

# Enhanced Expression and Activation of the Alternative Oxidase during Infection of Arabidopsis with *Pseudomonas syringae* pv *tomato*<sup>1</sup>

Bert H. Simons, Frank F. Millenaar, Lonke Mulder, Leendert C. Van Loon, and Hans Lambers\*

Graduate School of Functional Ecology, Department of Plant Ecology and Evolutionary Biology, Section of Plant Pathology (B.H.S., L.M., L.C.V.L.), and Section of Plant Ecophysiology (B.H.S., F.F.M., L.M., H.L.), Utrecht University, P.O. Box 800.84, 3508 TB Utrecht, The Netherlands; and Plant Sciences, Faculty of Agriculture, The University of Western Australia, Nedlands, WA 6009 Australia (H.L.)

---

Cyanide-resistant (“alternative”) respiration was studied in *Arabidopsis* during incompatible and compatible infection with *Pseudomonas syringae* pv *tomato* DC3000. Total leaf respiration increased as the leaves became necrotic, as did the cyanide-resistant component that was sensitive to salicylhydroxamic acid. Infiltration of leaves with an avirulent strain rapidly induced alternative oxidase (AOX) mRNA, whereas the increase was delayed in the compatible combination. The increase in mRNA correlated with the increase in AOX protein. Increased expression was confined to the infected leaves, in contrast to the pathogenesis-related protein-1, which was induced systemically. Virtually all of the AOX protein was in the reduced (high-activity) form. Using transgenic *NahG* and mutant *npr1-1* and *etr1-1* plants, we established that the rapid induction of the AOX was associated with necrosis and that ethylene, but not salicylic acid, was required for its induction. Increased pyruvate levels in the infected leaves suggested that increased substrate levels were respired through the alternative pathway; however, in the control leaves and the infected leaves, respiration was not inhibited by salicylhydroxamic acid alone. Increased respiration appeared to be associated primarily with symptom expression rather than resistance reactions.

---

Mitochondrial respiration provides the energy necessary to drive metabolic and transport processes in cells. During electron transport along the Cyt pathway a proton gradient is generated across the inner mitochondrial membrane, which allows ATP synthesis. Plant mitochondria contain an additional alternative electron-transport pathway that branches off from the Cyt pathway at the level of ubiquinone; it is not inhibited by cyanide and does not contribute to the formation of a proton gradient. This pathway is therefore not coupled to ATP synthesis (Siedow and Umbach, 1995). Electron transport along this alternative pathway involves only a single quinol oxidase, termed the AOX. The energy of electron flow through the alternative pathway is mainly lost as heat (Moore and Siedow, 1991; Siedow and Umbach, 1995). This process seems wasteful and the physiological significance of the alternative path-

way in the metabolism of these plants is still unclear. One example of alternative pathway activity during an essential part of the life cycle is in the reproduction of Araceae. The spadix is heated 10°C or more to volatilize odoriferous compounds that attract pollinators (Meeuse, 1975; Raskin et al., 1987). The alternative pathway is also found in most other plant species and plant organs and thus must serve other functions as well.

Cyanide-resistant plant respiration is increased during various stress conditions, e.g. low temperature, wounding, and plant diseases (Uritani and Asahi, 1980; Hiser and McIntosh, 1990; Vanlerberghe and McIntosh, 1992; Purvis and Shewfelt, 1993). AOX protein levels are also increased after wounding, infection, and low temperature conditions (Hiser and McIntosh, 1990; Vanlerberghe and McIntosh, 1992; Lennon et al., 1997), suggesting a role for the alternative respiration in stress alleviation. However, we do not know whether or to what extent the cyanide-resistant pathway also contributes to respiration under these conditions. Enhanced operation of the alternative pathway might relieve the Cyt pathway and prevent overreduction, thus reducing the formation of harmful radicals (Purvis and Shewfelt, 1993; Wagner and Krab, 1995). Nevertheless, the significance of the alternative respiratory pathway during stress remains to be elucidated. It is interesting that increased expression of both the AOX in *Arum* lily spadices during flowering and of PRs during resistance responses in, for example, tobacco, requires SA as a signal (Raskin et al., 1987; Rhoads and McIntosh, 1992; Delaney et al., 1994; Lennon et al., 1997). The latter is associated with the occurrence of SAR against further infections. Addition of SA to cell suspensions or intact leaves of tobacco also induces *aox* gene expression (Rhoads and McIntosh, 1993; Lennon et al., 1997). Together, these results suggest that SA acts as a signal in inducing both AOX and resistance responses in infected plants and that the alternative pathway might be associated with the resistant state.

---

<sup>1</sup> This work was supported by grants from the Netherlands Organization for Scientific Research.

\* Corresponding author; e-mail [hlambers@cyllene.uwa.edu.au](mailto:hlambers@cyllene.uwa.edu.au); fax 61-8-9380-1108.

---

Abbreviations: AOX, alternative oxidase; CFU, colony-forming unit(s); HR, hypersensitive reaction; KCN, potassium cyanide; PR, pathogenesis-related protein; RT-PCR, reverse transcriptase-PCR; SA, salicylic acid; SAR, systemic acquired resistance; SHAM, salicylhydroxamic acid.

The observations described above prompted us to investigate the possible relationship between the induction of the alternative pathway and resistance expression in *Arabidopsis* infection and whether SA is involved as a signal in both responses. *Arabidopsis* infected with the leaf-spotting bacterium *Pseudomonas syringae* is a well-described plant-pathogen system in which several features of the resistance response, as well as the involvement of SA, have been established (Whalen et al., 1991; Uknes et al., 1993; Cameron et al., 1994; Delaney et al., 1994). Using an avirulent and a virulent strain of *P. syringae* we studied whether the resistance response of the plant was associated with induction of the alternative pathway. The expression of the *aox* gene was compared with the expression of the gene encoding PR-1, which is a good marker for SA-dependent expression of SAR in *Arabidopsis* (Uknes et al., 1993).

To study the significance of SA for the induction of AOX in infected plants, transgenic and/or mutant *Arabidopsis* plants were used. Plants expressing the bacterial *nahG* gene, encoding the enzyme salicylate hydroxylase, which converts SA to catechol, are unable to accumulate SA. These transgenics exhibit an increased susceptibility to pathogens and are unable to express SAR (Delaney et al., 1994). Mutant *npr1-1* plants that are unable to express PRs or SAR upon infection (Cao et al., 1994) are defective in the subsequent signaling pathway. Expression of AOX was monitored in these plants in response to infection. Because ethylene stimulates cyanide-resistant respiration in plant organs (Laties, 1982), we also investigated the possible involvement of ethylene. This hormone plays a role in the responses to infections in the plant and its production is strongly increased in infected plant tissues, coinciding with necrosis (Uritani and Asahi, 1980; De Laat and Van Loon, 1982, 1983; Brederode et al., 1991). Expression of AOX was monitored upon infection of the *Arabidopsis etr1-1* mutant, in which the perception of ethylene was strongly reduced (Schaller and Bleecker, 1995).

## MATERIALS AND METHODS

### Plant Material, Bacterial Strains, and Growth Conditions

Seeds of *Arabidopsis* ecotype Columbia (Col-0) wild-type, *nahG* transgenic (Delaney et al., 1994), *npr1-1* (Cao et al., 1994), *etr1-1* (Bleeker et al., 1988), and *rps2-201* (Kunkel et al., 1993) mutants were sown on quartz sand. After 2 weeks the seedlings were transferred to a mixture of autoclaved potting soil and sand (12:5). Plants were cultivated in a growth chamber with 9-h d (200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at 24°C) and 15-h night (20°C) cycles and 65% RH. Twice a week plants were supplied with water or modified one-half-strength Hoagland nutrient solution: 2 mM  $\text{KNO}_3$ , 5 mM  $\text{Ca}(\text{NO}_3)_2$ , 1 mM  $\text{KH}_2\text{PO}_4$ , 1 mM  $\text{MgSO}_4$ , and trace elements, pH 7.0 (Hoagland and Arnon, 1938), containing 10  $\mu\text{M}$  Sequestren (CIBA-Geigy, Basel, Switzerland).

*Pst* (*Pseudomonas syringae* pv *tomato*) (Dong et al., 1991; Whalen et al., 1991) strains DC3000 (pLH12 $\Omega$ ; virulent) and DC3000 (pLH12; avirulent) were provided by Dr. A.F. Bent (University of Illinois, Urbana). These strains were grown at 28°C on King's medium B (King et al., 1954), containing

40 mg L<sup>-1</sup> tetracycline. Nonpathogenic *Pseudomonas fluorescens* strains WCS417 and WCS374, originally isolated from the rhizosphere of wheat (Lamers et al., 1988) and potato (Geels and Schippers, 1983), respectively, were grown similarly in the absence of the antibiotic. *Escherichia coli* strain DH5 $\alpha$ , harboring pGEM-3Z constructs, was grown in Luria-Bertani medium supplemented with 100 mg L<sup>-1</sup> ampicillin.

Plants were inoculated for 5 weeks after sowing. For treatment of the plants, bacterial cultures were washed and resuspended in 10 mM  $\text{MgCl}_2$ . Sterile 10 mM  $\text{MgCl}_2$  was used as a control. The bacterial suspension or the control solution was then pressure infiltrated into the abaxial side of the leaves using a syringe without a needle (Swanson et al., 1988). For treatment of leaves with SA, leaves were infiltrated with neutralized SA solutions. Treatment of leaves with ACC was carried out by dipping the leaves in a solution of 1 mM ACC and 0.01% Silwet L77 (v/v) in water (Van Meeuwen Chemicals, Weest, The Netherlands). Controls were performed using 0.01% Silwet (v/v) in water.

### Respiration of Infected Leaves

Leaves were detached, cut into four pieces with a razor blade, and kept in the dark for 15 min before measurement. Pieces were transferred into an air-tight cuvette containing 20 mM Hepes (pH 7.2) and 0.2 mM  $\text{CaCl}_2$  (Atkin et al., 1993), and  $\text{O}_2$  uptake was measured as a decrease of  $\text{O}_2$  concentration using a Clark-type electrode (YSI, Yellow Springs, OH). Cyanide-resistant  $\text{O}_2$  uptake was measured in the presence of 0.5 mM KCN. To assess whether the cyanide-resistant component was due to the presence of the alternative pathway, we used an appropriate concentration (2 mM) of the inhibitor SHAM. The effect of SHAM was also assessed in the absence of KCN.

### Quantification of Transcript Levels of AOX and PR-1

The competitive RT-PCR (Siebert and Larrick, 1992) was used to semiquantitatively determine AOX and PR-1 transcript levels in leaves. Poly(A<sup>+</sup>)RNA was isolated from several leaves of three plants using the QuickPrep Micro mRNA Purification kit (Pharmacia Biotech, Uppsala, Sweden); 200 ng was used for reverse transcription with the Ready-To-Go T-Primed First-Strand kit (Pharmacia Biotech). Competitive RT-PCR was then carried out using two gene-specific oligonucleotides as primers in the amplification reaction, 0.8  $\mu\text{L}$  of the first-strand mixture containing the cDNA and 0.8  $\mu\text{L}$  containing 1, 5, 10, 50, 100, and 500 pg of competitor DNA. The gene-specific oligonucleotides were based on the published sequence of *Arabidopsis* AOX (Kumar and Söll, 1992) and yield a fragment of approximately 350 bp. A 400-bp heterologous competitor DNA fragment, competing for the same set of primers, was obtained as described by Siebert and Larrick (1992). After agarose-gel electrophoresis in the presence of ethidium bromide, the resulting PCR products were quantified upon UV illumination. The dilution of the competitor DNA, yielding an approximate equimolar amount of product as the target cDNA, was taken as a measure of target mRNA

level. Transcript levels were expressed as relative values, taking the level in noninfected control treatments as 1. A similar procedure was carried out to determine PR-1 mRNA levels using the primer set and competitor DNA as described by Pieterse et al. (1996). Northern blots, probed with the Arabidopsis AOX-specific PCR product described above, failed to show specific bands, probably because of low AOX transcript levels. Northern analysis using total RNA extracts, however, agreed with competitive RT-PCR results of PR-1 mRNA levels (Pieterse et al., 1996), which confirmed the validity of the RT-PCR results. All experiments were carried out at least twice.

### Western Blotting

Total leaf extracts were prepared from 100 mg (fresh weight) of frozen leaf material obtained from several plants. The material was ground in liquid N<sub>2</sub> using a mortar and pestle and suspended in a total volume of 400  $\mu$ L of a protein sample mixture (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, and 0.001% bromophenol blue). After the sample was centrifuged for 10 min at 14,000 rpm in an Eppendorf centrifuge to precipitate cell debris, the proteins were separated by SDS-PAGE according to the method of Laemmli (1970) and subsequently electrotransferred to nitrocellulose filters using a blot-transfer buffer (25 mM Tris, 192 mM Gly, and 20% [v/v] methanol). Immunodetection of the AOX protein was carried out according to the product protocol of the AOX monoclonal antibody, aminoxyacetic acid (GT monoclonal antibodies kindly provided by Dr. T.E. Elthon, University of Nebraska, Lincoln), which was used as a primary antibody (1:50). A conjugate of Fab fragments of anti-mouse IgG from sheep and peroxidase (Boehringer Mannheim) was used as the secondary antibody (1:25,000). AOX protein was then detected by chemiluminescence using a substrate (SuperSignal ULTRA, Pierce), according to the protocol provided by the manufacturer.

### Determination of Pyruvate Concentration in Leaves

For the determination of pyruvate concentrations in leaves, about 1 g of fresh leaves was ground as described above. Preparation of the samples and measurement of the pyruvate concentration was carried out essentially as described by Wagner and Wagner (1995), with the addition of an extra purification step in which the final sample mixture was mixed with active C and subsequently filtered. Conversion of NADH to NAD<sup>+</sup> in the presence of lactate dehydrogenase (Boehringer Mannheim) was recorded at a wavelength of 340 nm.

## RESULTS

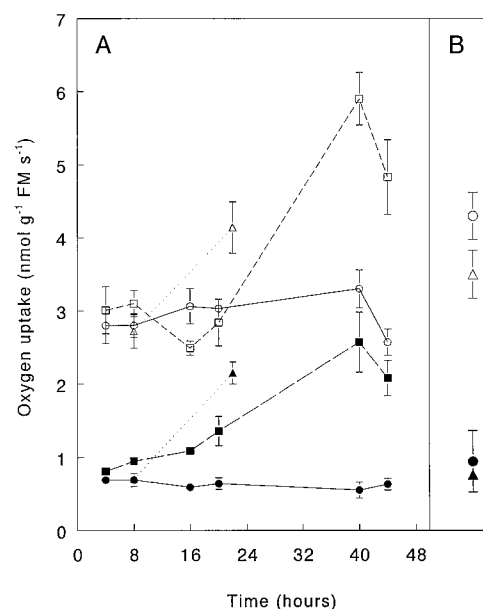
### Total and Cyanide-Resistant, SHAM-Sensitive Respiration in Leaves Infiltrated with Avirulent or Virulent Bacterial Strains

To determine whether infiltration of Arabidopsis leaves with either a virulent or an avirulent strain of *Pst* affected

respiratory activity, we measured rates of O<sub>2</sub> uptake of infected leaves and mock-infiltrated leaves. Leaves infiltrated with the avirulent strain collapsed within 1 d because of the HR, whereas those infiltrated with the virulent strain started to necrose 2 d after infiltration. Infiltration of the leaves with 10<sup>7</sup> CFU mL<sup>-1</sup> avirulent pathogen resulted in a rapid increase in total and in cyanide-resistant, SHAM-sensitive O<sub>2</sub> uptake (Fig. 1A). SHAM did not affect leaf respiration in the absence of KCN (data not shown). The compatible plant-pathogen combination showed a similar, but delayed, change in the total and cyanide-resistant, SHAM-sensitive O<sub>2</sub> uptake (Fig. 1A). Noninfected leaves of plants that were infiltrated with the avirulent strain did not show a systemic increase in cyanide-resistant, SHAM-sensitive respiration (Fig. 1B). The increase of total O<sub>2</sub> uptake in both the compatible and the incompatible combination coincided with an increase in the KCN-resistant component that was sensitive to SHAM (i.e. the alternative pathway). However, the lack of an effect of SHAM in the absence of KCN provides no information concerning the contribution of the alternative pathway to respiration in the absence of inhibitors.

### Induction of AOX in Response to Infection

AOX transcript levels were determined at intervals in leaves after infiltration with *Pst*, with AOX-specific primers yielding a 350-bp DNA fragment. To test whether this fragment was specific for AOX, the fragment was ligated



**Figure 1.** Total and cyanide-resistant, SHAM-sensitive O<sub>2</sub>-uptake rates of wild-type Arabidopsis leaves infiltrated with avirulent or virulent strains of *Pst*, expressed in units of fresh mass (FM). A, Leaves infiltrated with suspensions containing 10<sup>7</sup> CFU of the pathogens per mL. Circles, Control; triangles, avirulent pathogen; and squares, virulent pathogen. Open symbols indicate total O<sub>2</sub>-uptake of the leaves; closed symbols indicate cyanide-resistant, SHAM-sensitive O<sub>2</sub> uptake. B, Systemic, noninfiltrated leaves of mock plants infiltrated on lower leaves 3 d earlier. Results are mean values  $\pm$  SE; n = 4.

into a pGEM-3Z vector and transferred into *E. coli* strain DH5 $\alpha$ . From the resulting clones, 20 were subjected to a second amplification round, using degenerate *aox*-specific nested primers based on highly conserved sequences of plant AOX, as reported by Whelan et al. (1996). All clones gave a PCR product of the expected size (about 180 bp). Two of the clones containing the 350-bp fragment were also subjected to DNA-sequence analysis. The DNA sequence of the fragment was identical to the matching part of the published *aox* sequence from *Arabidopsis*, confirming that the amplification product of 350 bp corresponded to AOX.

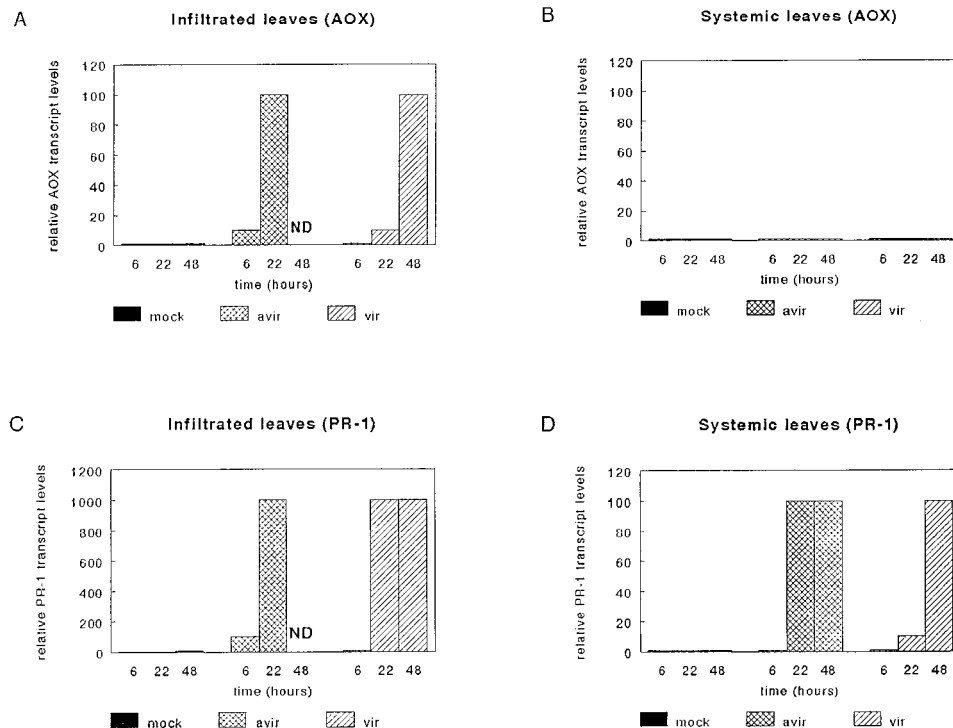
In the incompatible combination, AOX transcript levels were enhanced 10-fold within 6 h after infiltration (Fig. 2A). After 22 h, just before total collapse of the leaves (after which mRNA could no longer be isolated), AOX transcript levels were increased 100-fold. Leaves infiltrated with the virulent strain showed a similar increase of AOX transcripts; however, it was delayed by about 1 d (Fig. 2A). These differential time-dependent induction patterns were associated with the rate of necrosis development in the infiltrated leaves. Noninfected leaves of infected plants did not show any induction after infiltration with either the avirulent or the virulent *Pst* strain, indicating that there was no measurable systemic increase in AOX transcripts (Fig. 2B).

To compare the induction of AOX transcripts with that of SAR-associated PR-1 mRNA, we measured PR-1 transcript levels in the same samples. The results (Fig. 2, C and D) show that, like AOX transcripts, the PR-1 transcript levels

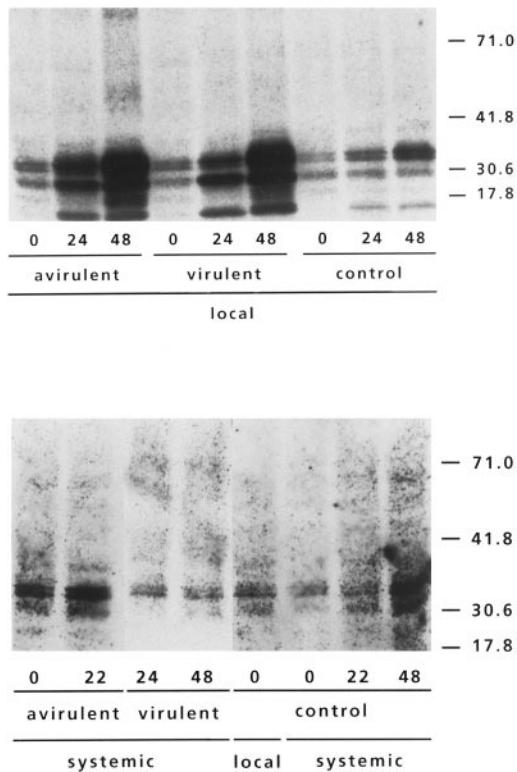
increased upon infiltration of the *Pst* strains into the leaves. Induction of PR-1 occurred earlier after infiltration with the avirulent strain than with the virulent strain, but it reached similar levels by 22 h. In contrast to the AOX transcript levels, however, PR-1 transcript levels were increased systemically. This increase developed more rapidly in the incompatible than in the compatible plant-pathogen combination (Fig. 3, C and D). Identical results were obtained in independent replicates.

Two *Pseudomonas fluorescens* strains (WCS374 and WCS417) were used as nonpathogenic controls to determine whether the induction of AOX was specific for an interaction with a pathogen. Neither of these two nonpathogenic strains induced AOX within 2 d after infiltration of the leaves with  $10^7$  CFU mL $^{-1}$  (not shown), nor did they cause any visible symptoms in the leaves for the duration of the experiment. These results indicate that AOX induction is associated with infection of either a susceptible host by a virulent strain or a resistant host by an avirulent strain of a pathogen rather than with bacterial infiltration.

It is well established that there is a linear relationship between the concentration of avirulent bacteria infiltrated into a leaf and the extent of necrosis (Turner and Novacky, 1974). It has been estimated that leaves collapse completely when about 25% of the plant cells are involved in the HR, which occurs upon infiltration with a concentration of about  $10^7$  CFU mL $^{-1}$ . To determine the extent of the correlation between the concentration of the bacterial suspen-



**Figure 2.** AOX and PR-1 transcript levels in *Arabidopsis* treated with avirulent (avir) or virulent (vir) *Pst*. Leaves were infiltrated with bacterial suspensions containing  $10^7$  CFU, and transcript levels were determined in the infiltrated leaves and in the unaffected, systemic leaves of the same plants. Values are given relative to the control level (set at 1) in mock-infiltrated leaves. ND, Not detectable.



**Figure 3.** Detection of AOX protein in Arabidopsis leaves treated with avirulent or virulent *Pst*. AOX was detected on western blots, using immunological staining of the protein. Top, Treated leaves: lanes 1 to 3, at 0, 24, and 48 h, respectively, after infiltration with the avirulent *Pst* strain; lanes 4 to 6, at 0, 24, and 48 h, respectively, after infiltration with the virulent *Pst* strain; and lanes 7 to 9, at 0, 24, and 48 h, respectively, after mock infiltration. Bottom, Nontreated leaves (except lane 5): lanes 1 and 2, at 0 and 22 h, respectively, after infiltration with the avirulent *Pst* strain; lanes 3 and 4, at 24 and 48 h, respectively, after infiltration with a virulent *Pst* strain; lane 5, control level in treated leaf immediately after mock infiltration (0 h); and lanes 6 to 8, at 0, 22, and 48 h, respectively, after mock infiltration.

sion and AOX expression, leaves were infiltrated with a range of bacterial concentrations. When  $5 \times 10^5$  CFU of the avirulent strain per mL was used, the leaves did not show visible symptoms, whereas a concentration of  $2$  to  $4 \times 10^6$  CFU  $\text{mL}^{-1}$  resulted in a delayed necrosis becoming visible 4 d after infiltration. Nevertheless, infiltration of no more than  $2 \times 10^6$  CFU of the avirulent pathogen per mL resulted in a rapid, substantial increase in AOX transcripts 6 h after infiltration. No increase in AOX transcripts was detectable in leaves infiltrated with  $5 \times 10^5$  CFU of the virulent pathogens per mL (Table I). Leaves infiltrated with less than  $10^7$  CFU of the compatible pathogen per mL also showed delayed disease symptoms, which was associated with a delayed AOX induction (Table I).

#### Immunodetection of AOX Protein Reveals That It Mainly Occurs in a Reduced (High-Activity) Form in Infected Plant Leaves

To study whether the induction of the AOX mRNA after bacterial infiltration is accompanied by a higher concentra-

tion of the AOX protein, AOX was analyzed by western blotting. Leaves infiltrated with avirulent or virulent *Pst* showed an increase in AOX protein (Fig. 3A) in accordance with the increase in AOX transcript levels. Two major bands were visible on western blots, one larger than the 30.6-kD marker and one with an apparent molecular mass that was smaller than the 30.6-kD marker. As was found previously (Elthon et al., 1989), a band that is smaller than the 17.8-kD marker appeared as well; we will not further discuss this smallest band. As suggested by Kumar and Söll (1992), the smaller protein might represent a partial degradation product of the larger protein. However, since AOX in Arabidopsis is encoded by a multigene family (Saisho et al., 1997), as it is in soybean (Whelan et al., 1996), the different bands may reflect the expression of different genes. The intensity of both larger bands was enhanced in the pathogen-infiltrated leaves, and two smaller bands became visible. No readily detectable increase of AOX protein was found in noninfiltrated leaves of treated plants, in agreement with the finding that AOX transcript levels were not increased systemically.

In vivo the AOX enzyme occurs as a dimer, the subunits of which are linked by disulfide bridges. The oxidized form, in which the two monomers are covalently linked, is much less active than the reduced form of the AOX dimer (Umbach and Siedow, 1993). A large pool of reduced AOX was detectable in the samples from leaves infiltrated with either avirulent or virulent *Pst* (Fig. 3), indicating that a large portion of the enzyme was in its activated form. As in the intact roots of *Poa annua* (Millenaar et al., 1998), the oxidized form of the protein could be visualized by this method only after a prolonged exposure of the film.

#### Induction of AOX in Compatible and Incompatible Plant-Pathogen Combinations Is Not Dependent on SA but Is Associated with Necrosis

The observation that PR-1 is strongly induced systemically, whereas AOX is not, indicates that the induction pathways are different or that the threshold level for the induction of AOX is not reached in systemic noninfected

**Table I.** Relative AOX transcript levels in leaves of Arabidopsis ecotype Col-0 infiltrated with different concentrations of avirulent or virulent *Pst*

The AOX transcript level in the control treatment (mock) is set at 1.

Treatment	Concentration $\text{CFU mL}^{-1}$	Time		
		6 h	22 h	48 h
Mock		1	1	1
Avirulent <i>Pst</i>	$10^7$	10	100	ND <sup>a</sup>
	$4 \times 10^6$	5	10	5
	$2 \times 10^6$	5	5	1
	$5 \times 10^5$	1	1	1
Virulent <i>Pst</i>	$10^7$	1	10	100
	$2 \times 10^6$	1	1	5

<sup>a</sup> ND, Not detectable. mRNA could no longer be isolated because of complete necrosis of the leaves.

leaves. To investigate a possible relationship between systemic effects of SA and the induction of AOX mRNA, leaves of an Arabidopsis *npr1-1* mutant that is unable to express PRs or SAR upon infection with avirulent *Pst* were infiltrated with the pathogen. AOX was normally induced in the infiltrated leaves (Table II), whereas, as expected, no PR-1 mRNA was detected (not shown). This result clearly demonstrates that the induction pathways of PR-1 (and SAR) and AOX after infection are different.

To investigate whether exogenously applied SA induces AOX in Arabidopsis, leaves were infiltrated with solutions containing 0.01, 0.1, 0.5, or 1 mM SA. Higher concentrations resulted in toxic effects. At the applied SA concentrations, there was no increase in AOX transcript levels after 6, 24, or 48 h (not shown). In contrast, 1 d after infiltration with 1 mM SA, PR-1 transcript levels had increased approximately 10-fold; 0.1 mM was the lowest SA concentration that gave a slight increase (5-fold) in PR-1 transcripts, which was detected 1 d after infiltration (not shown).

A possible involvement of SA in AOX induction during pathogenesis was further investigated in leaves infiltrated with the virulent pathogen using SA-degrading transgenic *nahG* plants. No major differences in disease development were observed compared with those in wild-type Arabidopsis. Determination of the AOX transcript levels showed that the AOX induction during the first 22 h was similar in wild-type and *nahG* plants (Table II). In contrast, there were clear differences in symptom development between wild-type and *nahG* plants that were infiltrated with the avirulent pathogen. In the transgenic *nahG* plants, symptoms appeared later and necrosis developed as in the compatible combination. Also, AOX induction in these leaves was delayed and similar to the compatible combination (Table II). PR-1 transcript levels remained very low in

*NahG* plants, in contrast to those in the wild type (Table II), confirming that SA levels in the transgenic plants do not increase after infection. Thus, the finding that AOX transcript levels were significantly increased during both the compatible and incompatible interaction indicates that SA is not essential for AOX induction during these infections.

On the other hand, the finding that in the *NahG* plants the "fast" AOX induction 6 h after infiltration with the avirulent pathogen was abolished (Table II) suggested that SA-dependent processes must be involved in AOX induction during the incompatible interaction. Because the HR was also delayed in the transgenic *NahG* (Delaney et al., 1994), HR might be associated with the fast AOX induction. To test this hypothesis, leaves of the Arabidopsis mutant *rps2-201* were infiltrated with the normally avirulent pathogen *Pst* DC3000 (*avrRpt2*). This mutant plant contains a defect in the *rps2* resistance gene (Kunkel et al., 1993; Yu et al., 1993) and is therefore compatible with *Pst* DC3000 (*avrRpt2*). Upon infiltration of the leaves with this pathogen, the fast AOX induction observed in the leaves of wild-type Arabidopsis was absent in the *rps2-201* plants. Instead, the induction of AOX in the mutant plant was similarly timed as that upon infiltration with the virulent pathogen (Table II). This result implies that the fast AOX induction, as with the incompatible interaction, is indeed associated with the rapid cell death that occurs during an HR. The comparatively slow induction of AOX in the compatible combinations is most likely associated with delayed necrosis of the plant tissue.

#### Total Respiration and Cyanide-Resistant, SHAM-Sensitive O<sub>2</sub>-Uptake Rates Are Not Affected in Leaves of *etr1-1* Plants Infiltrated with an Avirulent *Pst* Strain

To study the possible involvement of ethylene-dependent processes in AOX induction upon infection, O<sub>2</sub> uptake was measured using wild-type and *etr1-1* mutant plants. Leaves were infiltrated with the avirulent *Pst* strain, because the respiratory increase of infected leaves of the wild type was most predictable with this pathogen. To measure O<sub>2</sub> uptake during a prolonged period (2 d), leaves of wild-type plants were infiltrated with bacterial suspensions, containing from 5 × 10<sup>5</sup> to 10<sup>7</sup> CFU. With 5 × 10<sup>5</sup> CFU, no disease symptoms were visible until 6 d after infiltration; however, there was a considerable and rapid increase in cyanide-resistant, SHAM-sensitive O<sub>2</sub> uptake, which remained constant between 20 and 48 h (Fig. 4A). In contrast to wild-type Arabidopsis, leaves of *etr1-1* mutant plants, infiltrated with the same bacterial suspensions, did not show an increase in the total or cyanide-resistant, SHAM-sensitive rate of O<sub>2</sub> uptake with time (Fig. 4B). These results indicate that ethylene-dependent processes are required for the induction of AOX during infection of Arabidopsis with avirulent *Pst*.

#### Expression of AOX Is Dependent on Ethylene

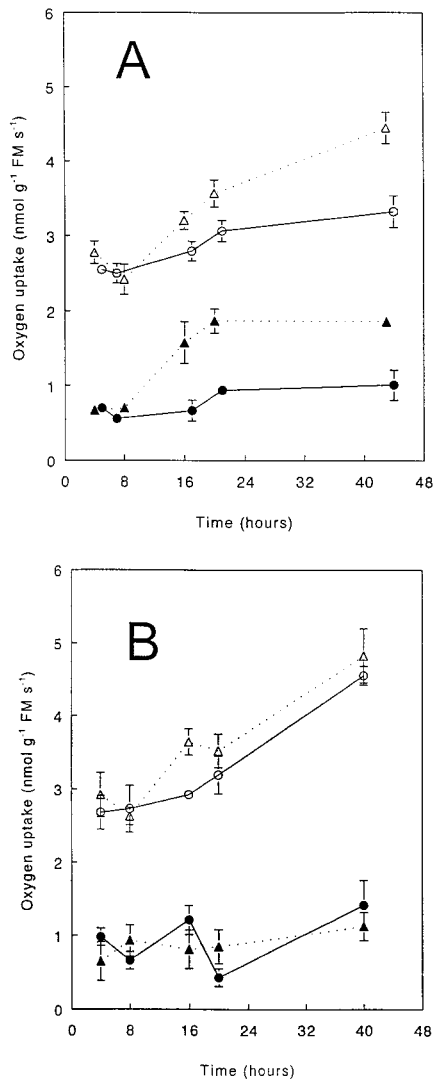
For a molecular analysis of the possible involvement of ethylene in the induction of AOX, AOX transcript levels were monitored in leaves of wild-type and *etr1-1* plants

**Table II.** Relative AOX transcript levels in leaves of different transgenic and/or mutant Arabidopsis plants infiltrated with avirulent (Avir) or Virulent (Vir) *Pst*

Leaves were infiltrated with 10<sup>7</sup> CFU per mL or, as a control, mock infiltrated with 10 mM MgCl<sub>2</sub>. For a description of the different transgenics and/or mutants, see the text. The AOX transcript level of the control (mock-treated) leaves of the wild type is set at 1. For wild-type and *NahG* plants, PR-1 transcript levels are presented in parentheses, with control (mock-treated) leaves of the wild type set at 1.

Arabidopsis	Type of Infiltration	Time		
		6 h	22 h	48 h
Wild type	Mock	1 (1)	1 (1)	1 (1)
	AvirPst	10 (10 <sup>2</sup> )	100 (10 <sup>3</sup> )	ND <sup>a</sup>
	VirPst	1 (1)	10 (10 <sup>3</sup> )	100 (10 <sup>3</sup> )
<i>NahG</i>	Mock	1 (10 <sup>-2</sup> )	1 (10 <sup>-2</sup> )	1 (10 <sup>-2</sup> )
	AvirPst	1 (10 <sup>-2</sup> )	10 (10 <sup>-1</sup> )	ND
	VirPst	1 (10 <sup>-2</sup> )	10 (10 <sup>-2</sup> )	ND
<i>npr1-1</i>	Mock	1	1	- <sup>b</sup>
	AvirPst	10	ND	-
	VirPst	1	10	-
<i>rps2-201</i>	Mock	1	1	-
	AvirPst	1	10	-
	VirPst	1	10	-

<sup>a</sup> ND, Not detectable. mRNA from this tissue could no longer be isolated because of complete necrosis. <sup>b</sup> -, Not determined.



**Figure 4.** Total and cyanide-resistant, SHAM-sensitive  $O_2$ -uptake rates of wild-type (A) and *etr1-1* (B) Arabidopsis leaves infiltrated with an avirulent strain of *Pst*. Leaves were infiltrated with suspensions containing  $5 \times 10^5$  CFU of the pathogens per mL. Circles, Control; triangles, avirulent pathogen. Open symbols, Total  $O_2$  uptake of the leaves; closed symbols, cyanide-resistant, SHAM-sensitive  $O_2$  uptake. Results are mean values  $\pm$  SE;  $n = 4$ . FM, Fresh mass.

infiltrated with the avirulent or virulent *Pst* strain. In the *etr1-1* plants the AOX transcript levels were only slightly increased upon infection with the avirulent *Pst* strain, compared with those in the wild type. The compatible combination did not result in a detectable increase in AOX transcript levels in *etr1-1* (data not shown).

#### Pyruvate Accumulates in Tissue Infiltrated with the Pathogenic Bacteria

Infected plant tissues accumulate hexoses (Farrar, 1992), which can be used in several defense-related biosynthetic pathways and/or converted into large amounts of respiratory substrate. When the Cyt pathway is unable to cope with the latter, one might imagine that the alternative

pathway is required. Flooding of the Cyt pathway with a respiratory substrate is likely to result in the accumulation of pyruvate (Vanlerberghe and McIntosh, 1996). Pyruvate is also a strong activator of AOX in vitro (Millar et al., 1993) and thus might be a feed-forward regulator of the alternative pathway in vivo. Therefore, levels of pyruvate in the leaf tissues infiltrated with the avirulent pathogen were determined. Necrotizing leaves showed a 4-fold increase of the pyruvate concentration 22 h after infiltration (Table III). It is unlikely that the bacteria contributed significantly to these increased pyruvate levels in the infected leaves, because their fresh weight was less than 0.01% of the total fresh weight of the infected leaves. These results suggested that the mitochondria was flooded with respiratory substrate and that the alternative electron-transport pathway was fully activated.

#### DISCUSSION

An increase in respiration is a widespread phenomenon in plant-pathogen interactions (Farrar, 1992; Lennon et al., 1997). The present study suggests that the alternative pathway might be a major contributor to the increase in respiration in Arabidopsis upon infection with avirulent and virulent *P. syringae* strains. This was evidenced by strongly increased AOX transcript levels, increased amounts of reduced (high-activity) AOX protein, and increased cyanide-resistant, SHAM-sensitive  $O_2$ -uptake rates in the infected leaves. Because SHAM did not affect leaf respiration in the absence of KCN, we have no evidence that the alternative pathway contributed to the enhanced leaf respiration. Infection with the tobacco mosaic virus in tobacco also enhances AOX protein levels, but it has no effect on the contribution of the alternative pathway to leaf respiration (Lennon et al., 1997).

Because SA acts as a signal in the induction of AOX in *Arum* lily flower stalks and in tobacco leaves and as a signal of PRs and SAR during resistance responses, the following questions have been addressed: Is AOX induced during plant diseases? Is such induction associated with the expression of PRs? Do SA and/or ethylene act as a signal(s) in the induction of AOX during infection in Arabidopsis leaves? In Arabidopsis AOX was strongly induced in the infected tissue with both the incompatible and the compatible combination. In treated leaves the expression of AOX and PR-1 mRNA upon infection was correlated in time, but in systemic leaves AOX transcript levels were not increased, in contrast to the transcript levels in PR-1. This

**Table III.** Pyruvate concentration in leaves of Arabidopsis 22 h after infiltration of the leaves with avirulent *Pst*

The data are from two independent experiments. The bacterial suspension contained  $10^7$  CFU mL<sup>-1</sup>.

Experiment	Control <sup>a</sup>	<i>Pst</i> (pLH12)	Percentage
<i>nmol g<sup>-1</sup> fresh wt</i>			
1	120	492	410
2	98	383	391

<sup>a</sup> Mock-infiltrated leaves (10 mM MgCl<sub>2</sub>).

is at variance with the results in tobacco, in which AOX protein levels increased both in tobacco mosaic virus-infected and in noninfected systemic leaves (Lennon et al., 1997). Application of SA did not induce AOX in Arabidopsis, and AOX induction was not abolished in *NahG* plants, indicating that SA accumulation is not essential in the induction of AOX due to bacterial infection in Arabidopsis. In contrast to AOX transcript levels, PR-1 transcript levels remained low in the *NahG* plants, even in the infiltrated leaves. Because PR-1 expression is associated with SA accumulation, AOX and PR-1 expression in Arabidopsis must be regulated by different signals. Direct evidence showing the involvement of different signaling pathways for induction of PR-1 and AOX came from experiments with the *npr1-1* mutant, which is unable to express PRs and SAR. Immediately upon infiltration of the leaves with either the avirulent or the virulent *Pst* strain, this mutant showed AOX-induction patterns (in time and place) similar to those in the wild-type plant.

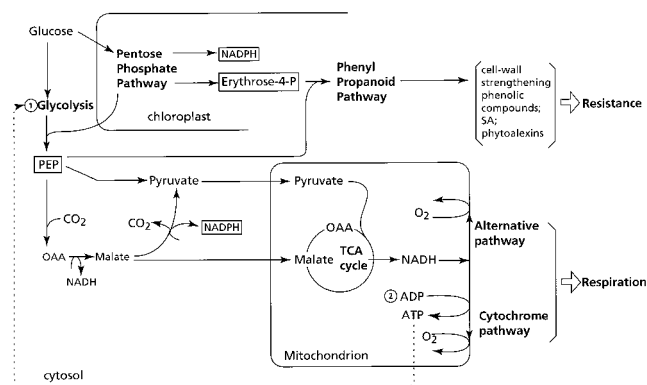
Although SA was clearly not essential for induction of AOX, fast induction of AOX during the avirulent plant-pathogen combination was abolished in both *nahG* and *npr1-1* plants, indicating that SA-dependent processes do play a role. Fast induction of AOX in the avirulent plant-pathogen combination was associated with HR. Lower amounts of the avirulent pathogen resulted in lower AOX transcript levels in the leaves (Table II), indicating a direct relationship between the level of induction and the amount of tissue affected. The delayed induction of AOX in the compatible combination suggests that HR accelerates but is not essential for the induction of the AOX. Apparently the AOX induction is associated with the development of necrosis. It is well known that respiratory changes occurring as a result of infection in plants are not limited to the infected cells but also take place in the surrounding tissue (Farrar, 1992). Therefore, the increases in AOX transcript levels, AOX protein, and total and cyanide-resistant, SHAM-sensitive respiration in Arabidopsis leaves infected with *Pst* most likely take place in the vicinity of necrotizing plant cells. Previous reports have shown that the increases in total and cyanide-resistant plant respiration can be stronger in a susceptible plant compared with those in a resistant one (Farrar and Rayns, 1987). Such increases are caused by the localization of the pathogen in the avirulent plant-pathogen combination; consequently, less tissue is affected. The major difference between the reaction of a resistant and a susceptible plant appears to be the speed at which respiration increases and AOX induction occurs.

Ethylene plays a major role in the induction of AOX upon infection. Based on the transcript levels, AOX induction in the *etr1-1* mutant plants was almost completely abolished in leaves infiltrated with either the virulent or avirulent pathogen. Moreover,  $O_2$ -uptake rates in the presence of cyanide were not increased in the leaves of the *etr1-1* plant infiltrated with the avirulent pathogen. Taken together, the results suggest that strong, local AOX induction during infections is associated with ethylene production at the site of infection. Ethylene is strongly increased at an early stage in an HR (De Laat and Van Loon, 1983;

Boller, 1991), indicating a functional role for the rapid action of AOX in the incompatible combination.

Although local increases of AOX transcripts corresponded well with increased AOX protein levels and increased cyanide-resistant, SHAM-sensitive respiration, the actual contribution of the alternative pathway to the respiration in infected tissue was not determined. This would require the use of the  $O_2$  discrimination technique (Day et al., 1996). The AOX pool in the plant tissue ultimately determines the maximum contribution of the alternative pathway to total respiration. The actual contribution can be strongly modulated, however, by the state of the disulfide bridge between two AOX subunits, because the reduced dimer is much more active than the oxidized, covalently bound form. Our western blotting results show that, at least during the later stage of infection, the amount of the reduced (high-activity) AOX dimer was increased. This suggests that AOX could significantly contribute to respiration in these tissues. Furthermore, pyruvate levels in the Arabidopsis leaves infiltrated with the avirulent pathogen were strongly increased. This suggests that the mitochondria are flooded with respiratory substrate and activator, a condition that is likely to favor full operation of the alternative pathway in the affected cells.

How can the resistance response and the energy-wasting alternative pathway be linked at the physiological level? A schematic representation of the main components and pathways involved in the physiological response of plant cells to infection and other stresses, such as wounding and chilling injury, are depicted in Figure 5. Infections and other stresses are associated with enhanced biosynthesis of aromatic compounds. The oxidative pentose-phosphate pathway provides erythrose-4-phosphate, which condenses with PEP into the precursor of numerous phenylpropanoids that are implicated in resistance reactions. The NADPH that is produced might act as a reductant in numerous stress-related reactions. Another source for



**Figure 5.** Major metabolic pathways involved in the resistance response to pathogens and its association with respiration. Important intermediates involved in the biosynthesis of several defense compounds are depicted in boxes. Numbers refer to major control points for glycolysis due to allosteric inhibition of phosphofructokinase by ATP (1) and inhibition of oxidative phosphorylation by limiting amounts of ADP (2). TCA cycle, Tricarboxylic acid cycle; OAA, oxaloacetic acid.



NADPH is the cytosolic NADP-malic enzyme, which catalyzes the oxidation of malate to pyruvate and CO<sub>2</sub>. This enzyme is induced upon the addition of elicitors, suggesting that it is involved in primary metabolism changes after infection (Schaaf et al., 1995). The pentose-phosphate pathway, which may account for 90% of the breakdown of Glc during infections (Shaw and Samborski, 1957), bypasses the allosteric adenylate control of glycolysis. In combination with the enhanced activity of the cytosolic NADP-malic enzyme, this can lead to an accumulation of pyruvate, particularly if the Cyt pathway in the affected tissue is somehow restricted. That the operation of the alternative pathway does not contribute to the formation of ATP might not be disadvantageous, because many defense-related reactions require NADPH rather than ATP, as shown in Figure 5.

It has been proposed that operation of the alternative pathway during environmental stresses might (partly) relieve the mitochondrial electron-transport pathway (Purvis and Shewfelt, 1993; Wagner and Krab, 1995). This could prevent overreduction that might result in the formation of harmful radicals. Formation of O<sub>2</sub> radicals is instrumental in necrotization, but limited lesion formation during avirulent reactions requires that surrounding tissues be protected. This is clearly illustrated by the occurrence of disease lesion mimics, in which spontaneous necrosis may spread because of mutations (Dietrich et al., 1994). During infections the formation of radicals might worsen disease symptoms. For a successful resistance response the production of radicals might be an important feature, because the performance of a plant during disease is determined not only by its ability to localize the pathogen but also by its capacity to minimize tissue damage. Because several other stress conditions induce AOX, we speculate that cyanide-resistant respiration is important to the plant for acclimation to adverse conditions. How much this enables the plant to cope with such stresses awaits experiments with plants that cannot express AOX.

#### ACKNOWLEDGMENTS

The authors wish to thank Dr. Patrick M. Finnigan (Australian National University, Canberra) for critically reading the manuscript, Dr. Jim Whelan (University of Western Australia, Netherlands) for providing advice and the AOX-specific degenerate primers, Dr. Thomas E. Elthon (University of Nebraska, Lincoln) for providing the AOX-specific monoclonal antibodies, and Dr. Andrew Bent (University of Illinois, Urbana) for providing the *Pst* strains. We would also like to thank Erik De Vlieger for his help with obtaining the data concerning the effects of SHAM on leaf respiration.

Received December 3, 1998; accepted February 23, 1999.

#### LITERATURE CITED

- Atkin OK, Cummings WR, Collier DE (1993) Light induction of alternative pathway capacity in leaf slices of Belgium endive. *Plant Cell Environ* **16**: 231–235
- Bleecker AB, Estelle M, Somerville C, Kende H (1988) Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. *Science* **241**: 1086–1089
- Boller T (1991) Ethylene in pathogenesis and disease resistance. In AK Mattroo, JD Suttle, eds, *The Plant Hormone Ethylene*. CRC Press, Boca Raton, FL, pp 293–314
- Brederode FT, Linthorst HJM, Bol JF (1991) Differential induction of acquired resistance and PR gene expression on tobacco by virus infection, ethephon treatment, UV light and wounding. *Plant Mol Biol* **17**: 1117–1125
- Cameron RK, Dixon RA, Lamb CJ (1994) Biological induced systemic resistance in *Arabidopsis thaliana*. *Plant J* **5**: 715–725
- Cao H, Bowling SA, Gordon AS, Dong X (1994) Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. *Plant Cell* **6**: 1583–1592
- Day DA, Krab K, Lambers H, Moore AL, Siedow JN, Wagner AM, Wiskich JT (1996) The cyanide-resistant oxidase. To inhibit or not to inhibit, that is the question. *Plant Physiol* **110**: 1–2
- De Laat AMM, Van Loon LC (1982) Regulation of ethylene biosynthesis in virus-infected tobacco leaves. II. Time course of levels of intermediates and in vivo conversion rates. *Plant Physiol* **69**: 240–245
- De Laat AMM, Van Loon LC (1983) The relationship between stimulated ethylene production and symptom expression in virus-infected tobacco leaves. *Physiol Plant Pathol* **22**: 261–273
- Delaney TP, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, Gaffney T, Gut-Rella M, Kessmann H, Ward E, and others (1994) A central role of salicylic acid in plant disease resistance. *Science* **266**: 1247–1250
- Dietrich R, Delaney T, Uknes S, Ward E, Ryals J, Dangi J (1994) *Arabidopsis* mutants simulating disease response. *Cell* **77**: 565–577
- Dong X, Mindrinos M, Davis KR, Ausubel FM (1991) Induction of *Arabidopsis* defense genes by virulent and avirulent *Pseudomonas syringae* strains and by a cloned avirulence gene. *Plant Cell* **3**: 61–72
- Elthon TE, Nickels RL, McIntosh L (1989) Monoclonal antibodies to the alternative oxidase of higher plant mitochondria. *Plant Physiol* **89**: 1311–1317
- Farrar JF (1992) Beyond photosynthesis: the translocation and respiration of diseased leaves. In PG Ayres, ed, *Pests and Pathogens*. BIOS Scientific Publishers, Oxford, UK, pp 107–127
- Farrar JF, Rayns FW (1987) Respiration of barley infected with powdery mildew: increased engagement of the alternative oxidase. *New Phytol* **107**: 119–125
- Geels FP, Schippers B (1983) Selection of antagonistic fluorescent *Pseudomonas* spp. and their root colonization and persistence following treatment of seed potatoes. *Phytopathol Z* **108**: 193–206
- Hiser C, McIntosh L (1990) Alternative oxidase of potato is an integral membrane protein synthesized de novo during aging of tuber slices. *Plant Physiol* **93**: 312–318
- Hoagland DR, Arnon DI (1938) The water culture method for growing plants without soil. *Calif Agric Exp Stn Bull* **347**: 36–39
- King EO, Ward MK, Raney DE (1954) Two simple media for demonstration of phycocyanin and fluorescein. *J Lab Clin Med* **44**: 301–307
- Kumar AM, Söll D (1992) *Arabidopsis* alternative oxidase sustains *Escherichia coli* respiration. *Proc Natl Acad Sci USA* **89**: 10842–10846
- Kunkel BN, Bent AF, Dahlbeck D, Innes RW, Staskawicz BJ (1993) *RPS2*, an *Arabidopsis* disease resistance locus specifying recognition of *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. *Plant Cell* **5**: 865–875
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680–685
- Lamers JG, Schippers B, Geels FP (1988) Soil-borne disease of wheat in The Netherlands and results of seed bacterization with pseudomonads against *Gaeumannomyces graminis* var. *tricici*. In ML Jorna, LA Sloodmaker, eds, *Cereal Breeding Related to Integrated Cereal Production*. Pudoc, Wageningen, The Netherlands, pp 134–139
- Laties GG (1982) The cyanide-resistant, alternative path in higher plant respiration. *Annu Rev Plant Physiol* **33**: 519–555
- Lennon AM, Neuenschwander UH, Ribas-Carbo M, Giles L, Ryals JA, Siedow JN (1997) The effect of salicylic acid and tobacco mosaic virus infection on the alternative oxidase of tobacco. *Plant Physiol* **115**: 783–791

- Meeuse BJD** (1975) Thermogenic respiration in aroids. *Annu Rev Plant Physiol* **26**: 117–126
- Millar AH, Wiskich JT, Whelan J, Day DA** (1993) Organic acid activation of the alternative oxidase of plant mitochondria. *FEBS Lett* **329**: 259–262
- Millenaar FF, Benschop J, Wagner AM, Lambers H** (1998) The role of the alternative oxidase in stabilizing the in vivo reduction state of the ubiquinone pool and the activation state of the alternative oxidase. *Plant Physiol* **118**: 599–607
- Moore AL, Siedow JN** (1991) The regulation and nature of the cyanide-resistant alternative oxidase of plant mitochondria. *Biochim Biophys Acta* **1059**: 121–140
- Pieterse CMJ, Van Wees SCM, Hoffland E, Pelt JA, Van Loon LC** (1996) Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *Plant Cell* **8**: 1225–1237
- Purvis AC, Shewfelt RL** (1993) Does the alternative pathway ameliorate chilling injury in sensitive plant tissues? *Physiol Plant* **88**: 712–718
- Raskin I, Ehmann A, Melander WR, Meeuse BJD** (1987) Salicylic acid: a natural inducer of heat production in *Arum* lilies. *Science* **237**: 1601–1602
- Rhoads DM, McIntosh L** (1992) Salicylic acid regulation of respiration in higher plants: alternative oxidase expression. *Plant Cell* **4**: 1131–1139
- Rhoads DM, McIntosh L** (1993) Cytochrome and alternative pathway respiration in tobacco. Effects of salicylic acid. *Plant Physiol* **103**: 877–883
- Saisho D, Nambara E, Naito S, Tsutsumi N, Hirai A, Nakazone M** (1997) Characterization of the gene family for alternative oxidase from *Arabidopsis thaliana*. *Plant Mol Biol* **35**: 585–596
- Schaaf J, Walter MH, Hess D** (1995) Primary metabolism in plant defense. Regulation of a bean malic enzyme gene promoter in transgenic tobacco by developmental and environmental cues. *Plant Physiol* **108**: 949–960
- Schaller GE, Bleecker AB** (1995) Ethylene-binding sites generated in yeast expressing the *Arabidopsis ETR1-1* gene. *Science* **270**: 1809–1811
- Shaw M, Samborski DJ** (1957) The physiology of host-parasite relations: the pattern of respiration in rusted and mildewed cereal leaves. *Can J Bot* **35**: 389–407
- Siebert PD, Larrick JW** (1992) Competitive PCR. *Nature* **359**: 557–558
- Siedow JN, Umbach AL** (1995) Plant mitochondrial electron transfer and molecular biology. *Plant Cell* **7**: 821–831
- Swanson J, Kearney B, Dahlbeck D, Staskawicz BJ** (1988) Cloned avirulence gene of *Xanthomonas campestris* pv *vesicatoria* complements spontaneous race change mutant. *Mol Plant-Microbe Interact* **1**: 5–9
- Turner JG, Novacky A** (1974) The quantitative relation between plant and bacterial cells involved in the hypersensitive reaction. *Phytopathology* **64**: 885–890
- Uknes S, Winter AM, Delaney T, Vernooij B, Morse A, Friedrich L, Nye G, Potter S, Ward E, Ryals J** (1993) Biological induction of systemic acquired resistance in *Arabidopsis*. *Mol Plant-Microbe Interact* **6**: 692–698
- Umbach AL, Siedow JN** (1993) Covalent and noncovalent dimers of the cyanide-resistant alternative oxidase protein in higher plant mitochondria and their relationship to enzyme activity. *Plant Physiol* **103**: 845–854
- Uritani I, Asahi T** (1980) Respiration and related metabolic activity in wounded and infected tissues. In PK Stumpf, EE Conn, eds, *The Biochemistry of Plants*, Vol 2. Academic Press, London, pp 463–485
- Vanlerberghe GC, McIntosh L** (1992) Lower temperature increases alternative pathway capacity and alternative oxidase protein in tobacco. *Plant Physiol* **100**: 115–119
- Vanlerberghe GC, McIntosh L** (1996) Signals regulating the expression of the nuclear gene encoding alternative oxidase of plant mitochondria. *Plant Physiol* **111**: 589–595
- Wagner AM, Krab K** (1995) The alternative respiration pathway in plants: role and regulation. *Physiol Plant* **95**: 318–325
- Wagner AM, Wagner MJ** (1995) Measurements of in vivo ubiquinone reduction levels in plant cells. *Plant Physiol* **108**: 277–283
- Whalen MC, Innes RW, Bent AF, Staskawicz BJ** (1991) Identification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. *Plant Cell* **3**: 49–59
- Whelan J, Millar AH, Day DA** (1996) The alternative oxidase is encoded in a multigene family in soybean. *Planta* **198**: 197–201
- Yu G-L, Katagiri F, Ausubel FM** (1993) *Arabidopsis* mutations at the *RPS2* locus result in loss of resistance to *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. *Mol Plant-Microbe Interact* **6**: 434–443