SCLEROTINIA SCLEROTIORUM: MOLECULAR ASPECTS IN PLANT-PATHOGENIC INTERACTIONS

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SUMMARY

This year, 2016, marks the 130th anniversary of Anton de Bary's detailed reports summarizing his observations and thoughts on the plant pathogenic fungus *Sclerotinia sclerotiorum*. As the most famous pioneer of plant pathology across the globe, de Bary made many intelligent observations that still hold true today, such as the secretion of enzymes and oxalic acid. As scientists advanced the story of *S. sclerotiorum* pathology through the following decades, they confirmed the identity of numerous plant cell-wall-degrading enzymes and oxalic acid, and they clearly demonstrated the importance of these virulence factors in establishing disease, plus they added more details. This review summarizes some of these findings that have occurred in the last 130 years on the molecular aspects of *S. sclerotiorum*-host interactions.

1. Introduction

Anton de Bary, the father of plant pathology, was an amazingly observant individual. His extraordinary curiosity and attention to detail led to many discoveries and understandings of multiple plant pathogen life cycles and behaviors. He studied many plant pathogens and wrote extensively on several, such as *Sclerotinia sclerotiorum*, much of which he summarized in a series of weekly submissions to a journal he co-edited with L. Just, the Botanische Zeitung, in 1886 (de Bary, 1886; available for free download at http://www.biodiversitylibrary.org/item/ 105850# page/36/mode/1up) and in his comprehensive treatise summarizing his many observations of plant pathogens (de Bary, 1887). Although he was light years away from the molecular revolution that would blossom nearly 100 years after his death, de Bary successfully identified the two main molecular factors affecting the ability of *S. sclerotiorum* to be a successful pathogen: the release of oxalic acid (OA) and ferments (enzymes) that could macerate plant host tissue. Thus started the studies on the molecular interactions of *S. sclerotiorum* and its hosts--over 130 years ago. Questions asked then, are still being asked today. Why do the fungal hyphae release OA, and what is in the fungal 'ferment' that could dissolve plant host cell walls? And similarly, how is the plant responding to this aggressive, uninvited visitor and these toxic substances to which it i exposed? What other molecules are involved? How, and what, are the genes used by the pathogen and host to create and control these molecular interactions?

Anton de Bary was far ahead of his contemporaries, and it would be several decades after his death in 1888 before scientists would make any further substantial progress on the questions he exposed. One of these reports of progress was a very nice microscopic study by Boyle in 1921 (Boyle) where he noted that the growing hyphae of *S. libertiana* (a synonym of *S. sclerotiorum*) were coated with a thick mucilaginous/gelatinous sheath which he described

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of OA in solution, as oxalate at pH levels above 1.2 readily binds calcium ions and precipitates from solution (i.e., adding known amounts of calcium and weighing the dried precipitate to estimate oxalate molarity).

2. Investigations on *S. sclerotiorum* plant cell-wall-degrading enzymes (PCWDEs)

As mentioned previously, since at least the 1800's, scientists have known that some cell-free extracts, termed 'ferments' and later 'enzymes', could stimulate chemical reactions to occur, but the link between enzymatic activities and actual protein molecules was not made until the 1920's and 1930's, with the 1946 Noble Prize in Chemistry awarded to scientists who helped make this connection. Therefore, in the 1950's enzymology was a popular research focus for scientists, and many biologists, including plant pathologists, were looking for enzymes. By the 1960's, the plant pathologists studying *S. sclerotiorum* had clearly identified the release of peptidiolytic enzymes in cultures and *in planta*.

In 1957, Echandi and Walker (Echandi and Walker, 1957) were the first to report the identification of such PCWDEs from *S. sclerotiorum*. These scientists identified both pectin methylesterase and polygalacturonase activities from *S. sclerotiorum* grown on wheat bran. These enzymatic activities were destroyed by heating at 55°C for 10 minutes. The activity of these pectolytic enzymes was highest at pH levels between 3 and 5, and the most optimal at pH 4. Enzyme activities were reduced to 44% and 20% at pH 6 and pH 7, respectively. This work was supported *in planta*, by analyzing *S. sclerotiorum* infected tissues (Hancock, 1966). Hancock documented pectin methylesterase and polygalacturonase activities in infected stem tissue, together with an increase in stem acidity, with the pH dropping from about 6.2 to 4.5 during his infection studies.

A very interesting study came out in 1965 by Bateman and Beer (Bateman and Beer, 1965). Working on *S. rolfsii*, they noted the benefits of a pathogen releasing both pectolytic enzymes and OA, as they found that the effects of the enzyme function and acid were synergistically enhanced in the presence of each other. They estimated that *S. rolfsii* culture filtrates contained up to 30 mM OA (80 mg/30 ml) after 6 days growth on carboxymethyl cellulose (which they found to be a much better inducer of OA than glucose). Researchers found that the activity of purified polygalacturonase was nonfunctioning if the substrate was calcium pectate, the form that is commonly found in plant cell walls, but if OA was added to the assay, the pectate was degraded, presumably due to the removal of calcium from the calcium pectate by OA's proven ability to chelate calcium. In addition, OA lowers the pH (estimated as 2.8 in their cultures), and they found that the optimal pH for the polygalacturonase activity was between 3 and 4, a pH optimum similar to what Echandi and Walker (1957) noted (see the previous paragraph). They also noted that pH values below 4 could kill plant tissue, without the presence of PCWDEs, and that fluids from diseased lesions was near 4.0. They therefore concluded that OA was most likely having its negative effect on plant hosts due to both 1. binding and removal of calcium out of calcium pectate to allow the polygalacturonases to function at maximal efficiency; and 2. to the general toxic effects of a lowered pH.

The search for extracellular enzymes that could factor into molecular plant-pathogen interactions, and their detailed characterizations, continued to be popular in the 1960's and for decades beyond. Evidence for cellulose degrading enzymes was found using paper, cotton or carboxymethyl cellulose as growth substrates in the media (Hancock, 1967). Marciano et al. (1983) detailed the roles of three types of PCWDEs, polygalacturonase, cellulose, and xylanase in virulence, together with the enhanced effect of OA and its low pH. A survey of the ability of extracellular fluids of a *S. sclerotiorum* culture to degrade cell-wall polysaccharides was conducted by Riou et al. (1992) in which enzymatic activity was detected for the degradation of cellulolytic, hemicellulolytic, and pectinolytic polysaccharides. Gel analysis confirmed the presence of at least one pectinase, beta-xylosidase, and cellobiosidase. Subsequent studies focused on characterizing the most effective class of plant-cell-macerating enzyme released by *S. sclerotiorum*: the endo-polygalacturonases (Fraissinet-Tachet, et al., 1995; Cotton, et al., 2002; Cotton et al., 2003). The work of Favaron et al. (2004) suggested that there are at least two different endo-polygalacturonases in *S. sclerotiorum*, each with a different pH optimum for activity, with endo-PGa being more active at lower pH values (from 3.6-5.0), and endo-PGb more active at pH 4.5-5.0. In addition

to the endo-polygalacturonases, researchers also conducted further detailed characterization of exo--polygalacturonases (Li et al., 2004). Other enzymes found to be released by *S. sclerotiorum* include at least two endo-beta-1,4-glucanases (Waksman, 1991; Chahed et al., 2014), at least two beta-glucosidases (Issam et al., 2003) and at least one beta-galactosidase (Waksman, 1989; Riou, et al., 1992a). The search for cutinases had been elusive, and there were conflicting reports from microscopic studies as to whether or not the cuticle was being enzymatically degraded. Although the microscopy work of Boyle (1921) did not show any evidence for cuticle degradation, de Bary reported that he had seen evidence of cuticle degradation, and in 2012, a cutinase was finally identified (Bashi et al., 2012). PCWDEs have complex roles and regulation, and Hegedus and Rimmer (2005) wrote an excellent mini-review proposing how activation and repression of PCWDEs, as well as sclerotia development, might be controlled by a tri-phasic model for infection involving glucose, cAMP, and pH levels that change during infection.

3. Mechanisms of OA and plant-Sclerotinia interaction

This present review focuses on the effects of *S. sclerotiorum*-released molecules, such as OA and secreted enzymes (and their related genes), on the plant host. Other reviews on *S. sclerotiorum* are also available to the reader. Two excellent reviews were published in 1979 as part of an APS Symposium of Sclerotinia. Purdy (1979) described the various, somewhat confusing, nomenclatures used to name *S. sclerotiorum*, and gave a superb summary of 'big picture' aspects of this disease such as histology, disease development and host range; Lumsden (1979) summarized details on the physiological and histological changes that occur during *S. sclerotiorum* pathogenesis. Lumsden put an emphasis on the role of OA chelating important cations and producing a gradient of lowering pH values, in conjunction with the plant PCWDEs, and how these factors would make the cells at the infection front more permeable, and how that would lead to greater leakage of nutrients for the fungus, thus weakening the host. Another very good, comprehensive review (Dutton and Evans, 1996) on the role of large quantities of OA in plant diseases caused by numerous fungi, not only explained how OA could enhance the efficiency of PCWDEs, but also emphasized the importance of the ability of OA to strongly chelate biologically important cations such as calcium, iron, manganese, magnesium, nickel, aluminum, and copper, affecting their solubility and thus availability; also of importance is the effect of OA on destabilizing cytoplasmic and chloroplastic membranes, which would aid in tissue maceration. For additional perspectives on the molecular interactions between plants and *S. sclerotiorum*, readers should also refer to the very recent mini review by Mbengue et al. (2016).

3.1. Phenotypes of various OA-deficient mutants

Looking at the pathogen side of this host--pathogen relationship, the reader can find several very good papers on the effects of *S. sclerotiorum*-produced OA on the fungus itself. As discussed below, *S. sclerotiorum* produces and metabolizes OA, which affects various aspects of fungal vegetative growth, sclerotia development and pathogenesis. To investigate the critical role of OA throughout the life cycle of *S. sclerotiorum*, mutagenesis was used. Mutants induced by ultraviolet light (UV) were screened for reduced OA production and these mutants were found to be non-pathogenic on common bean (Godoy et al., 1990). These mutants also did not produce sclerotia, and had low expression of pectinases and cellulases, showing that sclerotia formation and some PCWDEs, all required OA. In addition, the OA deficient mutants provided some clues to the biosynthetic pathway for OA production, as the addition of succinate to culture media led to the production of some OA, showing the pathway could include succinate (i.e., the mutation might have occurred in an enzyme of the biosynthetic pathway upstream of succinate production).

An enzyme known to be involved in the synthesis of OA is oxaloacetate acetylhydrolase (OAH, EC 3.7.1.1), which is the enzyme of *S. sclerotiorum* that converts oxaloacetate to OA and carbon dioxide. OAH was knocked out by targeted gene replacement via homologous recombination, leading to a genetically defined mutant that accumulated no OA, even under highly inductive conditions (Liang et al., 2015). The radial growth rates of vegetative hyphae of these mutants were observed to be almost equal to wild type (WT) on potato dextrose medium buffered to pH 3.6, whereas growth was severely re-

duced when the medium was buffered pH 6.9. On all media tested, the *oah* mutant, similar to the *UV*-induced OA-minus mutants of Godoy et al. (1990), could not form sclerotia, but mycelia were noted to form some loose and unmelanized aggregates as if some sort of futile effort was being made to produce sclerotia. Moreover, the *oah* mutant was also defective in compound appressorium formation on artificial surfaces. Complementation using the WT *OAH* gene on a plasmid was able to fully restore the radial growth rate and partially restore sclerotia development at a low frequency, but failed to regain the compound appressorium phenotype. The authors suggested that this failure could possibly have resulted from inconsistences in the regulation of expression of the transgene *OAH* expressed from the plasmid, versus the WT native expression, or perhaps it was due to the non-reversing of an unknown epigenetic process that occurred in the *oah* mutants that did not allow for complementation. In another study where *oah* mutants were generated either through targeted gene replacement or T-DNA insertion, involving a different strain (WMA1), again almost no OA production was detected (Xu et al., 2015). Interestingly, for these OA-mutants, the authors observed some phenotypic behavior that differed from those described by Godoy or Liang that were generated using *Sclerotiorum* strain 1980. One difference was observed when the authors ran high-performance liquid chromatography and found that the OA-minus mutants accumulated fumaric acid at high levels compared to the WT. The production of fumaric acid was not tested in the other reports on OA--mutants; however, one could assume that, if they did produce fumaric acid, it would be at low levels since the pH of the media for these mutants did not go below pH 6 after 2 days growth, whereas the mutants of Xu et al. reduced the pH of the media to just above, or just below, pH 4 by 48 hours. Additionally, the two mutants in the Xu et al. study were able to produce sclerotia, (with some noted differences in color and texture), whereas the Godoy and Liang mutants could not. A third major difference between this study on the WMA1-derived mutants and the 1980-derived mutants was that the WMA1-derived mutants were quite effective in inducing necrosis on multiple hosts, whereas the OA-minus mutants of 1980 could not, or did so very poorly, and only if the plants were wounded first. It is not clear why these differences occurred, and how much of these differences are strain specific, but it does suggest that there could be some unknown complexity in OA metabolism and perhaps in OA-regulated genes, and that the various OA-minus mutants affect these factors in different manners.

Another enzyme involved in OA metabolism in *S. sclerotiorum* was also recently characterized--the OA degrading enzyme oxalate decarboxylase (ODC) (Liang et al., 2015). Deletion of one of the two putative ODC genes led to hyper-accumulation of OA and less efficient differentiation of compound appressoria, implying that fine control of OA levels regulates appressorium formation. No differences in vegetative growth or sclerotia development were reported for this *odc* mutant, probably because the expression of the gene for this enzyme was detected only at mid to late stages of compound appressorium formation. Although the physiological function of ODC in *S. sclerotiorum* still requires further investigation, it is highly possible that this enzyme is responsible for OA regulation at multiple developmental stages.

The various OA mutants allowed for more detailed studies of the role of OA in disease development. Since the deletion of the *oah* gene caused defective compound appressorium formation, which makes it difficult for the pathogen to penetrate the cuticle layer, Liang et al. (2015) performed wound inoculations on a variety of hosts (including tomatoes, soybeans and arabidopsis) to evaluate the role of OA in pathogenesis. The results showed that the *oah* mutant produced very limited leisions that were “brown, green” in color and restricted by a “thin, dark” border, while the WT produced “light brown, spreading” lesions. Moreover, GFP labeling, along with 3,3’-Diaminobenzidine (DAB) and aniline blue staining, was conducted to characterize the interaction occurring in these different types of lesions. It was observed that compared to WT hyphae, the GFP fluorescence of mutant hyphae faded at 5 dpi and the mutants elicited much more H_2O_2 accumulation and callose deposition, indicative of stronger host defense responses. These phenotypes indicated that OA was a determinant virulence factor dampening host defenses, and the critical role it played in pathogenesis was further confirmed by the observation that immersing the leaf petiole in OA (pH 5.8) par-

tially restored the virulence. However, in the study of Xu et al. (2015), the *oah* mutant they generated caused disease in a host-dependent manner, with the virulence almost the same as the WT on faba bean, yet only weakly pathogenic on soybean. They concluded that these differential disease developments were related to differences in the buffering capacity within the host plants. When inoculation of *oah* mutants was conducted on green beans infiltrated with citriate-phosphate buffer at pH 4.2 , or with non-buffered potassium oxalate (10 mM, pH 4.2), virulence was recovered under the former treatment but not the latter. The authors also identified a positive correlation between lesion size and decreased pH in the infected tissues, and a negative correlation between lesion size and host leaf tissue buffering capacity. Based on these evidences, the authors proposed that the major contribution of OA was due to its strong acidity rather than the oxalate. However, considering the chemical similarty between citric acid (used as a buffer and for lowering the pH in controls) and OA: both are small organic acids that strongly bind divalent cations at physiological pHs; citric acid $pKa_1 \approx 3.1$, $pKa_2 \approx 4.8$ and $pKa_3 \approx 6.4$, other functions of OA besides its acidity, such as the chelation of cations, cannot be dismissed. Also, the fact that the OA mutants produced excess fumaric acid clouds the interpretations.

3.2. Molecular and physiological studies on the functions of OA secreted into hosts

Although the fungal-released proteins, in the form of enzymes, effectors and necrosis-inducing proteins/peptides, have a real influence on disease development, many view that the most beneficial virulence factor of *S. sclerotiorum* to be OA, and therefore numerous studies have focused on the role of OA in the physiology of molecular plant-pathogen interactions. For example, it was shown that OA increased stomatal opening in Arabidopsis, presumably benefitting the pathogen by facilitating the entrance of OA into the host apoplast (Stotz and Guimara, 2004). As introduced above, the acidity of OA and its chelation properties can produce visual effects on the plant host, but what is it doing at the molecular level? A study in 1988 (Favaron et al., 1988) detailed a defense activation in soybean in response to purified polygalacturonase and OA. Within 20 minutes of treatment, measurable amounts of the phytoalexin glyceollin were induced by two of the four polygalacturonases tested, as well as by millimolar levels of OA, with 5 mM being the most effective; 10 mM OA actually gave a 20% reduction in the induction levels as compared to 5 mM. In an actual infection, OA concentration ranges from very low to high (80 mM to be the highest reported), thus a dynamic picture of how OA molecules impact the host cells across a given region of tissue, seems probable.

An essential role proposed for OA is to modulate the rapid host reactive oxidative burst (ROS), one of the earliest and most universal hallmarks of plant defense (Cessna et al., 2000). Inoculation of an OA-deficient strain on tobacco leaves led to considerable ROS as measured by oxidation of nitroblue tetrazolium, while there was no obvious ROS increase caused by the WT strain. The result suggested that OA has a role in suppressing ROS in the plant host. Further experiments were performed to explore the mechanism of this inhibition, revealing that the acidity and chelation of cations, two highly popular hypothetical roles for OA in *S. sclerotiorum* pathogenesis, did not appear to be largely accountable for inhibition of ROS. Several additional observations indicated a potential target site of OA was downstream of the defense-associated Ca^{2+} influx, but upstream of the oxidase complex that produces H_2O_2 and ROS. However, the exact molecular targets of inhibition remains unclear. Another study proposed a potential explanation for this inhibition using a plant-based redox sensing GFP system (Williams et al., 2011). It was determined that *S. sclerotiorum*, by secreting OA, creates a transient reducing environment rapidly in plant cells after infection, which compromises the host ROS burst and other host basal defense responses such as callose deposition. By contrast, an OA-deficient mutant produced hypersensitive--like lesions characterized by restricted growth and cell death. Consistent with the first study, this redox alteration of the host cells was also shown to be independent of acidity. The underlying mechanism of manipulation of host redox status was proposed by the authors as involving key redox molecules such as thioredoxins, leading to the inability to form normal conformational changes of many redox-sensitive signaling components in the activation of plant defenses.

The defining characteristic of a necrotrophic fungus like *S. sclerotiorum* is to kill plant cells

and then obtain nutrients from the dead host for its own use. In this sense, plant programmed cell death (PCD), which is a strategy that plants use to achieve complete resistance to some biotrophic pathogens, may be beneficial to the pathogenesis of *S. sclerotiorum*. OA was suggested to induce apoptotic-like PCD in hosts, thus facilitating the disease development (Kim, et al. 2008). DNA fragmentation, which is a feature of apoptotic-like cells in mammals, was possibly observed in DNA extracted from tobacco leaf discs 36h after treatment with *S. sclerotiorum* WT culture filtrate, and from infiltration with different formulations of oxalate, while it was not obvious in water and other acids such as citric acid, HCl and succinic acid. The previous study involving soybean suspension cells showed that OA inhibited ROS (Cessna et al. 2000); however, in contrast, for this study on tobacco discs, oxalate induced ROS generation and triggered PCD. The tobacco disc assay showed that OA could elicit PCD and ROS production only in a relatively higher pH (5 to 6) milieu, while at lower pH values (3 to 4), neither DNA laddering nor ROS production was significantly detected. The mechanisms of PCD were further explored (Kabbage et al., 2013) and was found that plant cell death in response to S. sclerotiorum may be triggered by either apotosis or autophagy, and the OA affects this outcome. OA by itself seems to induce apoptosic PCD (Kim et al., 2008), but in an OA minus mutant, PCD seemed to result from autophagy, and the presence of OA suppressed this autophagic cell death (Kabbage et al., 2013). Autophagy is being associated with plant host responses to other necrotrophic fungi as well (Lai et al., 2011).

A comparative transcriptomic study of soybean leaves infiltrated with 5 mM OA versus HCl-acidified water revealed that one of most significantly up-regulated genes at 2 hours post infiltration independent of acidity, was ferritin, which forms complexes to safely store toxic iron ions within cells (Calla, et al., 2014a). The fact that ferritin was so strongly induced could have indicated an increase in free iron, presumably released from iron-binding components in cells by OA, a strong chelator of iron. The authors proposed that the loss of iron from enzymes containing cytochrome co-factors, such as the cytochrome P450s involved in secondary metabolism, would greatly weaken the ability of the host to produce an active defense. They also suggested that the loss of iron from the electron transfer components of the mitochondria and chloroplasts, could lead to high accumulation of ROS, such as when light stimulates chloroplast photosystem centers under low electron flux (Zhu, et al. 2105; Zhu, et al., 2015). In spite of all the circumstantial evidence, precise mechanisms connecting OA and the observed inductions of ROS and PCD is still lacking.

If OA can both suppress the oxidative burst and elicit PCD through stimulating the generation of ROS, the question becomes 'How does OA work to coordinate these two functions which seem to contradict each other?' One possibility suggested by Kim et al. (2008) was that the induction of PCD was time and dose dependent, supported by the observation that no significant difference of cell viability of tobacco leaf disks was detected between the treatment of 20 mM potassium oxalate and water until 48 h post-treatment, with the time point being delayed at a lower concentration of potassium oxalate of 10 mM. With retrospect to the Cessna et al. study (2000) on the effect of OA on the oxidative burst, 4 mM was found to be the median inhibitory concentration, and the inhibition was detected within 10h of treatment, a time much earlier than 48h. Thus, it is tempting to speculate that in the early infection stage, when OA is at a lower concentration, OA is more responsible for reducing host oxidant production, but in the later infection stage and at a higher concentration, it is more responsible for inducing PCD. Another possibility is related to the pH dependency exhibited by OA manipulating ROS production. It was suggested that the extracellular pH pertubations had a fast and strong effect on vacuolar pH while the cytoplasmic pH remained relatively undisturbed (Horn et al., 1992). Moreover, histological investigation of *S. sclerotiorum* infection process revealed that OA could be metabolized or transferred to host vacuoles in early infection stage (Heller and Witt-Geiges, 2013). Combining this information, it is not unreasonable to postulate that dual functionality of OA could be executed associated with different cellular compartmentalization of OA during different stages of infection, in addition to varying concentrations in and near the infection zone. In the early infection stage, OA would presumably exist mainly in the apoplast or host vacuoles where the pH decreases rapidly, and the oxidative burst is inhibited. When the concentration of OA increases, OA gra-

dually accumulates in the cytoplasm where the pH is higher than apoplast and the vacuole, but within the optimal range for PCD induction. This speculative model would also explain, to some degree, why the PCD was elicited at a relatively later time during infection.

3.3. A proposed "hemibiotrophic" lifestyle for *S. sclerotiorum*

The secretion of OA from *S. sclerotiorum* initially suppresses host immune response and later enhances cell death, which suggests that for a historically defined necrotrophic fungus, its strategy of infection may be much more intricate than just brutally imposing toxins and PCWDEs. Recently it has been proposed that *S. sclerotiorum* is actually a polyphagous plant pathogen that transitions from biotrophy to necrotrophy during pathogenesis (Kabbage et al., 2015). It was observed that the primary hyphae of *S. sclerotiorum* killed tobacco cells during the initial 12 hours of infection, but soon thereafter the pathogen employed clever strategies to avoid host recognition and host resistance during this early infection period, similar to the behavior of biotrophic rust pathogens. Following this stealth period, after about 12 hours post infection, *S. sclerotiorum* entered a necrotrophic phase by triggering rapid cell death (and possibly PCD) of plant cells, while at the same time, the leading invasive hyphae still maintained a biotrophic state. By contrast, microscopy of the infection of a model necrotrophic fungus, *Botrytis cinerea*, revealed that the hyphae were always associated with dead cells. Moreover, the OA-deficient mutant was not able to suppress host defense effectively and instead induced host cell death rapidly, thus also leading to the restriction of hyphal growth only in the dead cell area. It was concluded by the author that the initial biotrophic-like stage was critical to establishment of *S. sclerotiorum* pathogenesis (Kabbage et al., 2015).

3.4. Evaluation of *S. sclerotiorum*-resistant transgenic lines

Another approach to study the effects of OA is to look at transgenic plants that degrade OA, such as the use of the addition of oxalate oxidase (OxO), an enzyme that catalyzes the degradation of OA. For example, an OxO soybean transgenic line carrying the wheat germin transgene (gf-2.8) coding for OxO, conferred resistance to *S. sclerotiorum* (Donaldson et al., 2001). When attached flowers were inoculated, an OxO line was able to reduce OA levels and greatly inhibit ingress of the fungus, and the disease symptoms largely remained at the flowers; whereas in the non-transgenic parent (WT), the disease progressed rapidly, spreading to the main stem and beyond (Davidson et al., 2016). The authors concluded that the barriers to fungal invasion in the OxO transgenic tissue appeared to be living green tissues, (i.e. the peduncle, petiole, stem and leaf tissue) where OA was required to condition the tissue for colonization. They also measured OA levels during the infection period, and found that fully infected WT flowers contained about 3 mM OA. Davidson et al. (2016) also used infected, detached flowers, fully covered with visible mycelia, to inoculate leaves in a manner that mimics how soybean plants might be infected in the field (i.e. ascospores infect flowers, and an infected flower that has landed onto a leaf leads to infection of that leaf). Interestingly, when detached flowers were inoculated (such as those to be used in the leaf inoculation studies), both OxO and the WT were equally fully infected with visible mycelia covering the flowers within 3 days, and yet when the inoculation was on a flower that was attached, it took about 6 days for this stage to be reached for the WT. This heavy mycelial growth was almost never seen for the attached flowers of the OxO inoculated plants, showing that, although the attached living flower was able to be infected, this tissue was more resistant than when this same floral tissue was detached and presumably dying. It was also shown that the reduced levels of OA in the OxO infected detached flowers was not effective in preventing the fungus from taking over the flower, whereas the reduced OA levels in the attached OxO flowers was effective, showing that the plants do have the ability to defend against this pathogen in healthy living tissue if the OA levels are reduced. Inoculated detached leaves behaved similarly to inoculated attached flowers in that the OxO transgenics showed strong resistance, whereas the WT did not. However, that resistance did not become apparent until the second day post inoculation as hyphae emanating from infected flowers spread in large networks above and below the leaf cuticle and infiltrated the

cortical tissue of both hosts. Following the initial 24 hours, the OA levels remained fairly constant, remaining at the day 1 level for the OxO transgenic, and the lesion did not spread much beyond 1-3 cm during the 6 day study. However, in the leaves of the non-transformed parent, the OA levels increased and the leaves were completely overtaken with fungal growth by 4-6 days post inoculation. The authors concluded that their results showed that fungal host interaction is a two-phase process. Phase I occurred on both hosts but Phase II proceeded only on the WT parent, indicating that host colonization requires high levels of OA in order to proceed, and conversely, Phase I may require a lower level of OA or possibly OA may not be involved and that factors other than OA have a role during primary lesion formation.

4. Secretory proteins from *S. sclerotiorum*

As discussed above, *S. sclerotiorum* has been shown to release large amounts (up to tens of mM) of toxic OA, as well as numerous PCWDEs, to aid in the colonization of its host. Although this seems like an abundance of weapons, apparently it is only a fraction of the possible arsenal released from this pathogen. In addition to the handful of well-characterized PCWDEs, it has been reported that *S. sclerotiorum* releases many other proteins that can influence disease progression. Several potential effectors were identified to facilitate necrotrophic colonization by causing host necrosis or plant cell death (PCD). Zuppini et al. (2005) discovered that an endo-polygalacturonase (endo-PG) could modulate host cytosolic Ca^{2+} signaling, in aequorin expressing soybean cells leading to PCD characterized by chromatin condensation, apoptotic nuclei and activation of the cytochrome c/caspase 9 pathway. However, this study did not provide evidence to show that the effects of endo-PG on host cells were independent of cell-wall -degrading abilities. Subsequently, a protein (IPG-1) containing a C2 domain (a Ca^{2+}-regulatory domain) in canola was proposed to be a potential target in host cells for sspg1d, one of the endo-PGs of *S. sclerotiorum,* since these two factors were observed to interact with each other both in vivo and in vitro during early infection (Wang et al., 2009). Moreover, this protein presented a dynamic subcellular localization from the plasma membrane to the cytosol, both before and after Ca^{2+} ionophore treatment. Although the mechanism of function of endo-PG to be an effector is unclear, the authors postulated that the interaction between the endo-PG and IPG-1 could interfere with the successful binding of IPG-1 and Ca^{2+}, thus promoting PCD. Two recent studies reported a secretory protein with a putative Ca^{2+}-binding EF-hand motif, Ss-caf1, and a cysteine-rich small secretory protein, SsSSVP1, to be possible effectors of *S. sclerotiorum* (Xiao et al., 2014; Lyu et al., 2016). Both of these proteins were closely associated with virulence, and their transient expression in tobacco leaves led to cell death. The Ss-caf1 gene was identified by T-DNA mutagenesis isolate Sunf-M, and the resulting mutant called Sunf-MT6. The mutation caused Sunf-MT6 to loose the ability to form compound appressoria, and the ability to induce lesions without first wounding. Sunf-MT6 produced fewer but larger sclerotia, and produced more OA than Sunf-M. Complementation studies supported that loss of Ss-caf1 was responsible for the changes in phenotype.

Some of the secretory proteins may play important roles in modulating host defense responses during different phases of infection or transition of lifestyles for *S. sclerotiorum*, similar to OA as discussed above. Zhu et al. (2013) found that SSITL, an integrin-like protein, could be a potential effector protein that suppressed host resistance during initial biotrophy-like stages of infection. The expression of this gene was characterized as being highly induced at 3 days when *S. sclerotiorum* was grown on PDA medium, and at 1.5 to 3.0 hours post inoculation (hpi) on *Arabidopsis*, suggesting that it could be involved both in sclerotial development and pathogenesis. Immunogold labeling suggested its secretion into both fungal cell walls and host extracellular matrix, while immunofluorescence showed its release through the hyphal tips, supporting its role as an effector for early infection. *SSITL* silenced transformants of *S. sclerotiorum* by RNAi technology exhibited a range of abnormal vegetative growth and significant reduction of virulence on *Brassica napus*. More interesting, further investigation of the mechanism showed that this potential effector could delay the induced expressions of *PDR1.2* and *PR1*, constituents of jasmonic acid (JA) and salicylic acid (SA) signaling pathways, respectively. Since the interference with host defense responses is critical for establishment of infection (Kabbage et al., 2015), it is probable that *S. sclerotiorum* possesses other effectors. This emer-

ging topic is worthy of further investigation.

Bioinformatic approaches can facilitate identification of putative effector candidates. A broad analysis of the *S. sclerotiorum* genome sequence data suggested over 600 secreted proteins, based on the N-terminal leader peptides predicted by SignalP and elimination of genes coding for carbohydrate-active enzymes and peptidases (Amselem et al., 2011). Using the same dataset, another study initially identified more than 700 proteins in the predicted secretome, but then narrowed the candidate list down to 486 proteins, based on their expressions *in planta,* by referring to the published EST and microarray data (Guyon et al., 2014). Moreover, looking for proteins that possessed conserved fungal effector domains, exhibited signatures of positive selection, had recent gene duplications, and were *S. sclerotiorum* specific yet showed analogies to known protein fold in predicted 3D structure, reduced this candidate list to just 78 effector candidates. The authors highlighted a predicted subtilisin inhibitor and three *S. sclerotiorum*-specific toxin-analogous proteins for further functional investigation. Considering the release of all these proteins with numerous functions, in addition to the release of the multi-functional OA, the host plant faces numerous challenges!

5. High-throughput gene expression studies on *Sclerotinia*-host interactions

In attempts to obtain a more comprehensive view on *Sclerotinia*-host interactions, several studies on high-throughput transcriptome expression were conducted as these analyses shed light on possible molecular and biological processes involved in both pathogenesis and plant defense. As early as 2007, two studies were published using an *Arabidopsis thaliana* microarray platform consisting of 26,000 *Arabidopsis* genes to characterize the transcriptomes of *Brassica napus* L. in response to *S. sclerotiorum* (Zhao et al., 2007; Yang et al., 2007). Although the genome sequence was not fully covered with these early microarrays, both studies identified more than 1,000 differentially expressed genes after infection. These results suggested the majority of genes significantly up- or down-regulated were involved in plant signal transduction, pathogenesis-related proteins, ROS metabolism and cell wall integrity. With the development of microarray slide libraries for many plants, the study of *S. sclerotiorum*-plant interactions was extended to other hosts. In 2009 a soybean-*S. sclerotiorum* study, profiled the transcriptomes of stem tissues from a partially resistant and a susceptible soybean genotype at 8 and 14 hpi using soybean cDNA microarrays (Calla et al., 2009). The results from this soybean study showed much overlap with the results from the *B. napus* study, suggesting a relatively conserved behavior of plants in response to *S. sclerotiorum*. Among the 1,270 genes that were significantly differentially expressed between time points, 30 genes were related to cell wall modification and 42 genes were related to direct defense processes including several apoptosis-related, putative R genes with LRR domains and pathogenesis-related genes. Notably, about 120 genes were identified as early signal transduction pathway components, with a significant number of them participating in G-protein mediated signaling, inositol signaling and the widely reported ethylene (ET) signaling pathways. However, it was pointed out that the inositol signaling genes were reduced in abundance between 8 and 14 hpi leading to suggestion that inositol signaling may be activated during the earliest stages of infection. Thirty-two genes coding for enzymes on the phenylpropanoid pathway showed significant up/down-regulation across the time points, suggesting this to be a major pathway for secondary metabolism in defense to *S. sclerotiorum* in soybean. An interesting pattern was found for genes involved in isoflavonoid and anthocyanin biosynthesis, as two of the three sub-pathways of the phenylpropanoid pathway were induced, while all the differentially expressed genes in lignin biosynthesis showed reduced expression levels. The authors proposed that reducing the lignin biosynthesis could divert substrates to the other two sub-pathways, thus contributing to defense. In addition to the responses similarly displayed in both genotypes, 105 genes were significantly differentially expressed between genotypes. The genes in this list suggested that partial resistance during early infection could be associated with cell wall reinforcement such as papilla formation and secondary metabolite including anthocyanins and anthocyanidins.

The OxO transgenic soybean material described above (Donaldson et al. 2001; Davidson et al. 2016) was used in two high-throughput gene expression studies to investigate the physiological basis of soybean defense to *S. sclerotiorum*. The first

study (Calla et al., 2014b) used soybean leaves of this transgenic genotype (OxO) and its susceptible non-transgenic parent genotype (AC) sampled at 12, 24 and 36 hpi using fully colonized detached flowers as inoculum. Although OA secretion was reduced in the transgenic line, the comparative transcriptomic data showed that many of the same sets of genes had changed similarly in the same direction across genotypes, such as genes related to the cell wall, ethylene and jasmonic acid signaling pathways, phenylpropanoid pathway components, and WRKY transcription factors indicating that a basal defense was activated similarly in both genotypes, but was somewhat more robust in the resistant OxO transgenic. This quantitative nature of difference between compatible and incompatible interactions was also documented for plant responses to *Pseudomonas syringae* (Tao et al., 2003; Zou et al., 2005). Despite large similarities, the OxO genotype showed a higher level of up-regulation for many genes from 12 hpi to 24 hpi compared to AC genotype, but then dampened off at 36 hpi. The genes that exhibited higher induction in OxO included several PR proteins, secondary metabolism-related genes and matrix metalloproteinases. Among genes with significantly reduced expression over time, the number of genes in the AC genotype was about five times as many as that in OxO. Evidence supporting this pattern came from photosynthesis-related genes, with 75% genes more reduced in AC than in OxO. Eight of the ten genes showing the highest difference in the direction of expression change across the two genotypes were associated with photosynthesis or the redox state of the cell; this suggested that OA had the capacity to modulate the plant cell redox environment (perhaps involving chloroplasts) for successful disease establishment, as also proposed by Williams et al. ,(2011). This study also investigated the differential expressions of critical enzymes in the lignin biosynthesis pathway and found gene expression was induced for lignin-related enzymes, a result conflicting with the stem-inoculation study (Calla et al., 2009). The opposing results could be due to different tissues (stem versus leaf) or different time points, but this requires further investigation.

Another study using AC and OxO soybean germplasm characterized the role of pure OA (no fungus) in the *S. sclerotiorum*-soybean interaction (Calla et al., 2014a). Soybean leaves from the two genotypes were infiltrated with 5 mM OA at pH 2.4, water at pH 2.4 (pH adjusted with HCl, an acid that does not have any chelating properties), and water at pH 5.5; the transcriptome was characterized at 2 hpi. In agreement with other studies on the importance of the low pH property of OA for successful *S. sclerotiorum* infection (Xu et al., 2015; Favaron et al., 2004), most genes (>1000) showed statistically significant expression changes in response to OA at pH 2.4 versus water at pH 5.5. Many genes induced by OA at pH 2.4 were closely related to basal defense and overlap with those identified in the *S. sclerotiorum*-infection study described above (Calla et al. 2014b). For example, the phenylpropanoid pathway, cytochrome P450 and glutathione S-transferases, peroxidases and PR proteins were all induced. When looking into the effects of OA independent of low pH (by comparing expression changes in leaves in response to OA at pH 2.4 versus water at pH 2.4) there were 78 genes considered significant, supporting that OA is affecting the host through its chemical nature, not just low pH. Notably, eight of the genes that changed independent of pH coded for ferritin or ferritin subunits. The induction of ferritins was confirmed by an additional non-replicated RNA-Seq experiment conducted to verify the microarray data. As RNA-Seq advanced the coverage of transcriptome, other iron-related genes were also identified as being induced by OA, such as a cytochrome b561 and ferric reductases/oxidases. Based on the observations of both datasets, and as mentioned above, the authors proposed that OA released by *S. sclerotiorum* might be chelating iron out of ferritin, as well as out of other iron-containing cellular components in the host, weakening defense, and enhancing cell death. Interestingly, the author noted that no ferritin genes were significantly induced by infection of the pathogen (Calla et al., 2009; Calla et al., 2014), suggesting that the OA is releasing iron; however, when the fungus is present, the fungus is taking up the iron before it accumulates to the threshold levels needed to trigger ferritin gene expression. These expression studies again show the complexities of the *S. sclerotiorum* – host interactions.

A higher-resolution and more comprehensive transcriptomic analysis using RNA-Seq was conducted a transcriptome analysis to compare a resistant (R) and a susceptible (S) line of *Brassica napus* (Wu et al., 2016). They identified more

than 9,000 genes showing significant differences between the two genotypes, consisting of about 6,000 genes up-regulated and 3,000 genes down-regulated in the R line compared to the S line. Gene ontology enrichment analysis indicated the potentially important roles of genes responding to chitin, cadmium ion and hydrogen peroxide in the defense response. In the R line, genes involved in glycolysis were significantly up-regulated while genes involved in starch biosynthesis were down-regulated, suggesting *B. napus* switched from the anabolic to the catabolic state to provide energy or substrates for defense. Taking advantage of the high coverage of RNA-Seq, this study also systematically and thoroughly characterized the regulation of a variety of biological pathways by searching the *B. napus* genome for homologs of genes identified for these pathways in Arabidopsis. Through

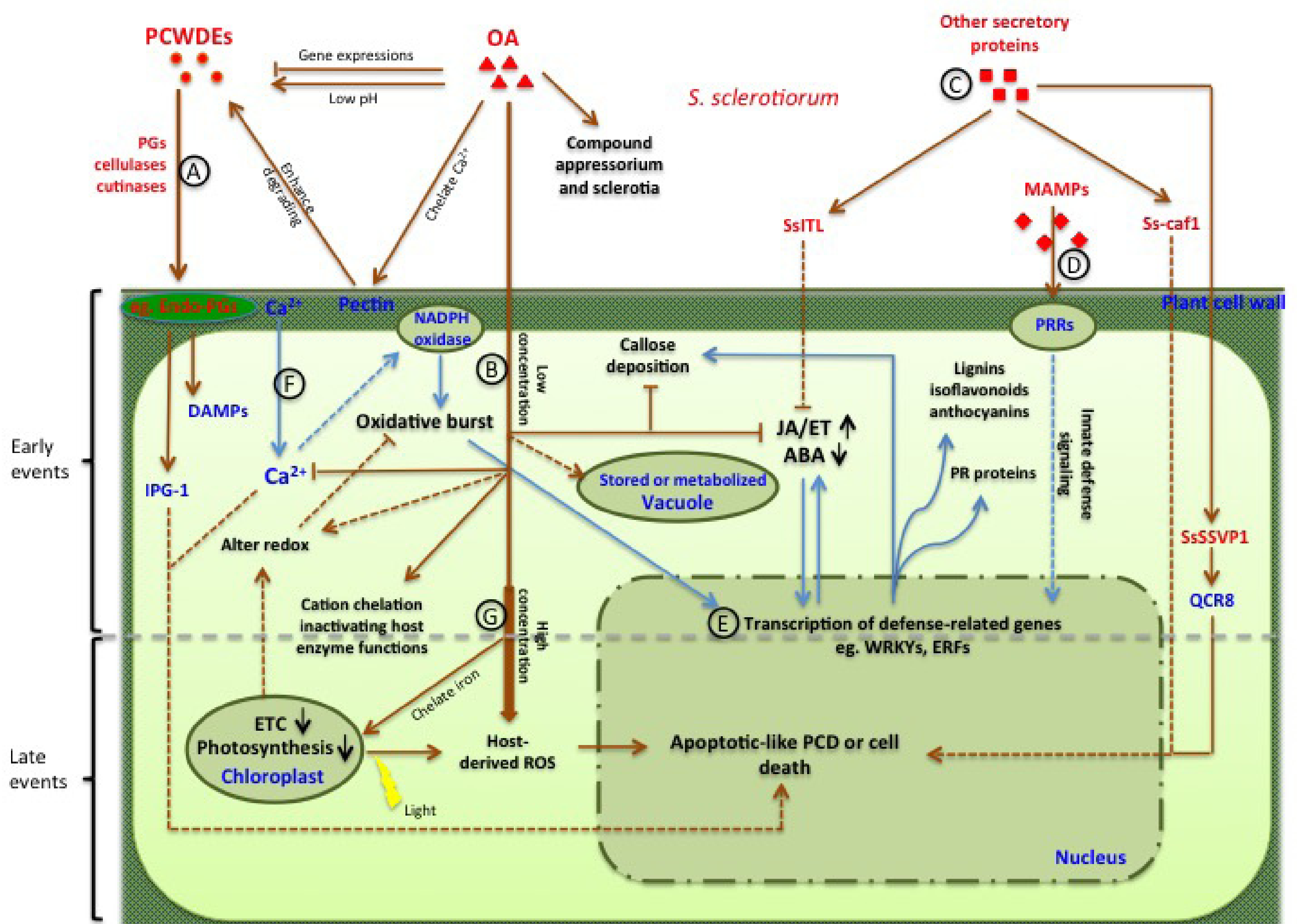

Figure 2. Postulated dynamic molecular events occurring in early and late host-*S. sclerotiorum* interaction stages. Most pathways shown here were also mentioned in the text, with some literature to support their assumption. Red: fungal molecules; blue: host molecules or components; brown arrows: fungal activities or negative impacts on host cells; blue arrows: host defense response that should benefit host defenses; dashed arrow: unknown pathways could be involved. PCWDE: plant cell-wall-degrading-enzymes; OA: oxalic acid; PG: polygalacturonase; DAMPs: damage-associated molecular pattern; MAMP: microbe-associated molecular pattern; PRR: pattern recognition receptors; ETC: electron transport chain; ROS: reactive oxygen species; PCD: programmed cell death. PCWDEs (A) PCWDEs are enhanced by OA in several ways. Endo-PGs, as one example, generates DAMPs by degrading cell walls and also interferes with IPG-1 and calcium signaling, contributing to PCD. In early events, under low OA conditions, the host initiates defense responses including MAMPs triggered defense signaling pathways (D), transcriptions of host defense-related genes and calcium influx (F) into cytoplast that induces host oxidative burst and possible autophagy. However even at low concentrations of OA, many of the diverse defense responses become more and more suppressed by OA (B) leading to reduced calcium signaling, inhibited callose deposition, modulated redox that suppresses the oxidative burst, and the chelation of various cations that are needed by numerous host enzymes for healthy metabolism. In later events, as OA concentrations continue to increase, not only is the host defense further weakened, but also host PCD is elicited by accumulated high concentrations of OA (G) perhaps stimulating ROS by chelating iron out of the ETC. Other secretory proteins (C) function by either suppressing host defense in early events or inducing cell death in later events.

this method, the networks of many genes were depicted and a more detailed comparison between two genotypes was presented, involving RLK-mediated pathogen recognition, MAPK signaling cascades, WRKY transcription regulation, glucanases and chitinases, hormone signaling pathways and glucosinolate synthesis. The authors noted a pattern where the R line induced a more dramatic basal defense response, in agreement with the conclusions of the soybean microarray study (Calla et al., 2014b). Interestingly, in addition to the large induction of JA and ET that were widely reported for plant defense to *S. sclerotiorum*, the SA, auxin, abscisic acid (ABA) and gibberellic acid (GA) pathways exhibited noticeable inhibition in both genotypes. However, the cross-talk between different hormone-related pathways may be more complex. Stotz (2007) and Zhu et al., (2013) evaluated the expression of *PR1* (an SA-responsive gene) in Arabidopsis after inoculation and both studies noted a negligible induction. Moreover, the Arabidopsis *npr1* mutant (defective in SA signaling) was found to be hypersusceptible. Several auxin-related genes were identified as significantly induced in response to OA infiltration or *S. sclerotiorum* infection (Calla et al., 2014a; Calla et al., 2014b). Thus there is a possibility that the plant defense to *S. sclerotiorum* may also involve the SA and auxin pathways, but the detailed mechanisms await further investigation.

6. Conclusions

From de Bary's keen observation of the secretion of PCWDEs and OA, studies on *S. sclerotiorum* continued to focus mainly on these traits for more than 100 years. This review presents the story of how scientists have succeeded to delve deeper towards understanding these two factors, with additional new revelations and details. The review also shows how pathologists working on *S. sclerotiorum*-host interaction kept up with the revolutions in science and utilized the new technologies as they emerged, including new methods from basic physiology, biochemistry, molecular biology and bioinformatics. Through these diligent efforts, a variety of PCWDEs have been identified, multiple functions of OA have been explored, a potential life phase transition for the pathogen has been proposed, and other disease-contributing factors have been characterized. On the host side, extensive defense responses at the gene level have been investigated to enhance our understanding of quantitative plant resistance to *S. sclerotiorum* and to provide options for better solutions in disease control. However, this review has also shown that many aspects of the *S. sclerotiorum*-host molecular interactions still remain unresolved and debated. Therefore, the research continues at a rapid pace to further understand these dynamic and complex host-pathogen systems. Figure 2 diagrams a proposed summary of some of the events that might be occurring during *S. sclerotiorum* infection, much of which was discussed in this review and is at least partially supported by the literature.

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as a substance released by the fungus that aided in the attachment of the mycelia to the host surface. He also observed, in disagreement with de Bary, that the fungal hyphae appeared to mechanically force their way past the plant cuticle. He further observed that once “the cuticle is broken the walls of the host cells near the point of penetration show signs of being chemically altered”. Interestingly, Boyle also noted that the cells adjacent to an infected cell could be negatively affected by the invading fungus as well, presumably by fungal- or plant-released enzymes or toxins:

> “Death of the cells extends some distance beyond the limits of the invading hypha, due either to enzymes secreted by the fungus or to the products of disorganized cells. The lethal substance or substances appear to diffuse more rapidly along the palisade cells of the mesophyll than into the spongy parenchyma, as the chloroplasts of the palisade cells for some distance on either side of the point of infection are swollen or disorganized, while those of the spongy parenchyma immediately underneath are still unaffected. This is evidently due to the fact that the palisade cells are in closer contact and hence allow a more rapid diffusion of the lethal substance or substances than the cells of the spongy parenchyma, which are in contact at comparatively few points.”

Although both de Bary and Boyle stated that their studies suggested the release of plant-cell-wall degrading ‘ferments’ or enzymes, the actual identification of these molecular weapons would not start until the late 1950’s and 1960’s. In the meantime, plant pathologists successfully confirmed de Bary’s assumption that *S. sclerotiorum* produced the toxin OA. In de Bary’s work, the fact that the liquid surrounding hyphae, or droplets released by sclerotia, was acidic and would precipitate with calcium, it was assumed that this liquid had to be OA (de Bary, 1886). In 1952, when Overell (Overell, 1952) found that spent culture filtrates of *S. sclerotiorum* could macerate carrot slices, he wanted to determine the cause. He noticed that the maceration ability was pH dependent and the macerating factor(s) was stable after autoclaving. Through the use of paper chromatography and similar calcium precipitation assays, he identified OA as being present, at concentrations in the 10’s of mM in cultures that had grown up to 35 days. Overell also found that similar maceration showed up when carrot slices were soaked in pure OA in a pH-dependent manner, with the best maceration activities occurring at pH levels between 2.6 – 4.0 for his studies. Therefore, he hypothesized that OA was the cause of the deterioration, and that the different ion forms of oxalate (Figure 1) were affecting the maceration, as OA would be mostly fully deprotonated as the pH levels rose past 4, and it would be mostly in the mono-basic ion form at pH 2.6 to 4.0 (oxalic acid $pKa_1 \approx 1.2$, and $pKa_2 \approx 4.2$).

Interestingly, the levels of OA found by Overell were fairly similar to the levels found in cultures of the close relative of *S. sclerotiorum, Sclerotinia rolfsii*, by Higgins 25 years earlier (Higgins, 1927). Higgins identified large amounts of OA released from *S. rolfsii*, reporting 0.3 – 3x more grams of OA as grams of mycelial dry when growing *S. rolfsii* in various liquid media for 31 days, and estimated the oxalate concentrations at 5-70 mM. Higgins, like Overell, also used precipitation with known amounts of calcium as a means to calculate the approximate amount

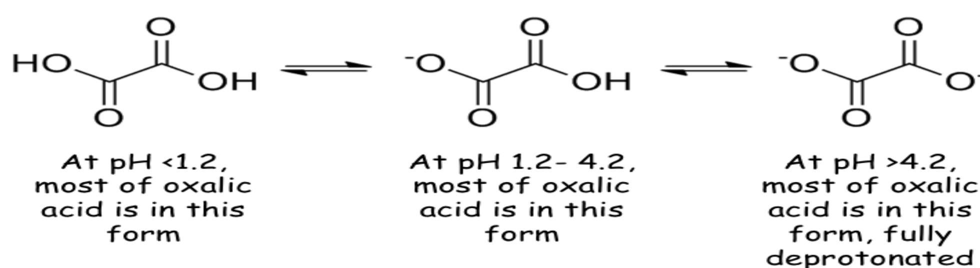

Figure 1. The molecular form of oxalic acid at different pH levels. The pH of the apoplast of plant cells is estimated to be about 5.6, so during initial *S. sclerotiorum* infection, most of the oxalic acid entering the plant tissue into the apoplost would be fully depronated. After continued fungal growth and acid release, the acidity could build up to levels that would overpower any buffering capacity of the apolplastic fluids, and the pH would lower. The pH of *S. sclerotiorum* infected tissue is often measured to be somewhere between 2 and 4, meaning that the oxalic acid would be mostly in the mono-basic (mono-protonated) form.

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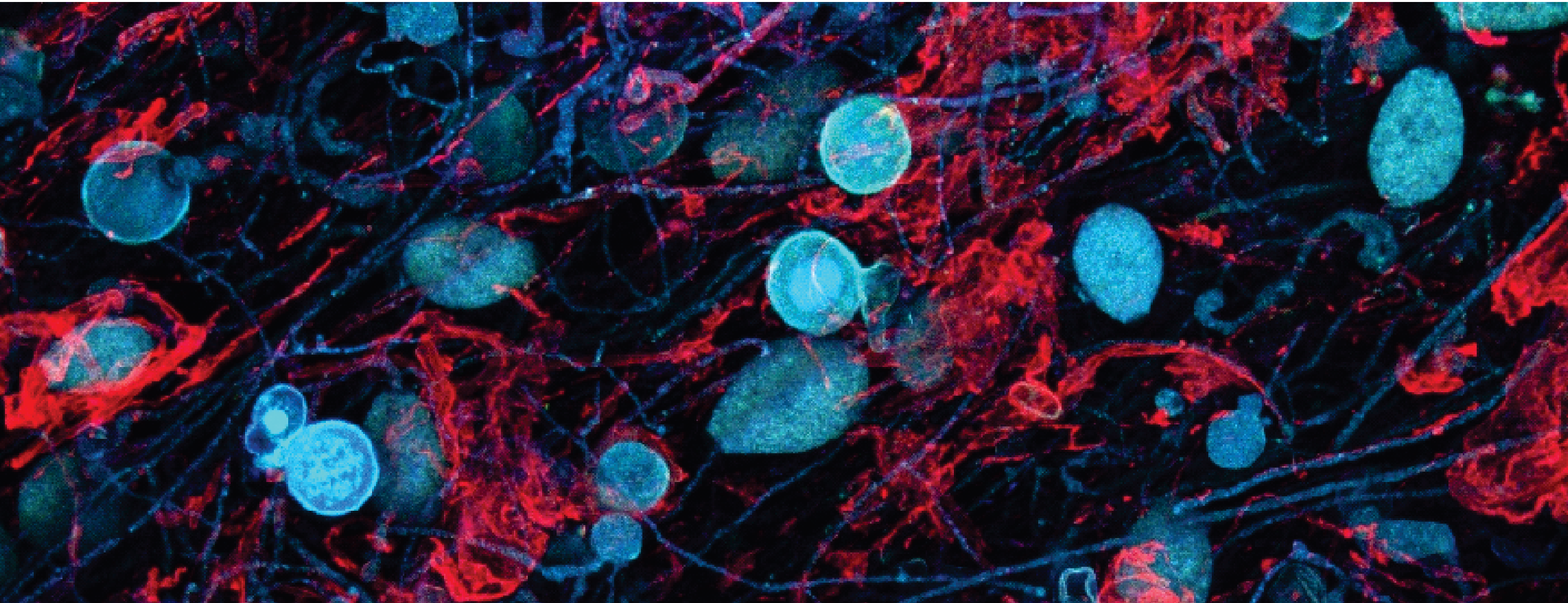

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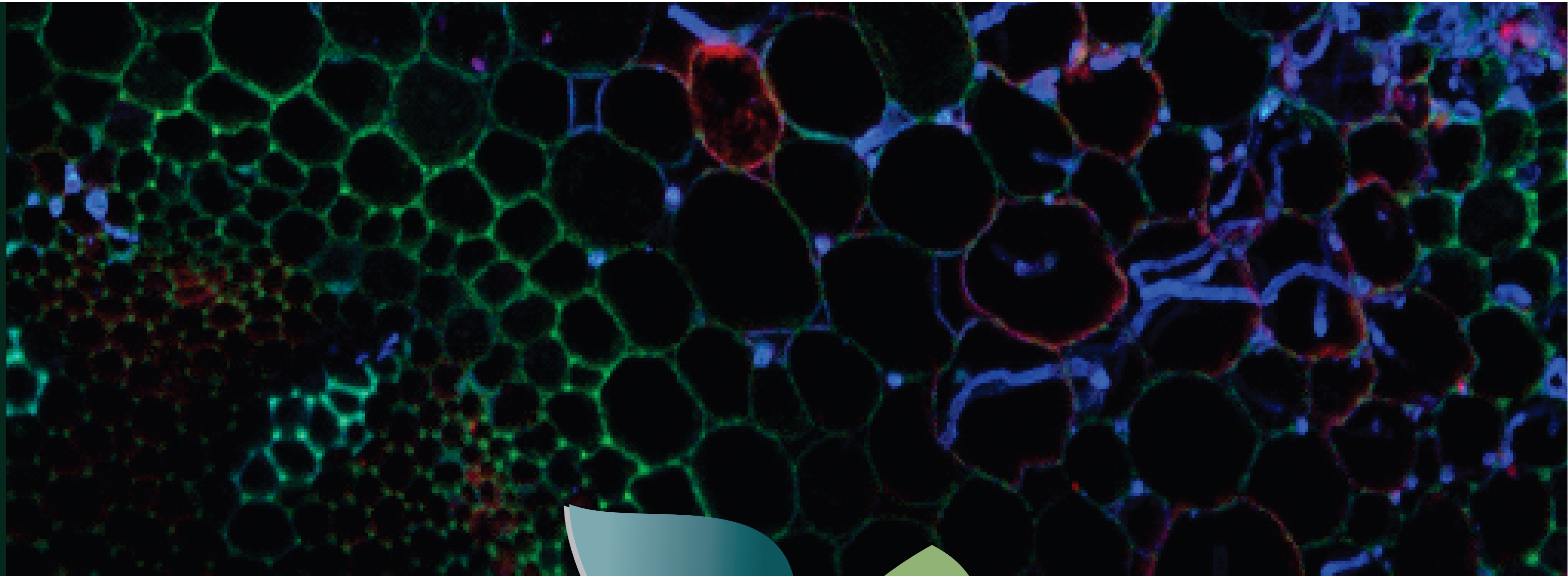

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EDITORIAL

Renovar para avançar

Toda a comunidade da fitopatologia brasileira concordará que a Revisão Anual de Patologia de Plantas se incorporou definitivamente como obra de referência para todos os profissionais e estudantes que atuam nessa área. Com seus capítulos atualizados e abrangendo não só assuntos específicos do Brasil, mas de toda a ciência da fitopatologia ela, juntamente com a Tropical Plant Pathology e a Summa Phytopathologica, consolidam e difundem informações científicas e tecnológicas essenciais para o avanço ainda maior da agricultura brasileira.

Ao ser definitivamente incorporada à Sociedade Brasileira de Fitopatologia a RAPP tornou-se institucional e passa a ter abrangência maior, sendo uma publicação que continuará consolidando assuntos atuais e importantes. Ao ser disponibilizada eletronicamente ela segue a tendência irreversível de publicações científicas. Quando se tornar completamente aberta seu índice de citação aumentará expressivamente.

Seu novo formato editorial demonstra leveza e alinha-se com outras publicações de revisões. Melhoria contínua no conteúdo e formato sinalizará que ela está no caminho de ser comparada, em futuro próximo, às melhores publicações do gênero. É evidente que a comunidade científica que mantém dinâmica e competitiva a Fitopatologia Brasileira está mais que habilitada a enriquecer e elevar o nível das revisões publicadas pela RAPP.

Como toda área da ciência, a Fitopatologia defronta-se com desafios crescentes, principalmente face às questões relacionadas a sustentabilidade de produção agrícola, aumento e agravamento de problemas fitossanitários e a problemas ambientais, principalmente aqueles associados a mudanças climáticas, cada vez mais determinantes na agricultura. A resposta a isso deverá vir na forma de novas tecnologias de manejo, novos cultivares e novas tecnologia de produção. O caminho para todos esses desafios passa necessariamente pela ciência da Fitopatologia. Avanços somente podem alcançados se o caminho da ciência for mantido e fortalecido. Fora disso não há milagres. A RAPP a medida que seguir o caminho da qualidade de suas revisões deverá contribuir em muito para que os avanços se concretizem.

A RAPP passa também a adotar o sistema de trabalho com revisão submetidas ao invés de revisões convidadas. Com isso espera-se que maior número de revisões serão submetidas para avaliação e eventual publicação, ampliando sobremaneira o número de colaboradores. Revisões convidadas podem sugerir que sejam revisões aceitas, o que nunca foi o caso. Somente mantendo sua qualidade editorial é que ela se fortalecerá como veículo importante na Fitopatologia Brasileira. Todas as frentes de avanço do conhecimento e da tecnologia devem ser priorizados. A diversidade e a amplitude da Fitopatologia permitem isso.

Toda a comunidade da Fitopatologia Brasileira está convidada a fazer com que a RAPP alinhe-se cada vez maisàs mais prestigiadas publicações brasileiras.

Dr. Marcos A. Machado
Membro do corpo editorial da RAPP e
Diretor do Centro de Citricultura Sylvio Moreira – IA- SP