. 1996). Meanwhile, *in situ* hybridization for transcripts of nine pea genes, revealed a severe reduction in the level of host gene expression for all genes studied only at the replication front (Wang and Maule, 1995). Previous to this study, virus-induced host gene shut-off had not been seen in plants only studied in detail in animal cell cultures (Aranda and Maule, 1998). Behind the replication front, host transcripts for many 'housekeeping' proteins were found to be elevated over the levels seen in uninfected tissue (Wang and Maule, 1995; Aranda et al. 1996). Similar effects were seen in other tissues infected with other viruses and in addition it was shown that levels of certain transcripts, notably those for heat shock protein 70-type proteins are elevated in cells supporting active virus replication (Aranda et al. 1999; Escaler et al. 2000). At the infection front, a concomitant reduction in the levels of product of eight of the genes was seen, although no effect was seen on the level of expression of the ADP glucose pyrophosphorylase protein, implying that virus infection may

perturb control of transcription and translation differentially between genes (Wang and Maule, 1995). Recent findings on the effect(s) of the potyviral HC-Pro protein and other viral counter-silencing factors on miRNA levels support the concept that some of these effects may be mediated at post-transcriptional levels (see Bartel, 2004).

However, the approaches taken by these workers could not allow any determination to be made of how essential these perturbations in host gene expression and consequent metabolic changes are in the establishment of infection.

Metabolic changes in the inoculated tissues: how important are they? In this and other systems, at least during the early stages of infection, the virus is probably not a general sink for host metabolites. For example, CMV accounts for about 1% of total protein in infected marrow tissues, insufficient by itself to explain the large-scale redirection of metabolism (Técsi et al. 1994b). However, at the very local level, that is, in the narrow band of cells in which the virus is replicating, the virus may be a very strong sink. This demand could be met by the localized changes observed in biosynthetic and respiratory pathways. It seems likely that the temporary carbon source for the enhanced biosynthetic pathways is the elevated level of starch found in the starch-ring as a consequence of enhanced photosynthesis. This starch is made from carbon fixed *in situ* rather than being manufactured from photosynthates imported from surrounding cells, because direct illumination of these cells was needed for starch levels to be raised (Técsi et al. 1994a).

However, it is questionable if localized starch build up is a prerequisite *per se* to support the demand for carbon-containing compounds. The rate of CMV accumulation was the same in marrow plants kept in a diurnal light regime or if kept covered for two days after inoculation (Técsi et al. 1994a). This was also true if black grids were placed on the inoculated leaf for two days. Virus accumulation was identical under the black bars and in the open spaces, yet starch only accumulated in those regions exposed to the light (Técsi et al. 1994a). Additionally, experiments with mutant lines of *A. thaliana* with specific mutations in starch metabolism showed that there is no simple relationship between the starch build-up in the inoculated tissue and susceptibility to virus infection (Handford, 2000). For example, the TC75 mutant is unable to accumulate starch (Caspar et al. 1985). In TC75 plants the development of starch rings in response to virus infection does not occur, yet several viruses, including CMV, the *Cauliflower mosaic virus* (CaMV), a tobamovirus, *Turnip vein clearing virus* (TVCV), and TMV U1 all accumulate to the same levels as in wild-type plants (Handford, 2000).

Thus, we are still left with an important question. Which of the complex changes in host metabolism seen in directly infected tissue are truly necessary to ensure a positive outcome for the virus, i.e. the successful establishment of viral infection within the inoculated tissue followed by its propagation throughout the host?

Salicylic acid-induced resistance to viruses: A possible role for alternative respiration in defensive signaling.

Potential relationships between salicylic acid-induced resistance, carbohydrate metabolism, and respiration. Salicylic acid (SA) is an important signal involved in the establishment of induced resistance (systemic acquired resistance: SAR) against viruses and other types of pathogen, as well as playing less well-understood roles in development and responses to abiotic stresses (Loebenstein and Akad, this volume; Martinez et al. 2004). In most, but not all, plants it is present at a low basal level and accumulates dramatically following an appropriate biotic or abiotic stimuli, such as the hypersensitive response (HR) or exposure to UV, respectively (Malamy et al. 1990; Métraux et al. 1990; Surplus et al. 1998). SA is a product of secondary metabolism and is made either from phenylalanine via cinnamic and benzoic acids, or from isochorismic acid in the plastid, the latter pathway likely to be the more important for establishment of SAR (Wildermuth et al. 2002; reviewed by Métraux, 2002).

The synthesis of SA and consequent stimulation of defense-related gene expression can be stimulated by perturbations in carbohydrate metabolism. This was shown by Herbers et al. (1996a) who disrupted the partitioning of photosynthates by constitutive expression of invertase in the apoplast or vacuole in transgenic tobacco plants. This engendered a lesion mimic phenotype in the transgenic plants, together with higher than normal basal SA levels, induction of pathogenesis-related (PR) proteins (see Loebenstein and Akad, this volume), and a decrease in susceptibility to *Potato virus Y*. Interestingly, transgenic plants expressing invertase in the cytosol did not exhibit these effects, leading to the suggestion that hexose-sensing signaling mechanisms associated with the secretory apparatus are able to cross-talk with the defensive signaling pathway (Herbers et al. 1996a). This data and other work from the same group on sugar-induced PR protein synthesis (Herbers et al. 1996b) provides further support for the idea that carbohydrate metabolism affects the resistance/susceptibility status of plants.

Our group became interested in the possibility of a relationship between respiratory metabolism and SAR because of the known connection between SA and a respiratory enzyme, the alternative oxidase (AOX).

The alternative respiratory pathway. The mitochondria of plant cells can utilize two respiratory electron transport chains. One of these, the cytochrome pathway, is also present in animal cell mitochondria, while the other, which is called the alternative or cyanide-insensitive respiratory pathway, is not. The alternative respiratory pathway branches from the conventional pathway at ubiquinol/ubiquinone (UQ). The alternative respiratory pathway consists of only one enzyme, AOX, which 'siphons' electrons out of UQ pool, reducing oxygen to water (see Murphy et al. 1999). Since this reaction is not coupled chemiosmotically to ATP synthesis it generates heat (Laties, 1982; Siedow and Moore, 1993; Affourtit et al. 2002). Indeed, for many years the only firmly established role for AOX was in thermogenesis in a specialized floral structure, the spadix, found in the inflorescences of plants belonging to the Araceae, such as the voodoo lily, *Sauromatum guttatum* (Raskin et al. 1987, 1989; Meeuse and Raskin, 1988), and the smaller 'cuckoo pints' or *Arum* lilies (ap Rees et al. 1976).

However, in most plants the major physiological role for AOX lies in the maintenance of mitochondrial homeostasis by maintaining a steady flow of reducing power into the respiratory chain (and indirectly regulate processes supplying the reducing power, such as the Krebs' cycle), and preventing reactive oxygen species (ROS) generation by components of the respiratory chain (Affourtit et al. 2001, 2002; Maxwell et al. 1999; Moore et al. 2002; Sakano, 2001; Yip and Vanlerberghe, 2001). AOX is encoded by a small family of nuclear genes, some of which are inducible. The AOX polypeptide (c. 35 kDa) is synthesised in the cytoplasm and translocated into the mitochondrion (Vanlerberghe and McIntosh, 1997). Once it reaches the inner mitochondrial membrane, AOX can form non-covalently bound enzymatically-active homodimers that can inter-convert to less active covalently linked homodimers held together by a disulfide bridge (Umbach and Siedow, 1993; Affourtit et al. 2001, 2002).

In thermogenic plants such as *S. guttatum* SA is the natural trigger for AOX-mediated thermogenesis (Raskin et al. 1987), and in both thermogenic and non-thermogenic plants SA stimulates AOX activity and *Aox* gene expression (Rhoads and McIntosh, 1992). Further underlining the potential connection between AOX and SA-mediated defensive signalling, it was found that a synthetic SAR-inducing chemical, 2,6-dichloroisonicotinic acid (INA), triggers thermogenesis in *Arum italicum*, and *Aox* gene expression in non-thermogenic plants (Chivasa and Carr, 1998; Chivasa et al. 1999).

Correlative evidence has also suggested an involvement of AOX in the HR. Thus, several groups have observed that *Aox* gene expression and AOX protein accumulation are elevated in plant tissue expressing the HR, further suggesting an association between AOX and pathogen resistance (Lennon et al. 1997; Chivasa and Carr, 1998; Lacomme and Roby, 1999; Simons et al. 1999). In a *Nicotiana sylvestris* cytoplasmic male sterility mutant with a defect in electron transport complex I there is an elevation in the basal level of AOX. Interestingly, when these plants were crossed with *NN* genotype tobacco, the progeny exhibited fewer lesions, suggesting they had an enhanced ability to localize TMV (Dutilleul et al. 2003).

Pharmacological and genetic modifications of the alternative respiratory pathway affect virus infection.

Pharmacological studies. We decided to test the apparent correlations between AOX SA and resistance. Initial experiments took a pharmacological approach, utilizing chemicals known to induce AOX activity, such as the cytochrome pathway inhibitors cyanide and antimycin A (AA), and an inhibitor of AOX, salicylhydroxamic acid (SHAM). Early on in this work it became apparent that there seemed to be no relationship between AOX and SA-induced resistance to bacterial and fungal pathogens (Chivasa et al. 1997; Simons et al. 1999). However, there did appear to be a relationship with induced resistance to viruses (Chivasa et al. 1997; Chivasa and Carr, 1998; Wong et al. 2002).

In tobacco, SA-induced resistance to the accumulation of TMV and PVX in inoculated tissue and to the systemic movement of CMV was inhibited by salicylhydroxyamic acid (SHAM) (Chivasa et al. 1997; Naylor et al. 1998). However, SHAM did not prevent SA-induced synthesis of PR proteins or prevent SA-induced resistance to fungal or bacterial pathogens (Chivasa et al. 1997). In later experiments, non-lethal concentrations of AA or cyanide were found to induce resistance to TMV in susceptible tobacco without inducing *PR1* gene expression (Chivasa and Carr, 1998). In *A. thaliana* these chemicals also induced resistance to TVCV and the DNA virus CaMV without any concomitant activation of PR gene expression (Wong, 2001; Wong et al. 2002). Based on this evidence, a model was proposed in which the signal transduction pathways involved in virus resistance separate downstream of SA: one branch (activated by SA or by AA or cyanide) leads to resistance to viruses, the other (activated by SA but not AA or cyanide) to the induction of PR proteins and to bacterial and fungal resistance (Murphy et al. 1999, 2001).

Work by our group, and that of Klessig and co-workers, using *Arabidopsis npr1* mutants provided support for this model. The product of the wild-type *NPR1* gene regulates induction of several PR proteins, is an important regulator of induced resistance to bacteria and fungi, and is considered to be an element of central importance in the establishment of SAR and certain other forms of induced resistance (see Durrant and Dong, 2004). It may also be important in restriction of TMV spread during the *N* gene mediated HR in Nicotiana species (see accompanying chapter by Dinesh-Kumar and colleagues). However, Kachroo et al. (2000) showed that *HRT* gene-mediated resistance to the *Turnip crinkle virus* (TCV), which is SA-dependent, could still occur in *npr1* mutants. Similarly, Wong et al. (2002) found that *npr1* mutant plants were uncompromised in their ability to express SA- or AA-mediated resistance to TVCV. Thus, SA-induced resistance to viruses in *A. thaliana* does not require NPR1 activity.

The pharmacological evidence, backed up by the work with *npr1* mutants, showed convincingly that regulation of PR gene induction and activation of resistance to viruses were activated by separate branches of the defensive signal transduction pathway. But since some of the chemicals used, particularly cyanide and SHAM, affect several enzymes other than AOX, this evidence could not show definitively that AOX is involved in signaling (Murphy et al. 1999, 2001; Singh et al. 2004). This provided the impetus to test the putative relationship between AOX and virus resistance using transgenic plants in which *Aox* gene expression has been modified.

Genetic modification of *Aox* gene expression. *Aox* gene expression can be altered in plants using constitutively expressed sense or antisense *Aox* cDNA sequences. However, some specific challenges are encountered when analysing the characteristics of *Aox*-transgenic plants.

Firstly, the best method for direct measurement of AOX activity *in vivo* uses specialized mass spectrometry equipment to determine the relative uptake of the ^{16}O and ^{18}O isotopes of oxygen during respiration (Robinson et al. 1992). But this is impractical to carry out on chemically treated, virus-infected tissue. Furthermore, it would not give valid results if additional oxygen consuming reactions were induced by exposure to viruses or chemicals, for example as during the HR, since the selective utilization of the two oxygen isotopes by AOX and cytochrome oxidase would be obscured (J. N. Siedow, personal communication). Therefore, lines of *Aox*-transgenic plants have been characterized in terms of their alternative respiratory pathway capacity (APC). APC is a measure of the maximum potential activity of AOX (Moore and Siedow, 1991) and is relatively straightforward to measure in plant cells and tissues using oxygen electrodes to measure oxygen consumption in the presence or absence of inhibitors of the cytochrome and alternative respiratory pathways.

A second challenge encountered with *Aox*-transgenic plants is that the changes in APC that can be achieved by constitutive expression of *Aox*-derived constructs are relatively modest (two to threefold above or below wild-type levels). This is probably because alterations of APC to any greater extent would interfere drastically with mitochondrial homeostasis and render such transgenic plants unviable. Support for this idea was obtained by Murphy et al. (2004) who used a TMV-derived vector (TMV.AOX) to drive AOX protein synthesis up to levels ten-fold or greater than those seen in unmodified plants. They found that in the highly susceptible host *N. benthamiana* infection with TMV.AOX resulted in systemic necrosis.

In initial experiments it was found that increasing or decreasing *Aox* gene expression and APC to the extent possible in *Aox*-transgenic plants did not alter either the overall susceptibility of plants to TMV-induced systemic disease, or their ability to resist the systemic spread of the virus following treatment with SA (Gilliland et al. 2003; Ordog et al, 2002). However, when accumulation of the virus was examined in the directly inoculated tissues of these *Aox*-transgenic plants, it was found that in plants with increased APC, AA-induced resistance to TMV was compromised. Meanwhile, in plants with decreased APC, SA or AA-induced resistance was transiently enhanced (Gilliland et al. 2003). We have suggested that the differential effect of altering *Aox* gene expression and APC on SA- vs. AA-induced resistance to TMV may be explained if SA, but not AA, can trigger more than one signalling pathway leading to the induction of mechanisms that limit virus accumulation (Gilliland et al, 2003; Singh et al. 2004). In this model, AA and SA can both induce signaling via the mitochondrion, which is influenced by AOX and can be to some extent disrupted in *Aox*-transgenic plants, while SA can trigger an additional antiviral mechanism that is not affected by AOX (below).

The degree to which *Aox* gene expression or APC can be altered in transgenic plants is, as noted above, rather limited. More recently, our group has found that by using TMV-derived transient expression vectors to express *Aox* sequences, far higher levels of expression of wild type or mutant AOX protein can be produced *in planta* (Murphy et al. 2004). When TMV vectors were used to drive very high levels of expression of either AOX, or AOX mutated in its active site (AOX-E), virus spread was enhanced and thereby induced larger HR lesions than those produced by the 'empty' viral vector after inoculation onto *NN*-genotype tobacco. Consistent with this, in the highly susceptible host *N. benthamiana,* systemic movement of TMV vectors expressing AOX or AOX-E was at least as fast as that of the empty vector and faster than that of TMV constructs bearing sequences of comparable length (the green fluorescent protein sequence or antisense *Aox*).

These results suggest that expression of either AOX or AOX-E is allowing the viral vector to overcome, at least to some extent, a pre-existing or basal resistance to the spread of TMV. This contrasts with some of our own earlier results and conclusions (Gilliland et al. 2003) in which we found that although altering *Aox* gene expression in stably transformed tobacco could affect certain aspects of induced resistance it did not affect the basal resistance or susceptibility to infection with TMV. We have suggested that the far higher levels of *Aox* expression (achievable with the viral vectors) are negatively affecting the operation of basal resistance to the spread of TMV and that this basal resistance is at least partly controlled by mitochondrial signaling mechanisms (Murphy et al. 2004).

Interestingly, we recently found that although TMV.AOX can readily spread and form lesions in the *NN*-genotype Xanthi-nc tobacco, it does not form HR lesions on plants of another *NN*-genotype cultivar, Samsun NN, unless they are transgenic for the bacterial salicylate hydroxylase encoding gene, *nahG,* and therefore cannot accumulate normal levels of SA (unpublished data). This suggests that Samsun NN tobacco possesses a basal resistance to the spread of TMV that is stronger than that in Xanthi-nc. Although this basal resistance must be, in part, dependent on SA, it cannot be overcome by high-level expression of *Aox* sequences from the virus. The result suggests that tobacco varieties vary widely in their basal resistance to virus infection independently of whether they possess a major single gene resistance such as that controlled by the *N* gene.

Salicylic acid-induced resistance to viruses: Induction and modes of action.

How does AOX function in resistance induction? As mentioned above, one of the functions of AOX is to negatively regulate the accumulation of ROS within the mitochondrion. These ROS are generated as a by-product of the activity of the respiratory electron transport chain, particularly when flow through the chain is constricted by, for example, the presence of metabolic inhibitors like cyanide or by other stresses.

We recently suggested a model in which changes in ROS levels in the mitochondrion act as signals controlling a subset of the antiviral resistance mechanisms induced by SA (Gilliland et al. 2003; Singh et al. 2004). Similar mechanisms have also been proposed for the coordination of defensive and stress-induced signaling via the mitochondrion (Dutilleul et al. 2003; Maxwell et al. 2002; Norman et al. 2004). One element of the mechanism put forward by Dutilleul et al. (2003) is that redox-responsive proteins in the

mitochondrion would detect these variations in ROS level. We suggest that these could transmit information into the cytosol and then, probably *via* further intermediates, to the nucleus to regulate changes in gene expression such as those described by Maxwell and colleagues (2002). We have suggested that some of the genes affected in this way may encode host factors affecting virus replication and movement (Singh et al. 2004).

If defensive signaling in the mitochondrion were transduced *via* alterations in ROS, this would offer an explanation for the modified responses of the *Aox*-transgenic plants to SA and AA. In non-transgenic plants AA and SA can, even at low concentrations, constrict electron flow through the respiratory chain, which will lead to a transient increase in ROS (Maxwell et al. 1999; Xie and Chen, 1999; Norman et al. 2004). However, in transgenic plants with an increased APC the mitochondrion would be 'buffered' against the build up of ROS, thus 'damping' the signal. This can explain why AA-induced resistance is seen to be inhibited in these transgenic lines but not why SA-induced resistance to TMV can still be detected (Gilliland et al. 2003). This has led us to propose that there is at least one additional mechanism, most likely involving gene silencing that contributes to SA-inducible resistance but which is not regulated *via* the mitochondrion and inducible by AA (the evidence for this is reviewed in Singh et al. 2004 and in the accompanying chapter by Gilliland et al.).

The nature of SA-induced virus resistance mechanisms is not the same for all viruses, plant cells or plant species. One of the problems with many models for defensive signal transduction pathways in plants is that they can give the misleading impression that a single signaling pathway regulates all resistance processes. In fact, SA-induced resistance appears to manifest itself in different ways in different cell types and involves resistance to at least three phases of the viral infection process: replication, cell-to-cell movement, and long distance movement.

Recent studies with TMV revealed that SA induces inhibition of both replication and cell-to-cell movement of this virus but these effects were found to be cell-specific (Murphy and Carr, 2002; Carr, 2004). When SA-treated tobacco plants were inoculated with TMV engineered to express the jellyfish green fluorescent protein (TMV.GFP), virus cell-to-cell movement was inhibited in the cells of the epidermis. Meanwhile, in protoplasts derived from mesophyll cells accumulation of the virus was dramatically decreased. In studies of directly inoculated leaf tissue it was found that the ratio of genomic RNA to coat protein mRNA and the ratio of plus- to minus- sense RNAs were affected by SA. Taken together, these results suggested that SA induces interference with the activity of the TMV RdRp complex (Chivasa

et al. 1997; Naylor et al. 1998; Murphy and Carr, 2002; Carr, 2004). However, taking into account the possible role of RdRP1 mentioned above, the decrease in TMV accumulation in these cells may also be due in part to an increase in the rate of viral RNA turnover (Gilliland et al. 2003).

Another reason that it is difficult to generalize about SA-induced virus resistance mechanisms is that not all viruses are affected in the same way in all plants. Thus, in tobacco SA does not inhibit the replication or movement of CMV in directly inoculated tissue but it does inhibit the systemic movement of this virus (Naylor et al. 1998; Murphy and Carr, 2002). In tobacco, SA-induced resistance to CMV is antagonized by SHAM, indicating that the resistance mechanism is controlled via the AOX-influenced mitochondrial-signaling pathway (Naylor et al. 1998). A similar situation occurs in *A. thaliana* where inhibition of CMV systemic movement is also induced by SA as well as by AA (Mayers et al. 2005). These results indicate that the mechanisms underlying induced resistance to CMV in tobacco and *A. thaliana* are very similar. However, not all plants combat CMV in this way.

In squash (*Cucurbita pepo*) SA-induced resistance to CMV results from inhibition of virus accumulation in directly inoculated tissue and this is due predominantly to inhibition of cell-to-cell movement. Furthermore, neither of the AOX inducers AA or KCN induced resistance to CMV in squash and AOX inhibitors, which can inhibit SA-induced resistance to CMV in tobacco, did not inhibit SA-induced resistance to the virus in this plant (Mayers et al. 2005). In *Nicotiana*, the ability of CMV to evade SA-induced resistance to movement and replication is conditioned by the 2b counter-defense protein (Ji and Ding, 2001; Palukaitis and García-Arenal, 2003). Evidently, the 2b protein is not able to subvert this type of resistance in this cucurbit host, possibly because the AOX-regulated signaling pathway is not involved in resistance induction.

We have suggested that the evolution of different resistance mechanisms to CMV in cucurbits *versus Nicotiana* and *A. thaliana* may have been driven to some extent by differences in plant anatomy and the photosynthate translocation mechanisms utilized by these hosts (Mayers et al. 2005). Broadly speaking, virus systemic movement follows the translocation of photosynthates, predominantly sucrose, from carbon source to carbon sink tissues (Nelson and van Bel 1997). However, the sucrose and virus may briefly part company during loading from the mesophyll cells into the phloem. This is because for viruses like CMV the entire route from the leaf mesophyll cells to the sieve element–companion cell complex must occur

via plasmodesmata, i.e. symplastically. However, the route taken by sucrose can be symplastic or apoplastic, depending upon the plant species (ap Rees 1994; Truernit 2001). Whether the transfer of sucrose occurs predominantly via the symplastic or apoplastic route depends upon the abundance of plasmodesmata linking the phloem tissue (sieve elements and companion cells) and the surrounding mesophyll cells (Truernit 2001). Detailed electron microscopic examination has shown, that in squash, which is a symplastic loader, these connections are abundant (Gamalei 1989). In contrast, the number of plasmodesmal connections per μm^2 of this interface is about 600-fold less in tobacco, and in *A. thaliana* there are between 37 and 10-fold fewer connections, depending upon the correction factor used (Gamalei 1991; Haritatos et al. 2000).

In tobacco and *A. thaliana*, where there are relatively few plasmodesmal connections between the mesophyll and phloem tissue, any inhibition of virus movement through the plasmodesmata at this interface will significantly compromise the ability of a virus to spread out of the primary inoculated leaf. But in squash the large number of plasmodesmal connections between the mesophyll and phloem cells may render any inhibition of virus movement at this interface to be less effective in preventing systemic virus movement from occurring. This may have placed a selective pressure on squash, and probably other cucurbits, for SA-induced resistance to CMV to target an earlier stage in virus invasion than we see in tobacco or *A. thaliana*.

These comparative studies of SA-induced resistance to CMV in different plant species show that different host species may use significantly different approaches to resist infection by the same virus. They also imply that caution may be required when attempting to apply findings on plant-virus interactions from model systems to a wider range of host species.

Summing up: Can plant biochemical studies help us to a better understanding of virus resistance and susceptibility?

At the beginning of this review we put forward the idea that the intimacy of the host-virus relationship could make the biochemical activity of the host a decisive factor in determining the outcome of the interaction in plants: resistance *versus* susceptibility. In the first section we examined the situation with regard to primary carbon metabolism in compatible interactions between viruses and plants. This has been an extensively studied area and a vast and complex array of metabolic changes occurring in time and space have been linked to virus infection. However, at the present time no direct causal relationship has been demonstrated between any virus-induced biochemical symptom and the success of the virus infection in a

susceptible host. This is unfortunate since if a biochemical change was identified as being required for successful virus replication and spread, the host enzymes involved could be targeted for chemical or genetic manipulation to induce resistance to the virus. In the future, increasingly powerful methods of large scale metabolic analysis combined with improved means of high-throughput genetic manipulation, such as virus-induced gene silencing, hairpin-mediated RNAi or the screening of libraries of T-DNA knockout plants, may allow us to identify unambiguously those virus-induced metabolic changes which are required for successful infection.

With respect to induced resistance, it does seem that certain elements of primary metabolism may affect the outcome of the virus-plant encounter. These elements include sugar sensing, which appears to play a role in regulation of SAR-related gene expression (Herbers et al. 1996a,b), and alternative respiration, which through its role as a negative regulator of ROS in the mitochondrion, modulates induction of some antiviral mechanisms in tobacco and *A. thaliana* (Singh et al. 2004). Thus, metabolic pathways are participating in resistance through signaling, rather than through, for example, redirecting metabolism to decrease levels of substrates needed for viral replication.

In conclusion, our understanding of biochemical changes in the virus-infected plant is still, for the most part, at the descriptive level. Nevertheless, studies have revealed roles for plant metabolic pathways in defensive signaling, and new approaches for the analysis and controlled perturbation of plant biochemical pathways hold the promise of yielding information useful in the design of future protection strategies.

Acknowledgements

Professor Richard Oliver and Dr Kerry Blissett formerly of the Carlsberg Laboratory, Copenhagen, Denmark are thanked for their assistance with the NMR studies. Work by the authors mentioned in this review was supported by The Gatsby Charitable Foundation and The British Council (M.G.H.), as well as the U.K. Biotechnology and Biological Sciences Research Council and The Leverhulme Trust (J.P.C). M.G.H. is a recipient of a Study Abroad Studentship from The Leverhulme Trust.

This review is dedicated to the memory of Professor Tom ap Rees.

References

Affourtit, C., Krab, K. and Moore A.L. (2001). Control of plant mitochondrial respiration. *BBA-Bioenergetics* 1504: 58-69.

Affourtit, C., Albury, M.S.W., Crichton, P.G. and Moore, A.L. 2002. Exploring the molecular nature of alternative oxidase regulation and catalysis. *FEBS Lett*. 510: 121-126.

ap Rees, T. 1994. Plant physiology. Virtue on both sides. *Current Biology* 4: 557-559.

ap Rees, T., Fuller, W.A. and Wright, B.W. 1976. Pathways of carbohydrate oxidation during thermogenesis by the spadix of *Arum maculatum*. *Bioch. Biophys*. Acta 473: 22-35.

Aranda, M. and Maule, A. 1998. Virus-induced host gene shutoff in animals and plants. *Virology* 243: 261-7.

Aranda, M.A., Escaler, M., Wang, D. and Maule, A.J. 1996. Induction of HSP70 and polyubiquitin expression associated with plant virus replication. *Proc. Natl. Acad. Sci. U S A*. 93: 15289-93.

Aranda, M.A., Escaler, M., Thomas, C.L. and Maule, A.J. 1999. A heat shock transcription factor in pea is differentially controlled by heat and virus replication. *Plant J.* 20: 153-61.

Banerjee, N. and Zaitlin, M. 1992. Import of tobacco mosaic virus coat protein into intact chloroplasts *in vitro*. *Molec. Plant-Microbe Interact.* 5: 466-471.

Banerjee, N., Wang, J.-Y. and Zaitlin, M. 1995. A single nucleotide change in the coat protein gene of tobacco mosaic virus is involved in the induction of severe chlorosis. *Virology* 207: 234-239.

Barón, M., Rahoutei, J., Lázaro, J.J. and García-Luque, I. 1995. PSII response to biotic and abiotic stress. *In Photosynthesis: from Light to Biosphere*, Vol IV, pp. 897-900, Mathis, P. ed. Kluwer Academic Publishers, Netherlands.

Bartel, D.P. 2004. MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. *Cell* 116: 261-297.

Bedbrook, J.R. and Matthews, R.E.F. 1973. Changes in the flow of early products of photosynthetic carbon fixation associated with the replication of TYMV. *Virology* 53: 84-91.

Blua, M.J., Perring, T.M. and Madore, M.A. 1994. Plant virus-induced changes in aphid population development and temporal fluctuations in plant nutrients. *J. Chem. Ecol*. 20: 691-707.

Bolas, B.D. and Bewley, W.F. 1930. Aucuba or yellow mosaic of tomato: a note on metabolism. *Nature* 126: 471.

Carr, J.P. 2004. '*Tobacco mosaic virus*.' In *Plant-Pathogen Interactions* (Ed. N.J.Talbot). *Annu. Plant Rev.* 11: 27-67. Blackwell Publishers, Oxford.

Caspar, T., Huber, S.C. and Somerville, C. 1985. Alterations in growth, photosynthesis, and respiration in a starchless mutant of *Arabidopsis thaliana* (L.) deficient in chloroplast phosphoglucomutase activity. *Plant Physiol.* 79: 11-17.

Chivasa, S., Murphy, A. M., Naylor, M. and Carr, J. P. 1997. Salicylic acid interferes with tobacco mosaic virus replication via a novel salicylhydroxamic acid-sensitive mechanism. *Plant Cell* 9: 547-557.

Chivasa, S. and Carr, J. P. 1998. Cyanide restores *N* gene mediated resistance to tobacco mosaic virus in transgenic tobacco expressing salicylic acid hydroxylase. *Plant Cell* 10: 1489-1498.

Chivasa, S., Berry, J.O., ap Rees, T. and Carr, J.P. 1999. Changes in gene expression during development and thermogenesis in *Arum*. *Aust. J. Plant Physiol.* 26: 391-399.

Cohen, J. and Loebenstein, G. 1975. An electron microscope study of starch lesions in cucumber cotyledons infected with tobacco mosaic virus. *Phytopathology* 65: 32-39.

Diener, T.O. 1963. Physiology of virus-infected plants. *Annu. Rev. Phytopathol.* 1: 197-218.

Doke, N. and Hirai, T. 1970. Radioautographic studies on the photosynthetic CO_2 fixation in virus-infected leaves. *Phytopathology* 60: 988-991.

Dutilleul, C., Garmier, M., Noctor, G., Mathieu, C., Chetrit, P., Foyer, C.H. and de Paepe, R. 2003. Leaf mitochondria modulate whole cell redox homeostasis, set antioxidant capacity, and determine stress resistance through altered signaling and diurnal regulation. *Plant Cell* 15: 1212-1226.

Durrant, W.E. and Dong, X. 2004. Systemic acquired resistance. *Annu. Rev.Phytopathol.* 42: 185-209.

Ehara, Y. and Misawa, T. (1975) Occurrence of abnormal chloroplasts in tobacco leaves infected systemically with the ordinary strain of cucumber mosaic virus. *Phytopathol. Z.* 84, 233-252.

Escaler, M., Aranda, M.A., Thomas, C.L. and Maule, A.J. 2000. Pea embryonic tissues show common responses to the replication of a wide range of viruses. *Virology* 267: 318-25.

Funayama, S., Sonoike, K. and Terashima, I. 1997. Photosynthetic properties of leaves of *Eupatorium makinoi* infected by a geminivirus. *Photosynth. Res.* 53, 253-261.

Gamalei, Y.V. 1989. Structure and function of leaf minor veins in trees and herbs. A taxonomic review. *Trees* 3: 96-110.

Gamalei, Y.V. 1991. Phloem loading and its development related to plant evolution from trees to herbs. *Trees* 5: 50-64.

Gilliland, A., Singh, D.P., Hayward, J.M., Moore, C.A., Murphy, A.M., York, C.J., Slator, J. and Carr, J.P. 2003. Genetic modification of alternative respiration has differential effects on antimycin A-induced *versus* salicylic acid-induced resistance to Tobacco mosaic virus. *Plant Physiol.* 132: 1518-1528.

Goodman, R.N., Király, Z. and Wood, K.R. 1986. *The Biochemistry and Physiology of Plant Disease*. University of Missouri Press, Columbia, MO, USA.

Gunasinghe, U.B. and Berger, P.H. 1991. Association of potato virus-Y gene-products with chloroplasts in tobacco. *Mol. Plant-Microbe Interact.* 4: 452-457.

Handford, M.G. 2000. Host factors and virus infection of Arabidopsis thaliana*. Ph.D. Thesis, University of Cambridge.*

Haritatos, E., Medville, R. and Turgeon, R. 2000. Minor vein structure and sugar transport in *Arabidopsis thaliana*. *Planta* 211: 105-11.

Herbers, K., Meuwly, P., Frommer, W.B., Métraux, J.-P. and Sonnewald, U. 1996a. Systemic acquired resistance mediated by the ectopic expression of invertase: Possible hexose sensing in the secretory pathway. *Plant Cell* 8: 793-803.

Herbers, K., Meuwly, P., Métraux, J.-P. and Sonnewald, U.1996b Salicylic acid-independent induction of pathogenesis-related protein transcripts by sugars is dependent on leaf developmental stage. *FEBS Lett.* 397: 239-244.

Hodgson, R.A.J., Beachy, R.N. and Pakrasi, H.B. 1989. Selective inhibition of photosystem II in spinach by tobacco mosaic virus: an effect of the viral coat protein. *FEBS Lett.* 245: 267-270.

Holmes, F.O. 1931. Local lesions of mosaic in *Nicotiana tabacum* L. *Contrib. Boyce Thompson Inst.* 3: 163-172.

Honda, Y. and Matsui, C. 1974. Electron microscopy of cucumber mosaic virus-infected tobacco leaves showing mosaic symptoms. *Phytopathology* 64: 534-539.

Hršel, I. 1962. The electron microscopic investigation of changes in cytoplasmic structures in leaves of *Cucumis sativa* L. infected with cucumber virus 4. *Biol. Plantarum* 4: 232-238.

Hull, R. 2002. *Matthews' Plant Virology*. 4th Edition, Academic Press, NY.

Izaguirre-Mayoral, M.L., Carballo, O. and Gil, F. 1990. Purification and partial characterisation of isometric virus-like particles in *Kalanchoe* species. *J. Phytopathol.* 130: 303-311.

Jensen, S.G. 1967. Photosynthesis, respiration and other physiological relationships in barley infected with barley yellow dwarf virus. *Phytopathology.* 58: 204-208.

Ji, L.-H.and, Ding, S.-W. 2001. The suppressor of transgene RNA silencing encoded by *Cucumber mosaic virus* interferes with salicylic acid-mediated virus resistance. *Mol. Plant-Microbe Interact.* 14: 715-24.

Kachroo, P., Yoshioka, K., Shah, J., Dooner, H.K. and Klessig, D.F. 2000. Resistance to turnip crinkle virus in arabidopsis is regulated by two host genes and is salicylic acid dependent but *NPR1*, ethylene, and jasmonate independent. *Plant Cell* 12: 677-690.

Lacomme, C. and Roby, D. 1999. Identification of new early markers of the hypersensitive response in *Arabidopsis thaliana*. *FEBS Lett.* 459: 149-153.

Laties, G.L. 1982. The cyanide-resistant, alternative path in plant mitochondria. *Annu. Rev.Plant Physiol.* 33: 519-555.

Leal, N. and Lastra, R. 1984. Altered metabolism of tomato plants infected with tomato yellow mosaic virus. *Physiol. Plant Pathol.* 24: 1-7.

Lehto, K., Tikkanen, M., Hiriart, J.B., Paakkarinen, V. and Aro, E.M. 2003. Depletion of the photosystem II core complex in mature tobacco leaves infected by the *Flavum* strain of tobacco mosaic virus. *Mol. Plant-Microbe Interact.* 16: 1135-1144.

Lennon, A.M., Neuenschwander, U.H., Ribas-Carbo, M., Giles, L., Ryals, J.A. and Siedow, J.N. 1997. The effects of salicylic acid and tobacco mosaic virus infection on the alternative oxidase of tobacco. *Plant Physiol.* 115: 783-791.

Magyarosy, A.C., Buchanan, B.B. and Schürmann, P. 1973. Effect of a systemic virus infection on chloroplast function and structure. *Virology* 55: 426-438.

Malamy, J., Carr, J.P., Klessig, D.F. and Raskin, I. 1990. Salicylic acid- A likely endogenous signal in the resistance response of tobacco to viral infection. *Science* 250: 1002-1004.

Martinez, C., Pons, E., Prats, G. and Leon, J. 2004. Salicylic acid regulates flowering time and links defence responses and reproductive development. *Plant J.* 37: 209-217.

Matthews, R.E.F. 1991. *Plant Virology*, 3rd edition. Academic Press, NY.

Maxwell, D.P., Wang, Y. and McIntosh, L. 1999. The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. *Proc. Nat. Acad. Sci. USA* 96: 8271-8276.

Maxwell, D.P., Nickels, R. and McIntosh, L. 2002. Evidence of mitochondrial involvement in the transduction of signals required for the induction of genes associated with pathogen attack and senescence. *Plant J.* 29: 269-279.

Mayers, C.N., Lee, K.-C., Moore, C.A., Wong, S.-K. and Carr, J.P. 2005. Salicylic acid-induced resistance to *Cucumber mosaic virus* in squash and *Arabidopsis thaliana*: Contrasting mechanisms of induction and antiviral action. *Molec. Plant-Microbe Interact.* 18: 428-434.

Métraux J.-P., Signer, H., Ryals, J., Ward, E., Wyssbenz, M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W. and Inverardi, B. 1990. Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science* 250: 1004-1006.

Métraux, J.-P. (2002). Recent breakthroughs in the study of salicylic acid biosynthesis. *Trends Plant Sci.* 7: 332-4.

Meeuse, B.J.D. and Raskin, I. 1988. Sexual reproduction in the Arum lily family, with emphasis on thermogenicity. *Sexual Plant Reproduction* 1: 3-15.

Moore, A.L., Albury, M.S., Crichton, P.G. and Affourtit, C. 2002. Function of the alternative oxidase: is it still a scavenger? *Trends Plant Sci.* 7: 478-481.

Murphy, A.M., Chivasa, S., Singh, D.P. and Carr, J.P. 1999. Salicylic acid-induced resistance to viruses and other pathogens: a parting of the ways? *Trends Plant Sci.* 4: 155-160.

Murphy, A.M., Gilliland, A., Wong, C.E., West, J., Singh, D.P. and Carr, J.P. 2001. Signal transduction in resistance to plant viruses. *Eur. J. Plant Pathol.* 107: 121-128.

Murphy, A.M. and Carr, J.P. 2002. Salicylic acid has cell-specific effects on tobacco mosaic virus replication and cell-to-cell movement. *Plant Physiol.* 128: 552-563.

Murphy, A.M., Gilliland, A., York, C.J., Hyman, B. and Carr, J.P. 2004. High-level expression of alternative oxidase protein sequences enhances the spread of viral vectors in resistant and susceptible plants. *J. Gen. Virol.* 85: 3777-3786.

Naderi, M. and Berger, P.H. 1997. Effects of chloroplast targeted potato virus Y coat protein on transgenic plants. *Physiol. Mol. Plant Pathol.* 50: 67-83.

Naidu, R.A., Krishnan, M., Ramanujam, P., Gnanam, A. and Nayudu, M.V. 1984. Studies on peanut green mosaic virus infected peanut (*Arachis hypogea* L.) leaves I. Photosynthesis and photochemical reactions. *Physiol. Plant Pathol.* 25: 181-190.

Nelson, R.S. and van Bel, A.J.E. 1998. The mystery of virus trafficking into, through and out of the vascular tissue. *Prog. Bot.* 59: 476-533.

Norman, C., Howell, K.A., Millar, A.H., Whelan, J.M. and Day, D.A. 2004. Salicylic acid is an uncoupler and inhibitor of mitochondrial electron transport. *Plant Physiol.* 134: 492-501.

Ordog, S. H., Higgins, V. J. and Vanlerberghe, G. C. 2002. Mitochondrial alternative oxidase is not a critical component of plant viral resistance but may play a role in the hypersensitive response. *Plant Physiol.* 129: 1858-1865.

Palukaitis, P. and García-Arenal, F. 2003. Cucumoviruses. *Adv. Virus Res*. 62: 241-323.

Pennazio, S. 1996. Respiration in systemically virus-infected plants. *Petria* 6: 1-10.

Porter, C.A. 1959. Biochemistry of plant virus infection. *Adv. Virus Res.* 6: 75-91.

Porter, C.A. and Weinstein, L.H. 1957. Biochemical changes induced by thiouracil in cucumber mosaic virus-infected and non-infected tobacco plants. *Contrib. Boyce Thompson Inst.* 19: 87-106.

Raskin, I., Ehman, A., Melander, W.R., and Meeuse, B.J.D. 1987. Salicylic acid: A natural inducer of heat production in Arum lilies. *Science* 237: 1601-1602.

Raskin, I., Turner, I.M. and Melander, W.J. 1989. Regulation of heat production in the inflorescence of an *Arum* lily by endogenous salicylic acid. *Proc. Natl. Acad. Sci. USA* 86: 2214-2218.

Reinero, A. and Beachy, R.N. 1986. Association of TMV coat protein with chloroplast membranes in virus-infected leaves. *Plant Mol. Biol.* 6: 291-301.

Reinero, A. and Beachy, R.N. 1989. Reduced photosystem II activity and accumulation of viral coat protein in chloroplasts of leaves infected with tobacco mosaic virus. *Plant Physiol.* 89, 111-116.

Rhoads, D.M. and McIntosh, L. 1992. Salicylic acid regulation of respiration in higher plants- alternative oxidase expression. *Plant Cell* 4: 1131-1139.

Rhoads, D.M. and McIntosh, L. 1993. Cytochrome and alternative pathway respiration in tobacco- effects of salicylic acid. *Plant Physiol.* 103: 877-883.

Roberts, P.L. and Wood, K.R. 1982. Effects of a severe (P6) and a mild (W) strain of cucumber mosaic virus on tobacco leaf chlorophyll, starch and cell ultrastructure. *Physiol. Plant Pathol.* 21: 31-37.

Robinson, S.A, Yakir, D., Ribas-Carbo, M., Giles, L., Osmond, C.B., Siedow, J.N. and Berry, J.A. 1992. Measurement of the engagement of cyanide-resistant respiration in the crassulacean acid metabolism plant *Kalanchoe daigremontiana* with the use of on-line oxygen isotope discrimination. *Plant Physiol.* 100: 1087-1091.

Sakano, K. 2001. Metabolic regulation of pH in plant cells: role of cytoplasmic pH in defense reaction and secondary metabolism. *Int. Rev. Cytol.* 206: 1-44.

Samuel, G. 1934. The movement of tobacco mosaic virus within the plant. *Ann. appl. Biol.* 21: 90-111.

Seaton, G.G.R., Hurry, V.M. and Rohozinski, J. 1996. Novel amplification of non-photochemical chlorophyll fluorescence quenching following viral infection in *Chlorella*. *FEBS Lett.* 389, 319-323.

Selman, I.W., Brierley, M.R., Pegg, G.F. and Hill, T.A. 1961. Changes in the free amino acids and amides in tomato plants inoculated with tomato spotted wilt virus. *Ann. appl. Biol.* 49, 601-615.

Siedow, J.N. and Moore, A.L. 1993. A kinetic model for the regulation of electron transfer through the cyanide-resistant pathway in plant mitochondria. *Biochim. Biophys. Acta* 1142: 165-174.

Simons, B.H., Millenaar, F.F., Mulder, L., van Loon, L.C. and Lambers, H. 1999. Enhanced expression and activation of the alternative oxidase during infection of Arabidopsis with *Pseudomonas syringae* pv. tomato. *Plant Physiol.* 120: 529-538.

Singh, D..P, Moore, C.A., Gilliland, A. and Carr, J.P. 2004. Activation of multiple anti-viral defence mechanisms by salicylic acid. *Molec. Plant Path.* 5: 57-63.

Surplus, S.L., Jordan, B.R., Murphy, A.M., Carr, J.P., Thomas, B, and Mackerness, S. A.-H. 1998. Ultraviolet-B-induced responses in *Arabidopsis thaliana*: Role of salicylic acid and reactive oxygen species in the regulation of transcripts encoding photosynthetic and acidic pathogenesis-related proteins. *Plant, Cell Environ.* 21: 685-694.

Técsi, L.I., Maule, A.J., Smith, A.M. and Leegood, R.C. 1994a. Complex, localized changes in CO_2 assimilation and starch content associated with the susceptible interaction between cucumber mosaic virus and a cucurbit host. *Plant J.* 5: 837-847.

Técsi, L.I., Maule, A.J., Smith, A.M. and Leegood, R.C. 1994b. Metabolic alterations in cotyledons of Cucurbita pepo infected by cucumber mosaic virus. *J. Exp. Bot.* 45: 1541-1551.

Técsi, L.I., Smith, A.M., Maule, A.J. and Leegood, R.C. 1995. Physiological studies of a viral mosaic infection. In *Physiological Responses of Plants to Pathogens: Aspects of Applied Biology*, Vol. 42, pp. 133-140. Walters, D.R., Scholes, J.D., Bryson, R.J., Paul, N.D. and McRoberts, N. (eds.) Association of Applied Biologists, Wellesbourne, UK.

Técsi, L.I., Smith, A.M., Maule, A.J. and Leegood, R.C. 1996. A spatial analysis of physiological changes associated with infection of cotyledons of marrow plants with cucumber mosaic virus. *Plant Physiol.* 111: 975-985.

Truernit, E. 2001. The importance of sucrose transporters. *Curr. Biol.* 11:R169-R171.

Umbach A. L. and Siedow J. N. 1993. Covalent and non-covalent dimers of the cyanide-resistanct alternative oxidase protein in higher plant mitochondria and their relationship to enzyme activity. *Plant Physiol.* 103: 845-854.

Vanlerberghe, G. C. and McIntosh, L. 1997. Alternative oxidase: From gene to function. *Annu. Rev. Plant Physiol.* 48: 703-734.

Wang, D. and Maule, A.J. 1995. Inhibition of host gene expression associated with plant virus replication. *Science* 267: 229-231.

Welkie, G.W., Yang, S.F. and Miller, G.W. 1967. Metabolite changes induced by cucumber mosaic virus in resistant and susceptible strains of cowpea. *Phytopathology.* 57: 472-475.

Wildermuth, M.C., Dewdney, J., Wu, G. and Ausubel, F.M. 2002. Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature* 414: 562-5. Erratum in: Nature 2002, 417: 571.

Wong, C.E. 2001. Signalling in salicylic acid-induced resistance to viruses in *Arabidopsis thaliana. PhD Thesis, University of Cambridge*.

Wong, C. E., Carson, R. A. J. and Carr, J. P. 2002. Chemically induced virus resistance in *Arabidopsis thaliana* is independent of pathogenesis-related protein expression and the *NPR1* gene. *Mol. Plant Microbe Interact.* 15: 75-81.

Xie, Z.X. and Chen, Z.X. 1999. Salicylic acid induces rapid inhibition of mitochondrial electron transport and oxidative phosphorylation in tobacco cells. *Plant Physiol.* 120: 217-225.

Yip J. Y. H. and Vanlerberghe G. C. 2001. Mitochondrial alternative oxidase acts to dampen the generation of active oxygen species during a period of rapid respiration induced to support a high rate of nutrient uptake. *Physiologia Plant.* 112: 327-333.

Zaitlin, M. and Hull, R. 1987. Plant virus-host interactions. *Annu. Rev. Plant Physiol.* 38, 291-315.

Zechmann, B., Müller, M. and Zellnig, G. 2003. Cytological modification in zucchini yellow mosaic virus (ZYMV) infected Styrian pumpkin plants. *Arch. Virol.* 148, 1119-1133.