The PhytO3 Tech Crop Protection Technology for Microorganism and Insect Control Using Ozone, UV, and Dipole-Electrical Air Jet Spray Technologies

Technical Basis and Chemistries Involved

Hanspeter Steffen[1] and Rip G. Rice[2]

[1] Hanspeter Steffen, SwissFood Tech Management, Inc., Baar, Switzerland, hp.steffen@swissfoodtech.com

[2] Rip G. Rice, RICE International Consulting Enterprises, 1710 Hickory Knoll Road, Sandy Spring, MD 20860, RipRice4ozone@cs.com

Abstract

Plants can't walk away when they are attacked by pathogenic microorganisms and insects, or if they are exposed to any form of stress. They do not have a central nervous system that allows them to defend themselves or to ask for help! In the early part of the 20th century, it was discovered that growing plants can be stimulated to respond to stresses by developing a Systemic Acquired Resistance (SAR) to microorganisms and insects. During the last 50 years, and especially during the past decade, significant advances in this agronomical technology have been made. SARs have been proven to result from the application of many types of chemical formulations. Plants respond by generating their own chemicals internally that subsequently prevent attacks by microorganisms and insects.

Recently it has been shown that sequential treatment of growing plants with (1) an aqueous spray of high voltage, pulsed negatively charged water, followed immediately with (2) a spray of ozonated water containing 8 mg/L of ozone generated from oxygen, and that followed immediately by (3) high energy UV-C radiation, also causes plants to develop SARs to microorganisms and insects, but without the use of chemicals. The primary advantages of this new ozone-UV-based technology are (a) there are no harmful effects on the plants, (b) no toxic chemical residues remain on the plants, (c) the technology can be used in rainy weather, when crop protection is most necessary, (d) the technology is environmentally friendly (no chemical residues), and (e) the technology is cheaper for crop growers compared to current chemical approaches.

In this paper will be summarized the technical background of Systemic Acquired Resistance, how it is believed the 3-step PhytO3 Tech approach performs to stimulate SARs in crops, a description of field-operating equipment in use today in several European countries, and a cost analysis in comparison to current chemical treatments.

Key Words

Ozone; UV-Radiation; Agricultural Crops; Systemic Acquired Resistance; Negatively charged Water

Introduction

Just as the human body can be immunized to certain microorganism attacks and can recover from physical wounds, so growing agricultural plants have evolved a number of inducible defense mechanisms against attacks by pathogens (disease-causing agents) and physical wounding (by sucking and chewing insects) by producing and distributing resistance-inducing chemicals within the plants themselves. Ross (1961) showed that tobacco plants challenged with tobacco mosaic virus (TMV) subsequently developed increased resistance to secondary infection in localized and distal tissues (tissues farthest from the point of origin). This spread of resistance throughout the plant's tissues was termed "Systemic Acquired Resistance" (SAR).

Since the discovery by Ross (1961) of the SAR-triggering effect of TMV in tobacco plants, many other materials have been shown to produce similar SAR triggers. These agents include microorganisms, pathogens, some chemicals, some reactive oxygen species (ROS) such as hydrogen peroxide (Alvarez et al., 1998) and recently ozone (Mahalingam et al., 2003) and UV-C radiation (Nawrath et al., 2002). The resistance conferred is long-lasting, sometimes for the life of the plant, and is effective against a broad band of pathogens, including viruses, bacteria, fungi, and oomycetes (oospore-forming, non-photosynthetic fungi) (Ryals et al., 1996; Sticher et al., 1997).

Kachroo et al. (2003) introduced the subject of SAR with the statement that plants resist pathogen infection by inducing a defense response that is targeted specifically to combat invasion by the pathogen (Keen, 1990; Van der Hourn et al., 2002). In many cases, the induction of these responses is accompanied by localized cell death at the site of pathogen entry, which often is able to restrict the spread of pathogens to cells within and immediately surrounding the lesions. This phenomenon, known as the Hypersensitive Response (HR), is one of the earliest visible manifestations of induced defense response, and resembles programmed cell death in animals.

Concurrent with hypersensitive response development, defense mechanisms are triggered locally and in parts distant from the site of primary infection. This phenomenon, known as Systemic Acquired Resistance (SAR), is one of the most studied induced defense responses and is accompanied by a local and systemic increase in endogenous salicylic acid and a concomitant up-regulation of a large set of defense genes, including genes that encode pathogenesis-related (PR) proteins (Ward et al., 1991; Gaffney et al., 1993; Uknes et al., 1993; Dong, 2001).

Although the stimulation of SAR in growing agricultural plants has been known to occur for many years, a complete understanding of the whys and hows of SAR has not yet been revealed. Research efforts have increased, and within the past decade significant strides have been made. The most recent review article on the subject was published by Durrant and Dong (2004).

Nature of Systemic Acquired Resistance

Early experiments indicated that an infected plant leaf produces a systemic signal for SAR, and that this signal is not species-specific (Dean and Kuc, 1986; Jenns and Kuc, 1979). The nature of the systemic signal has been a subject of controversy for years, with evidence to support many theories. As of this writing, the most simplistic explanation for the observed and effective observation of systemic acquired resistance in plants involves the affected plant responding to attack by generating certain chemicals

internally. These chemicals then are distributed rapidly throughout the plant, where they provide a defense against further attack by outside forces.

Plant-Secreted SAR - Causing Chemicals

Chemicals that are secreted internally in major amounts by plants and that appear to have an effect in developing and procreating SAR include salicylic acid (o-hydroxybenzoic acid), ethylene ($H_2C=CH_2$), and jasmonic acid (3-oxo-2-pent-enylcyclopentylacetic acid), but additional chemicals are found to be produced in more recent studies. Research studies on these three major chemicals are numerous. A study by Maldonado et al. (2002) suggests that a lipid-based molecule might be the mobile signal for SAR. Kumar and Lessig (2003) found that SAR activity of lipid molecules is increased several times by the presence of salicylic acid.

Two additional chemicals are known to be secreted in response to attacks on growing plants by various agents, including ozone, hydrogen peroxide, and UV-C radiation – these are ethylene, $H_2C=CH_2$, a gas that is quite reactive with ozone, and jasmonic acid (3-oxo-2-pentenylcyclo-pentylacetic acid). These two pathways, plus those involving salicylic acid, may occur individually, or concurrently.

Steffen (2005a,b) points out that as of this date, more than 41 genes are known in plants responding to the elicitor ozone via the ethylene pathway. Ethylene as well as salicylic acid is used by the exposed plant as a messenger and elicitor of pathogenic defense gene expression, activating the same defense systems as salicylic acid.

The jasmonic acid generated in a plant in response to ozone and other elicitors, triggers the secretion of defense genes that are especially important in root zones of plants (Steffen, 2005a,b).

Elicitors can be generated by physical attack (chewing or sucking insects), microbiological (pathogenic) microorganisms, or chemicals (pesticides, herbicides, reactive oxygen species).

Transport of The Systemic Signal

How does the SAR signal travel throughout the plant? Again, the complete answers are not known, and the topic is the subject of much research. Girdling experiments have suggested that the SAR signal produced in inoculated leaves travels through the phloem (plant sieve tubes that conduct synthesized food substances from leaves to plant parts where they are needed) to upper leaves (Guedes et al., 1980; Ross, 1966). Later research has indicated that the phloem is the major conduit for SAR signals, but some fraction of the signal also may be able to move by a different route (Durrant and Dong, 2004).

Roles of Ozone, H_2O_2, And Other Active Oxygen Species

Numerous studies indicate the importance of “Active” or “Reactive” Oxygen Species in causing SAR as well. Alvarez et al. (1998) found that H_2O_2 accumulates in small groups of cells in uninoculated leaves of *Arabidopsis* after infection with a virulent strain of *P. syringae*. These “microbursts” of active oxygen species are produced within two hours after an initial oxidative burst in the inoculated tissue and are followed by the formation of microscopic hypersensitive response lesions. Using catalase to scavenge the peroxide, it was demonstrated that both the primary and secondary oxidative bursts are required for the

onset of SAR. Alvarez et al. (1998) proposed that microbursts of reactive oxidative species may activate defense responses at a low level throughout the plant, and that this contributes to the SAR-induced state.

A study by Paolacci et al. (2001) showed that exposure to gaseous ozone could elicit plant defense responses abiotically in leaves of bean plants. These authors suggested that these effects might rest on a sequence of molecular events leading to the hypersensitive response during plant-pathogen incompatible interactions. In that study, Paolacci et al. (2001) also refer to several earlier studies demonstrating the ozone-induced synthesis of phenylpropanoid molecules, of related isoflavanoid and stilbene phytoalexins (of which salicylic acid is a member), as well as of catechin (a flavin allelochemical and antioxidant that is synthesized downstream of other reactions) in several plant species.

The transcription of certain of the corresponding genes (following exposure to ozone) also has been studied [reviewed by Kangasjärvi et al., 1994; Langebartels et al., 1997; Schraudner et al., 1997; Sandermann et al., 1998)]. On ozone exposure, the activity of chalcone synthase (CHS) was found to increase in soybeans (Keen and Taylor, 1975) and in pine (Rosemann et al., 1991). The expression of phenylalanine lyase and CHS, together with those of other defense genes, enzymes and metabolites (among these flavone glycosides), was found to be enhanced in parsley after three to eight hours from the beginning of ozone exposure (Eckey-Kaltenbach et al., 1994a,b). Such defense responses were found to overlap at least in part with those elicited by UV-irradiation and by pathogens (Sandermann, 1996).

A review by Fermin-Muñoz (2000) found that the production of active oxygen species, such as superoxide anions, hydroxyl free radicals, and hydrogen peroxide (all formed when ozone is dissolved in water and exposed to UV-C light) have been observed in many plant-pathogen interactions, and are known to play an important role in plant defense (Wu et al., 1997). Plants have been engineered to continuously produce active oxygen species, for example, expression of a defective calmodulin gene (Oh et al., 1999) or a less active catalase (Chamnongpol et al., 1998) in transgenic tobacco led to increased accumulation of H_2O_2 and to an activated expression of pathogenesis related (PR) proteins.

Levine et al. (1994) showed that hydrogen peroxide plays an important role in establishment of both localized and systemic defense responses in plants. It has been recognized for some time that when plant leaves are contacted by hydrogen peroxide, their stomata react by closing. Very recently, however, Desikan et al. (2005) have discovered that a previously uncharacterized function for the *Arabidopsis* (*Arabidopsis thaliana*) ethylene receptor (ETR1) is that of mediating H_2O_2 signaling in stomatal guard cells, allowing the stomata to remain open, at least for a longer period than had been observed.

It should be borne in mind, however, that over-exposure of growing plants to ozone can cause cell death to occur (Rao and Davis, 1999; Rao et al., 2000a,b). Rao et al. (2002) discuss this eventuality, but proposed a schematic model to illustrate that ozone-induced, Hypersensitive Response cell death in *Arabidopsis* is the net result of extensive cross-talk between multiple acting signaling pathways that converge to modulate the type and magnitude of ozone-induced defense responses. Mechanisms in this model involve salicylic and jasmonic acids, as well as ethylene.

According to the Rao et al. (2002) model, upon entering leaf tissue through stomata, ozone generates excess active oxygen species (including H_2O_2), resulting in increased biosynthesis of signaling molecules (such as salicylic acid), which, in turn, potentiates the feedback amplification loop of runaway cell death cycle that induces the biosynthesis of signaling molecules such as ethylene. This chemical (ethylene) has been shown to induce lipases known to promote senescence, a slow form of cell death (Hong et al., 2000). As yet, it is unclear whether salicylic acid alone is sufficient to induce the production of ethylene.

However, ozone, either by reacting directly with membrane lipids (Mudd, 1997) and/or by generating excess active oxygen species, induces the biosynthesis of jasmonic acid or methyl jasmonate, which has been shown to reduce ozone-induced lesions both by attenuating SA-dependent lesion initiation (Rao et al., 2000b) and ethylene-dependent lesion propagation processes (Overmyer et al., 2000).

The Role Of Salicylic Acid In SAR

Salicylic acid (o-hydroxybenzoic acid) and its possible roles in developing and transporting SAR throughout plants has been the subject of a number of review articles (Dempsey et al., 1999; Dong, 2001; Ryals et al., 1996; Shah and Klessig, 1999). In many plants, but not all, SAR is preceded by an increase in salicylic acid within the plant. It is generally conceded today that salicylic acid is an essential signal for SAR across a range of plants, although the mechanism by which this acid induces SAR might differ with the plants. Salicylic acid is believed to be synthesized internally by the plant in response to attacks or wounds. Bacterial enzymes and proteins in the plants are believed to be responsible for generating salicylic acid, while many other biochemicals within the plant take part in controlling its synthesis (Durrant and Dong, 2004).

Enhancement of the salicylic acid signal also occurs through a signal amplification loop involving reactive oxygen species (Shirasu et al., 1997). Salicylic acid has been observed to bind the H_2O_2-scavenging enzymes catalase and ascorbate peroxidase, and inhibits their activity. This finding has led to the proposal that increases in H_2O_2 were responsible for signal transduction leading to pathogenesis-related gene induction and resistance (Chen et al., 1993; Darner and Klessig 1995). Later studies have suggested that H_2O_2 functions upstream of salicylic acid (León et al., 1995; Neuenschwander et al., 1995). Low concentrations of salicylic acid also have been shown to potentiate the production of reactive oxygen species and cell death. In soy bean cells inoculated with *P. syringae*, the addition of salicylic acid dramatically enhanced the oxidative burst and cell death (Shirasu et al., 1997; Tenhaken and Rübel, 1997). It is therefore hypothesized that in systemic tissues, the accumulation of low levels of salicylic acid, together with the development of microbursts of reactive oxygen species, could amplify responses to secondary infections (in plants) and contribute to SAR (Draper, 1997; Shirasu et al., 1997).

There is ample evidence to indicate that SAR is conferred by expression of a collection of genes (Durrant and Dong, 2004). The sequencing of the *Arabidopsis* genome has allowed global analysis of gene expression changes during SAR to be conducted using DNA microarray technology. A promoter analysis study conducted on 1058 genes induced by pathogen infection, salicylic acid, methyl jasmonate, or ozone, suggested a role for all of these materials in stress responses, but did not identify which were important during SAR (Mahalingam et al., 2003).

Interaction between SAR And Other Plant Defense Pathways

Durrant and Dong (2004) point out emphatically that it is impossible to understand SAR fully without studying its interaction with other biological processes that occur within growing plants. It has been hypothesized that plant defense pathways interact synergistically or antagonistically to fine-tune responses according to challenging organism(s). Different responses may confer resistance to the same pathogen. On the other hand, activation of one pathway may lead to cross-talk inhibition of another that is less effective against the challenging pathogen. Cross-talk between different defense pathways is reviewed by Bostock (1999).

Examples of cross-resistance have been found wherein insect feeding can induce aspects of SAR (Heil and Bostock, 2002). This has been observed in response to aphids and whiteflies (sucking insects, which therefore do minimal damage to plant tissues). Plants perceive some insects as pathogens rather than herbivores, and this concept is supported by the identification of a gene that confers resistance to aphids and nematodes (Milligan et al., 1998; Rossi et al., 1998). Evidence for coregulation by salicylic and jasmonic acids comes from a gene expression profiling study in which 55 genes were induced by treatment by either salicylic or jasmonic acid (Schenk et al., 2000).

Besides synergism between the three chemical pathways to SAR (salicylic acid, jasmonic acid and ethylene), there is also evidence for antagonism between the three. The induction of SAR has a negative effect on the jasmonic acid and ethylene pathways, normally induced by chewing insects and wounding of the plants (Felton et al., 1999). A challenge for the future will be to understand how the three responses are coordinated and to unravel the downstream signaling network in each case (Durrant and Dong, 2004).

Figure 1 (page 21) shows the sequence of events that take place in a plant from the time of recognition of the pathogen to defense gene induction, based on current knowledge (Durrant and Dong, 2004). Note the prominent role of salicylic acid, and the alternate role of reactive oxygen species (including H_2O_2).

Our understanding of SAR has increased considerably over recent years as we have begun to elucidate the molecular mechanisms underlying this response. Many of the processes contributing to SAR are clearly required in both local and systemic tissues and contribute to basal disease resistance. These include the synthesis of salicylic acid, changes in redox status, and the induction of defense gene expression. ..... There is evidence for negative and positive feedback of salicylic acid signaling and cross-talk between different signaling pathways, adding to the complexity of the defense response. .... Induction of SAR to control infection of crop plants is already being used in the field by application of BTH. Better understanding of the SAR signaling pathway will certainly lead to new environmentally friendly methods of crop protection (Durrant and Dong, 2004).

Durrant and Dong (2004) conclude their excellent review of SAR with the following comments:

> *The current authors conclude that, given the known SAR-stimulating effect of hydrogen peroxide, H_2O_2, and of ozone (Mahalingam et al., 2003), there is potential for a procedure that can provide low levels of H_2O_2 and/or ozone, to agricultural crops. If such a system can be developed, even though the chemistries and biochemistries that must occurring within plants are still many years away from being understood, the most significant advantages of a successful SAR-triggering system would be increased crop yields without resorting to chemicals that leave residues on the crops or in the soil.*

Such a system is the recently-developed PhytO3 Tech technology, described in the next section, and field-tested is several European countries.

The PhytO3 Tech Crop Protection System

System Description for Field Use

Application of the PhytO3 Tech Crop Protection Technology to fields of growing agricultural crops involves equipment uniquely designed to provide the rapid, sequential, but almost simultaneous application of three discrete crop treatment steps. The trick is to apply sufficient ozone and UV light to stimulate the development of SAR protective responses within the growing plants, without allowing

excess ozone or its reactive oxygen metabolites to be present for longer periods of time so as to cause cell death.

Step #1: Application of Electro-Shock. By means of a 38-ft boom sprayer (Figure 1), the plants of a crop are first wetted with a spray of high voltage, pulsed, negatively-charged water, which may also contain a wetting agent. The water is pumped to the spray boom from a water tank (mounted on the front of the tractor, and containing a ring-plate electrode mounted on the floor) to which is applied 1,000-2,000 volts (0.5-1.5 amp) of pulsed DC current (100-1,500 pulses per second – variable, depending on the crop).

Figure 2. The PhytO3 Tech Crop Protection boom sprayer (Steffen, 2005b).

From the tank, the water is pumped via a membrane pump to the backside of the tractor via PVC pipes to the lateral boom, where it is sprayed via 36 separate air jet nozzles. These nozzles are positioned under the boom protection cover at a slight angle toward the front in the driving direction. The spray fluid in the piping system is always in contact with the electrodes so that conductivity of electrons through the system is guaranteed.

The spray nozzles are compressed air-assisted to produce droplets no larger than 50 microns in size, and are driven by the Jet-Stream blower deep into the plant canapé, to guarantee an even water film as well on the underside of the leaves. Nam et al. (2004) used electrostatic spraying to treat beef carcasses to ensure that the electrically charged water droplets were attracted to the targets and they moved upwards against gravity to coat hidden surfaces of food.

Through this very dense spray mist, so dense as to serve as a conductor, the electrons march through the plants into the ground. With this treatment, the plants receive an electron shower, which temporarily stuns the plants, similar to the effects on fish during electro-fishing, or on pigs in a slaughterhouse receiving an anaesthetic electro-shock. The plants literally fall into a state of shock anaesthesia, opening their leaf stomata wide. At the same time, cell membranes of pathogens that may be present become soft and permeable. Insects also become paralyzed and are unable to keep themselves on the plants, and are thereby washed off by the mist stream and also shaken off by the whirling effects of the air jets.

This “Tsunami wave” of electrons prepares the plants for the immediately following steps two and three.

Step #2: Application of Ozonated Water. Also mounted in the boom is a second built-in spray pipeline also covered by the boom protection cover, which is connected to tank at the rear of the tractor. This rear tank contains ozonated water containing approximately 8 mg/L of ozone. The ozone generation and contacting is also mounted on the rear of the tractor, and produces ozone from oxygen by corona discharge. This ozonated water also is pumped via a membrane pump via a second pipeline that contains 36 additional electrostatic nozzles. However, these 36 nozzles are directed somewhat in the backwards direction so that the two spraying lines do not interfere with each other. This means that there is spraying in both forward (electrostatic water) and backward (ozone-containing water) directions.

Ozonated water sprayed onto the plants by means of air-assisted electrostatic nozzles is in the form of droplets 30-40 microns in size. These droplets, also negatively charged, have an attraction dipole power of up to 75 G. Therefore, some of these droplets flow upwards during spraying, thereby guaranteeing wetting of the plants on the undersides of the leaves, as well as all other portions of the plants (see Nam et al., 2004, cited earlier).

Step #3: Application of Ultraviolet Radiation. High Energy UV-C radiation (~254 nm) is produced by medium-pressure mercury UV lamps (600-1,000 Watts) with radiation energy of approximately 10,000 mW sec/cm^2, applied at a distance from the plants of approximately one foot. The UV bulbs mounted under the boom protection cover in such a manner that the UV light is irradiating from the top of the spray mist cloud immediately as the spray mist cloud exits the spray nozzles. The 38 UV bulbs (mounted under a 38-ft boom) are air-cooled by the air jet blower and are water-cooled by the spraying mist. The UV-254 nm radiation destroys most of the ozone applied, forming hydrogen peroxide and other reactive oxygen species, and destroying microbes present on the plants.

In a technical variation, 3000 W Xenon lamps are used, whereby the UV-light is generated over a wider spectrum from 160 to 2000 nm (75% in the 240-260 nm range) with an ignition power of ca 25,000 Volts and pulsed (3-4 pulses/second) with a peak power duration of 20-40 nanoseconds. This technology provides better penetration power and lamp cooling is simpler. With this system, more UV-light power can be supplied in a very short period of time, and this allows spraying speeds of the tractor of up to 5 miles/h. The UV light energy supplied to the plants per second corresponds to more than 40,000 times the power of sunlight.

The UV radiation has a penetration power of approximately three feet from the ground, which provides sufficient UV radiation to function both as a bactericide and to decompose ozone simultaneously. Because of the unique positioning of the UV bulbs under the boom, and because of the force of the spray mist from spray nozzles also under the boom), plants are moved as the boom passes over them, thus assuring that all portions of the plants are exposed to the electro-shock, water, ozone, and UV radiation. However, the spray mist is very dense – so dense that the UV radiation cannot penetrate the mist completely. Consequently, there are water droplets containing ozone that reach the plant leaves without being exposed to the ozone-decomposing UV radiation. This means that water droplet contain a multitude of active oxygen species, including ozone, hydrogen peroxide, and their free radical decomposition products.

Even though the three steps are described as being discrete and sequential, all three steps are applied rapidly is a single pass of the boom, and all three steps are essentially simultaneous. The overall effect appears to be synergistic, that is, the three processes applied (aqueous electro-shock, application of ozone-containing water, and exposure to UV radiation) provide the desired performance effects (opening of stomata by aqueous electro-shock, application and decomposition of ozone, destruction of excess ozone, formation of SAR-triggering active oxygen species, bactericidal disinfection, and creating an environment

that is discouraging to chewing insects) that cannot be attained as conveniently nor as effectively if the three processes were to be applied single, in multiple passes of the boom.

The total microbial load on the plants is reduced by 3-5 logs, depending on the crop.

Chemistries Occurring During Treatment

The level of ozone contained in the ozonated water (ca 8 mg/L) is very high, relatively speaking. If solutions containing this much ozone were to remain in contact with growing agricultural plants, they would soon sustain considerable oxidative damage. Fortunately, the nearly simultaneous exposure to high energy UV radiation (Step #3) guarantees the rapid destruction of excess ozone, and minimizes the time period that the plant leaves are exposed to ozone.

As UV radiation destroys ozone in water, the first intermediate product formed is hydrogen peroxide, H_2O_2 (Peyton and Glaze, 1988).

$$h<$$

$$O_3 + H_2O \;6\; O_2 + H_2O_2$$

Hydrogen peroxide is a weak acid. When combined with water, it partially dissociates into hydroperoxide ions:

$$H_2O_2 + H_2O \;\Xi\; HO_2^- + H_3O^+ \qquad k_a = 10^{-11.6}$$

The hydrogen peroxide molecule itself reacts very slowly with ozone (Taube and Bray, 1940), whereas the hydroperoxide anion is very reactive. As a result, the ozone decomposition rate by H_2O_2 increases with increasing pH.

Under the continued influence of UV radiation, both ozone and H_2O_2 decompose, the former rapidly, the latter much more slowly. The products of these decompositions are a multiplicity of very reactive oxygen-containing free radicals, the most prevalent being the hydroxyl free radical, (HO≅) (Hoigné, 1988). All of these free radicals have extremely short (nanoseconds) half-lives, and are destroyed quickly. However, the hydroxyl free radical itself is a more powerful oxidant than is molecular ozone. The oxidation potential of molecular ozone, O_3, is 2.07 eV, while that of the hydroxyl free radical, HO≅, is 2.83 eV.

Returning to Step #1 – the electro-shocking of the growing plants causes the plants to fall into a state of shock anaesthesia, opening their leaf stomata (the pores through which the plants breathe and through which respiration occurs). When the subsequent spray of ozone-containing water is applied nearly simultaneously with exposure to high energy UV radiation, H_2O_2 is produced as ozone in the water is decomposed. It is likely that some dissolved ozone and some of the dissolved hydrogen peroxide enter the plant through the more widely opened stomata, where neither chemical can be exposed to the decomposition action of the applied UV radiation. Although any dissolved ozone that enters the plant through the stomata in this manner can be expected to decompose rapidly, by reaction with plant internal

components, the hydrogen peroxide, being much less reactive than ozone, can pass much further into the plant internal system before decomposing.

Having H_2O_2 inside the plant's circulation system will be quite beneficial, because it is a known stimulant of SAR (Alvarez et al., 1998; Sandermann, 2000).

Postulation

From these chemical considerations, it is postulated that the three-step PhytO3 Tech technology is really a sophisticated procedure for uniformly introducing small concentrations of H_2O_2 (via decomposition of ozone) through the stomata of plant leaves, into the plant internal system. In turn, the development of SAR is triggered in each plant thus ingesting H_2O_2.

An immediate comment on this postulation will be, "If peroxide is the SAR-triggering agent, then why not simply spray an aqueous solution of H_2O_2 onto the plants and avoid the use of ozone in the first place?" The answer to that argument is that by the ozone-decomposition route, only a few molecules of peroxide with be produced to enter each available stomata.. This means that high concentrations of peroxide inside the plants are avoided, which is desired for rapid triggering of SAR without detrimental side effects due to excessive levels of peroxide.

Additionally, were concentrations of peroxide to be sprayed onto the plants, excesses of the chemical would surely find their way into the soil, with unknown, but probably detrimental effects.

As well, the use of hydrogen peroxide as a spray would require the storage and handling of this chemical, which is known to decompose during storage, liberating oxygen and leaving water. In addition to initial cost for this chemical, its decomposition poses storage problems that farmers will not want to take on.

Another comment on this hypothesis is "Exposure of growing plants to 8 mg/L of ozone in aqueous solution can cause detrimental effects on the plants". Certainly true, but only if such ozone levels are maintained for many minutes. The exposure of ozone-containing water to UV radiation is well-known to immediately (within seconds) reduce the measurable concentration of ozone to zero. Thus, a second beneficial effect of the UV radiation applied, in addition to forming the SAR-triggering hydrogen peroxide, is to destroy crop-damaging excess ozone almost as soon as it comes in contact with the plants.

A third beneficial effect of UV-radiation is the fact that UV-light, by photon absorption, induces SAR as a stressor, similar to ozone.

Still a fourth beneficial effect of the UV radiation (254 nm) is its disinfection effect on surface microorganisms that it may contact. This is also true of excess molecular ozone, and of the short-lived, reactive, free radicals produced by the mixture, including the hydroxyl free radicals.

Finally, the environment created by an aqueous spray of negatively-charged ions, water containing 8 mg/L of dissolved ozone, plus exposure to high energy UV radiation (that produces hydrogen peroxide plus hydroxyl and other very active free radicals), is one that any chewing or sucking insects that may be present will surely want to avoid.

It may be that molecular ozone (O_3) and/or its free radical decomposition products (as well as the free radical decomposition products of hydrogen peroxide) inside of the plant also trigger SAR. Since all are "active oxygen species", that is a likely possibility that must be proven by rigorous scientific research.

Thus, the PhytO3 Tech Crop Protection system, although applying a substance (ozone) that normally damages plants if allowed to be present in high concentrations, does not harm the growing plants, because the levels of ozone applied are eliminated almost instantaneously (by UV decomposition), leaving no toxic residues on the plants, or the harvest, or in the soil, thereby eliminating the need for the chemical sprays that are in use today.

The genius of this delivery system is that it seems to provide just enough H_2O_2-producing ozone (via decomposition with UV radiation) to generate just enough peroxide either within the plant after injection of some ozone, or outside the stomata, prior to the entry of water containing ozone, H_2O_2 and other reactive oxygen species into the stomata, to evoke the SAR-producing salicylic acid, without providing sufficient ozone to cause cell death.

Cost Comparisons

Table 1 compares estimated costs for PhytO3 Tech technology vs costs for conventional chemical spraying on a per acre basis. There is a distinct financial benefit to the farmer to abandon chemical spraying in favor of installing PhytO3 Tech equipment. Not only are the operating costs per acre significantly lower, but the initial capital cost investment can be returned quickly, sometimes after only the first crop has been brought to market.

The PhytO3 Tech equipment will be depreciated after approx. 160 Hectares (395 acres) of crop production. This means that for a farmer with 160 Hectares (395 acres) production per year, there will be a savings of $42,000 annually in the second and following years.

The PhytO3 Tech boom equipment is capable of treating 6 acres per hour at a tractor speed of 4 mi/hr. Using it 6 hours/day, 36 acres can be treated per day. Thus, a 500 acre farm will require 14 days for treatment. Larger farms will need multiple boom-sprayers on hand, since some crops require spraying every 14 days.

Table 2 shows the cost savings that can be expected by typical-sized American farms – small (1,000 acres), medium (2,500 acres) and large (3,500 acres).

Development of the PhytO3 Tech technology is indeed promising, not only for the promise of higher quality, chemical-free produce, but also for higher yields, and lowered production costs.

Field Tests on Crops in Spain

During 2003-2005, crops listed in Table 3 were sprayed using the PhytO3 Tech equipment, with generally acceptable produce qualities. In total, 10,368 microbiological tests were performed. Control plots were detached from the trial plots at a distance of about < 50 meters (< 165 feet). Both plots were fenced with plastic bands and planted on the same day with the same variety and watered and fertilized in identical manners.

Table 1. Cost Evaluation: PhytO3 Tech Crop Protection Technology vs Conventional Chemical Spraying

Cost Factors	PhytO3 Tech	Conventional Spraying
Capital Investment -- Spray Equipment, 39.4 ft-boom sprayer, tractor mounted, complete with ozone, UV, and electrical generation equipment (PhytO3 Tech equipment only)	$ 42,000	$ 25,000
Energy Consumption, Tractor + Sprayer with 2.1 miles/h speed (3.3 ft/sec) and 12-13 min working time per acre, 5 treatments per acre and crop	100 kWh	60 kWh
1 h working time/acre; $0.12/kWh cost	$ 12	$ 7
Wetting Agent	$ 5	$ 5
Water (gallons)/acre	1,000 (5 sprays)	50
Water Cost (@ $3.00/1000 gal)	$ 3.00	$ 0.15
Chemicals	$ 0	$ 121.50 (est)
OPERATIONAL COSTS per acre (includes UV bulb replacement, spray nozzle replacement, and depreciation of equipment and interest)	$ 20	$ 15
TOTAL COSTS PER ACRE	$40.50	$ 209.25
Difference per acre per crop = $168.75 in favor of PhytO3 Tech		

Table 2. Projected annual cost savings for various sizes of farms

Farm size, acres	**265**	**500**	**1,000**	**2,500**	**3.500**
Savings @ $169/acre	$44,785	$84,500	$169,000	$422,500	$591,500
No. Boom Sprayers *	1	1	2	5	7
Capital cost of sprayers	$42,000	$42,000	$84,000	$210,000	$294,000
Payback time, months	12	6	6	6	6
* Based on spraying every 14 days					

However, control plots did not see any PhytO3 Tech treatment. Harvesting of the control plots was always later than for the trial plots because the quality of the harvested control crops had no saleable value. Fruits were marked by insect bites, microorganisms were present throughout the crops (*monilia, botrytis, ervinia, alternaria, phytoflora, fusarium*, early and late blight *oidium*), as well as insects, caterpillars, white flies, several varieties of aphids, red spiders, moths, nematodes in the soil, etc. Only some of the control root crops produced some quality crops – onions, carrots, and potatoes.

A primary criterion between the controls and trials was whether the first crop was saleable. All PhytO3 Tech trial plots delivered during the three years of field testing were high quality in appearance and were readily saleable. PhytO3 Tech crops compared to chemically-treated crops during then three year testing period always were of higher quality, except in one instance of beans that had been sprayed too frequently with PhytO3 Tech. This had resulted in some dwarfism occurring, resulting in about 15% less yield of the PhytO3 Tech crop, although the quality of the beans still was high.

Typical results are as follows:

BEFORE SPRAYING – 800,000 TO 35,000,000 Total Microbial Counts (TMC)/cm^2 in Stomacher-substrate-solution 1:10 in peptone on agar after 24 h at 34EC in an incubator.

AFTER SPRAYING – 2,600 to 15,400 TMC/cm^2

6 HOURS AFTER SPRAYING – 800 to 3,200 TMC/cm^2

24 HOURS AFTER SPRAYING – 200 to 6,100 TMC/cm^2

Spraying intervals were between 7 and 14 days. Spraying was performed in the mornings (06.00 - 10.00 am) and evening (17.00-21.00 pm), using 2-8 mg/L ozone in water and a spray speed of 1 m/sec. UV-C lamps of 35 W output (35,000 mW/cm^2/sec) were 5-90 cm from the plants.

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Table 3. Crops sprayed with PhytO3 Tech spray booms during 2003-2005 in Spain

Crop	Surface (ha)	Control (m^2)	No. Treatments	Crop Quality
2003- White asparagus	21	1000	15	OK
2004-Tomatoes	7	1000	7	OK
Sweet peppers	3	1000	8	OK
Hot peppers	15	1000	8	OK
Broccoli	7	1000	8	OK
Cauliflower	10	1000	10	OK
Leak	4	1000	10	OK
Onions	2	50	11	OK some neck rot
Sweet corn	0.3	50	8	OK
Squash	0.3	50	7	OK
Iceberg lettuce	0.25	50	5	OK
Lollo Rosso lettuce	0.25	50	5	OK
Escarole lettuce	0.25	50	5	OK
Spinach	0.25	50	4	OK
French beans	4	1000	6	OK
Peas	5	1000	6	OK
Brussels sprouts	3	1000	10	OK
Wheat	10	1000	4	OK
Potatoes (Amandine, Charlotte)	4	1000	8	OK - some nematodes
Strawberries	0.25	20	15	OK
Sweet melons Watermelons	10	1000	10	OK - some fusarium
2005-Peaches	15	1000	4	OK
Carrots	10	1000	7	OK - some nematodes
Celery	0.3	50	5	OK
Red beets	0.3	50	8	OK
Baby leaf lettuce	0.3	50	5	OK
Borlotti beans	0.3	50	6	OK
Tobacco	2	50	5	OK
Grapes	1	20	10	OK
Wheat	10	1000	5	OK

Crop	Surface (ha)	Control (m^2)	No. Treatments	Crop Quality
Lettuce, Iceberg, Romaine Cicorino	0.3	20	5	OK
Sugar Peas	0.3	20	6	OK
Cherry tomatoes (Greenhouse)	0.3	15	8	OK
Potatoes	4	1000	8	OK
Giant garlic	0.5	20	10	OK
Garlic	0.5	20	8	OK
Tomatoes	4	1000	8	OK

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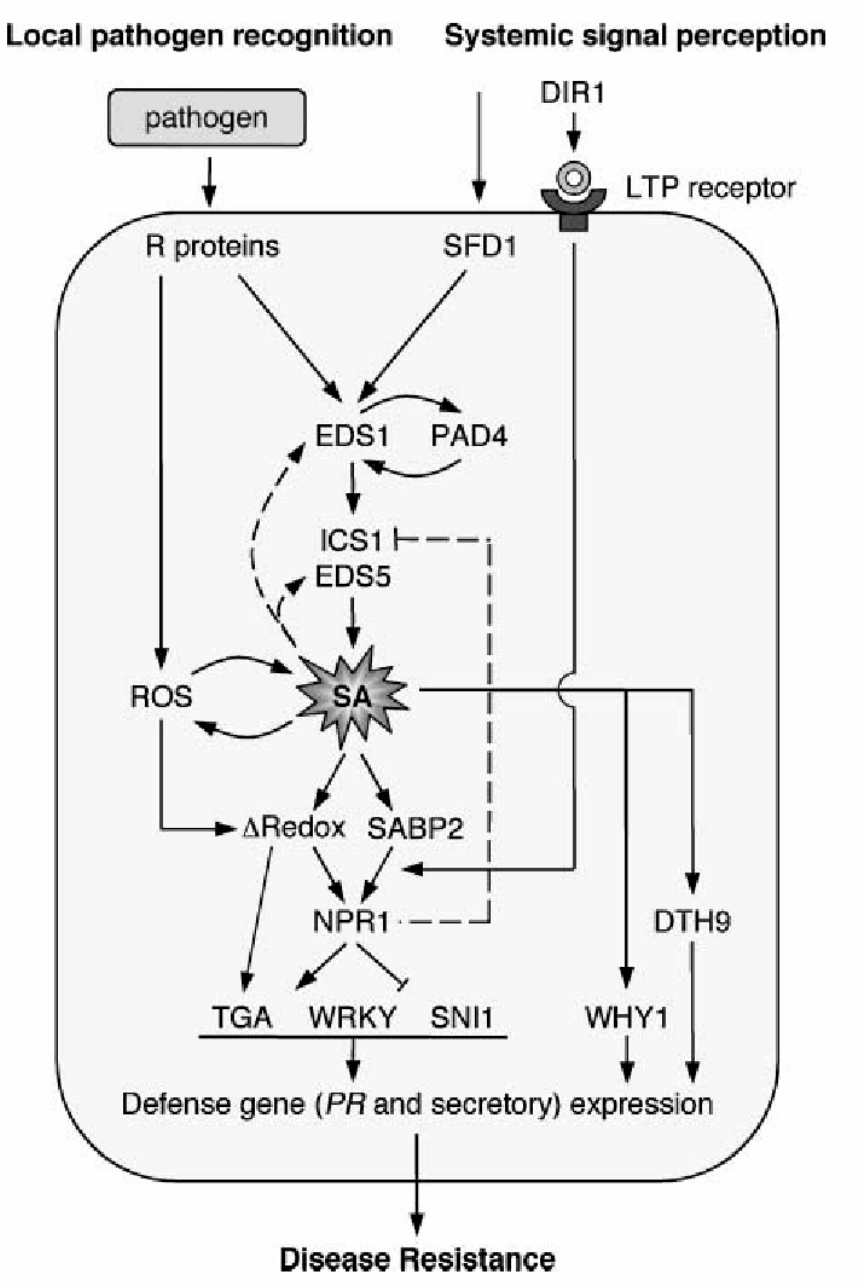

Figure 1. shows the sequence of events that take place in a plant from the time of recognition of the pathogen to defense gene induction, based on current knowledge (Durrant and Dong, 2004). Note the prominent role of salicylic acid, and the alternate role of reactive oxygen species (including H_2O_2).