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Title

Victoria Blight, Defense Turned Upside Down.

TJ Wolpert¹ and JM Lorang

Department of Botany and Plant Pathology and Center for Genome Research and Bioinformatics, Oregon State University, Corvallis OR, 97301-2902

¹wolpertt@science.oregonstate.edu

Abstract

Genes that confer disease resistance to biotrophic pathogens typically encode nucleotide-binding, leucine-rich-repeat proteins (NB-LRRs). These proteins confer resistance by detecting the presence of virulence effectors secreted by biotrophic pathogens. Recognition triggers NB-LRR activation and subsequently, the defense response which often includes localized host cell death. The fungus, *Cochliobolus victoriae*, is a necrotrophic pathogen that causes a disease called Victoria Blight. Virulence of this fungus is dependent on its production of a peptide called "victorin" that has been traditionally described as a toxin. Only plants that respond to victorin are susceptible to *C. victoriae* whereas those that do not are resistant to the fungus. Genetic and molecular analyses have revealed that victorin functions like a biotrophic effector recognized by a NB-LRR resistance protein in Arabidopsis. Further, numerous plant species express victorin sensitivity suggesting there are numerous NB-LRRs that recognize victorin. Thus, through expression of victorin, *C. victoriae* is able to exploit plant defense to cause disease and is capable of evoking this response in an array of different plants.

Keywords

Resistance, Susceptibility, Effector, Host Selective Toxin, Victoria Blight, *Cochliobolus victoriae*

Introduction

Victoria Blight is a disease originally described on oats and is caused by the necrotrophic pathogen, *Cochliobolus victoriae*. The fungus is pathogenic by virtue of its production of a family of unusual peptides called victorin. Victorin has historically been referred to as a host-specific toxin and is absolutely required for virulence. Isolates that make victorin are fully pathogenic whereas isolates or outcrosses that do not are non-pathogenic (1). On the host side, sensitivity to victorin is conditioned by a single dominant gene called *Vb*. Only oats that are sensitive to victorin are susceptible to the pathogen while oats that are insensitive, (homozygous recessive for *Vb*) are completely resistant to the pathogen.

The molecular features of Victoria Blight are reminiscent of classically described gene-for-gene interactions except that the predominant phenotypes are reversed. In Victoria Blight, virulence (i.e. victorin production) and host susceptibility are the dominant phenotypes as contrasted with classic gene-for-gene interactions where avirulence and disease resistance are genetically dominant. This association is particularly interesting when considering the origin of Victoria Blight.

Victoria Blight arose as a consequence of the widespread introduction of the *Pc2* gene for resistance to the crown rust pathogen, *Puccinia coronata* (2). Crown rust conforms

to a classic gene-for-gene interaction where *Pc2* confers genetically dominant disease resistance presumably through recognition of the effector, AvrPc2. Because of its utility for controlling crown rust, cultivars containing *Pc2*, derived from the oat cultivar "Victoria", were widely planted throughout North America (2). Soon after, a new disease arose on oats (3). Because of its exclusive association with cultivars containing Victoria-derived resistance, this disease was called "Victoria Blight". Efforts to retain Victoria-type resistance to crown rust while removing susceptibility to Victoria Blight have failed, suggesting that *Vb* and *Pc2* could be the same gene (4,5). Further, various physiological studies suggest that victorin induces a defense-like response in sensitive oats (6) and thus reinforce a potential connection between *Vb* and *Pc2*. The association of Victoria Blight susceptibility with crown rust resistance implies that a defense gene to one disease can confer susceptibility to another and suggest that some pathogens can exploit defense for susceptibility.

Thus Victoria blight presents a nexus between two seemingly opposite host responses and suggests that pathogens can evoke susceptibility by "intentionally" activating defense. This has serious implications because it means that at least some genes deployed to limit disease can actually lead to increased disease with potentially devastating consequences. Recent molecular characterizations of this disease interaction provide compelling support for this possibility.

Victorin Structure

Structural analyses revealed that victorin activity is not due to a single compound but actually derives from a group of closely-related, cyclized pentapeptides all of which contain glyoxylic acid and a cyclic combination of five unusual amino acids (7,8,9). Structure-activity studies of the predominant form, victorin C, revealed that the aldehyde moiety of the glyoxylate residue is essential to victorin's mode-of-action. In addition, it was found that the free ϵ -amino group of the hydroxylysine residue can be derivatized while retaining host-selective activity (10). This allows the production of a variety of derivatives that can be used for "tracking" victorin both *in vivo* and *in vitro*. *In vivo* binding studies with labeled victorin reveal that it binds covalently to a low molecular weight protein of about 13-15 kDa in both sensitive and insensitive oat genotypes and to the 100 kDa P-protein of the glycine decarboxylase complex (GDC) only in sensitive genotypes (11). However, numerous studies confirmed that the GDC is not the primary site of action and that binding to the P-protein likely occurs due to genotype-specific changes in mitochondrial permeability following victorin treatment (12,13).

Identification of a Genetic Model for Victoria Blight

Molecular characterization of the *Vb* gene would undoubtedly clarify whether *Vb* and *Pc2* are the same gene. However, due to the large genome size and paucity of molecular characterization, a molecular genetic approach in *Avena sativa* (oats) is only recently becoming a possibility. Therefore, to initiate a molecular genetic analysis of victorin sensitivity, a screen of *Arabidopsis thaliana* ecotypes was conducted (14). This screen originally identified six ecotypes that clearly display victorin sensitivity. Genetic analyses of individuals selected from these ecotypes demonstrated that victorin sensitivity in *Arabidopsis*, as in oats, is conferred by a single dominant gene which was allelic in all the sensitive ecotypes tested (14). We named this locus "*LOV*" for locus orchestrating victorin

effects. Thus, the genetics of the response to victorin in Arabidopsis is similar to oats. Also similar to oats, it was found that the victorin response is exclusive to genotypes carrying *LOV1*. Concentrations at least 1000 times higher (the highest concentrations tested) than required for a response in sensitive Arabidopsis do not affect insensitive (*lov1 / lov1*) Arabidopsis genotypes. Also like oats, victorin induces electrolyte leakage only in sensitive Arabidopsis and the Arabidopsis victorin response involves Rubisco cleavage, DNA degradation and victorin binding to the mitochondrial P-protein of the glycine decarboxylase complex (15). This latter response is associated with a mitochondrial permeability transition in oats and indicates a significant role for mitochondria in the regulation of victorin-induced cell death in both oats and Arabidopsis. Also similar to oats, where ethylene inhibitors reduce victorin sensitivity (16), it was found that the ethylene response mutant *ein2* attenuates victorin sensitivity in Arabidopsis (17). Additionally, victorin induces rapid expression of *PR1*, salicylic acid accumulation, and camalexin production in Arabidopsis (17). All of these responses are consistent with the resistance-like responses associated with victorin treatment of oats.

Because *LOV* clearly mediates victorin sensitivity, *LOV* was tested for its ability to confer susceptibility to *C. victoriae* (14). Plants were spray-inoculated with *C. victoriae* spores that had been washed to remove residual victorin and evaluated for disease symptoms. Toxin-insensitive Columbia plants do not develop symptoms. However, victorin-sensitive plants show leaf chlorosis and necrosis. Microscopic examination revealed that while conidia germinate and form appressoria on insensitive Columbia leaves, little or no penetration by the fungus is evident. In contrast, on leaves of victorin-sensitive plants, hyphae penetrate tissue and proliferate in the intercellular spaces of the mesophyll. In completion of Koch's postulates, infected tissue, following surface sterilization and plating on water agar, displayed extensive sporulation of *C. victoriae* from tissue of victorin-sensitive Arabidopsis.

These analyses demonstrate that victorin-sensitive Arabidopsis display a susceptible phenotype whereas insensitive Arabidopsis are resistant to *C. victoriae*. Thus, Arabidopsis can be a host of *C. victoriae* and victorin remains, by definition, a "host-selective" compound. Consequently, the Arabidopsis *LOV* gene, as is the case with the *Vb* gene in oats, appears to be a true disease susceptibility gene and apparently functions by conditioning the victorin response.

A map-based cloning effort (17) identified *LOV1* as At1g10920. *LOV1* encodes a CC-NB-LRR protein homologous to members of the RPP8 resistance gene family in Arabidopsis. This gene family includes *RPP8*, *RCY1* and *HRT* (18,19,20). *LOV* shows approximately 70% identity and greater than 86% similarity to *RPP8* and the other family members. *RPP8* confers gene-for-gene type resistance to the oomycete, *Hyaloperonospora arabidopsidis* (62), whereas *RCY1* and *HRT* confer resistance to the yellow strain of cucumber mosaic virus and contribute to resistance to turnip crinkle virus, respectively (19,20). Thus an NB-LRR gene, a type of gene normally associated with disease resistance and belonging to a family of known resistance genes, confers victorin sensitivity and disease susceptibility in Arabidopsis. In addition, evaluation of victorin sensitivity in a survey of 30 ecotypes (21) revealed that sensitivity and therefore functional *LOV* is the predominant phenotype in Arabidopsis with sensitivity occurring in 28 of the 30 ecotypes. Sequence analyses of the *LOV* locus among the ecotypes revealed very limited allelic variation and suggested the possibility of a recent selective sweep. Given that positive selection would not likely occur for a role of *LOV* in conferring victorin sensitivity and disease susceptibility, these data

suggest that *LOV* very likely functions as a disease resistance gene to a widespread, common pathogen of Arabidopsis. Thus, victorin sensitivity and disease susceptibility in Arabidopsis is conditioned by a NB-LRR-encoding gene closely related to genes known to confer disease resistance and possibly has a role in conferring disease resistance to an unknown pathogen. Collectively, this set of conditions is highly reminiscent of what has been described in oats and suggests that Arabidopsis is a relevant model for examining the Victoria Blight disease interaction.

Defining the Nature of *LOV*

Even though *LOV* encodes a NB-LRR and data suggest a role in conferring resistance to a presently unidentified pathogen, in the absence of the identity of the pathogen, an unambiguous role in defense cannot be assigned to *LOV*. In other words, it is currently impossible to define *LOV* as a resistance gene. Thus, the identification of *LOV* in Arabidopsis does not unequivocally prove that a resistance gene can confer disease susceptibility and that pathogens can activate defense as a form of virulence. This is a critical implication of Victoria Blight that needs clarification. In the absence of the identification of the resistance specificity of *LOV*, it remains possible that *LOV* is an atypical NB-LRR that confers disease susceptibility through a mechanism distinct from that of typical NB-LRRs in conferring defense. To resolve this possibility, efforts were directed at structural, mechanistic and functional evaluations of *LOV* to determine if *LOV* displays properties shared by other defense proteins or is an atypical NB-LRR.

Structural analysis of *LOV*

Ethyl methanesulfate (EMS)-mutagenesis of victorin-sensitive lines carrying the *LOV1* allele was performed and offspring screened for a loss of victorin sensitivity (21). Fifty-nine loss-of-sensitivity mutants identified were genetically demonstrated to be allelic to *LOV1*. These mutants were further characterized by sequence analyses of their *LOV* genes. The coding region of the 59 mutants were each found to carry a single point mutation except for two alleles, which each contained two point mutations. Discovery that the number and type of mutants found were very similar to those found in comparable studies of EMS-induced, loss-of-function mutants of the CC-NB-LRR encoding resistance gene, *RPM1*, facilitated a comparison of structural elements inferred for each proteins' function (21). Direct comparisons showed a very similar spectrum of mutations. Remarkably, alignment of the spectrum of mutations in *LOV1* and *RPM1* demonstrated that fully 20% of the induced amino acid changes occurred in precisely aligned amino acid residues. Additionally, the remaining amino acid changes all occurred in regions of the *LOV* protein known to be important for resistance protein function such as the P-loop, the LRR domain and the coiled-coil domain at the N-terminus of the protein. Overall, the distribution of mutations is consistent with the idea that the *LOV* protein functions similarly to *RPM1* and other NB-LRR proteins that confer disease resistance.

Analysis of *LOV* Activation

In addition to identifying EMS loss-of-function mutations in the *LOV* gene, EMS mutagenesis also revealed four additional mutants that were not allelic to *LOV* but were allelic to each other. Thus, a second gene was discovered as necessary for victorin sensitivity (22). The gene was mapped and cloned and identified as At1g45145, which encodes thioredoxin h5 (*TRXh5*). All of the four EMS mutants identified could be complemented by introduction of the wildtype *TRXh5* gene. Additional confirmation of gene identity was provided by demonstrating that a SALK insertional mutant in *TRXh5* also

causes loss of victorin sensitivity (22). Discovery of the requirement for TRXh5 was key to understanding how victorin activates LOV1.

Thioredoxins (TRXs) are typically small (~13-15 kDa) proteins that act as protein disulfide oxidoreductases and are found in all free-living organisms. TRXs contain two cysteine residues in their active site that either form a disulfide bond or exist as thiols, depending on the oxidation state of the protein. Reduced TRXs can help protect cells from oxidative stress by providing reducing power to thiol-containing antioxidant proteins. However, their primary function is to regulate the redox state of disulfide bonds in target proteins, typically by reducing a disulfide to the free sulfhydryl state. In this role, TRXs act as redox-dependent regulators of the target protein's activity.

Mammals have one cytosolic TRX and one mitochondrial TRX, which are both essential for survival (23,24). However, plants have greatly expanded this gene family. For example, the *Arabidopsis* genome encodes 19 thioredoxins belonging to six major groups that localized to various subcellular compartments. The largest group and the group to which TRXh5, the thioredoxin identified as necessary for victorin sensitivity belongs, consists of eight "h"-type thioredoxins (24). Previously, there had been no specific, non-redundant functions assigned to any of the h-type thioredoxins in *Arabidopsis*. Typically, a mutation in a thioredoxin h does not exhibit a phenotype, presumably because a different family member can compensate for the loss of function. Consequently, it was very unusual to identify such a strong phenotype (loss of victorin sensitivity) associated with the loss of a specific thioredoxin h (TRXh5).

Because the other catalytically redundant, h-type thioredoxins do not compensate for a mutation in TRXh5-mediated victorin sensitivity, mutagenesis results suggested that catalytic activity may not be the property of TRXh5 necessary for victorin sensitivity. Two separate approaches confirmed this (22). In the first, mutation of the two *Arabidopsis* thioredoxin reductases (required for recycling the oxidized protein following catalysis back to its reduced, enzymatically-active form) had no effect on victorin sensitivity (22). In an additional more direct approach, it was found that expression of TRXh5 mutated at the second, essential, active-site cysteine to a serine (TRXh5C42S), which abolishes enzymatic activity, does not compromise the ability of the protein to complement victorin sensitivity (22). These results each demonstrate that enzymatic activity of TRXh5 is not necessary for victorin sensitivity. Interestingly, however, it was found that mutation of TRXh5 at the first, active-site cysteine (TRXh5C39S) does eliminate the ability of the protein to support victorin-induced cell death (22).

Binding studies of radiolabelled victorin in oats identified an ~15 kDa protein that binds victorin in both sensitive and insensitive oats (26). Given that TRXh5 is a similar sized protein; is identical in both sensitive and insensitive *Arabidopsis*; and that some property other than catalytic activity is required for its ability to support victorin sensitivity; it appeared possible that TRXh5 could, like the 15 kDa protein in oats, be a victorin binding protein and perhaps this property is necessary for supporting victorin sensitivity (27).

Victorin has a mass of approximately 1 kDa. Immunoblot analyses following victorin treatment of *Arabidopsis* demonstrated a victorin-dependent, ~1 kDa shift in TRXh5 electrophoretic mobility (27). Furthermore, analysis of proteins extracted from plants treated with biotinylated victorin, a biologically active derivative (28), revealed that the larger, immuno-reactive band also reacted with anti-biotin antibody (27). Lastly, covalent

binding was confirmed by Mass Spectral analyses of purified TRXh5 following *in vitro* incubation with victorin (27). Thus, TRXh5 is a victorin binding protein.

Several lines of evidence support that victorin binds to TRXh5 at the active-site C39 residue and is essential for cell death. As previously indicated, while both mutants, TRXh5C42S and the TRXh5C39S, lose catalytic activity, only TRXh5C42S, but not TRXh5C39S, retains its ability to bind victorin and complement sensitivity. This indicates that the active site, C39 is necessary for binding (27). Also, sulfhydryls form covalent thio-hemiacetyl bonds with aldehydes, and victorin's aldehyde moiety is essential to its toxicity (28), indicating that a cysteine residue provides a suitable site for binding. Third, victorin binds TRXh5 under reducing conditions, where cysteines exist as free sulfhydryls but not under non-reducing conditions or in the presence of the sulfhydryl blocker N-ethyl-malimide (NEM) (27), again supporting cysteine as a binding site. Additionally, mutation of the only other cysteine in TRX-h5 other than the active-site cysteines does not affect victorin-induced cell death or victorin binding. Collectively these data suggest victorin binds TRXh5 at the active site C39 and that this binding is required for victorin induced cell death (i.e. LOV activation).

Basal defense or PTI (pathogen-triggered immunity) is triggered by recognition of conserved molecules present in the pathogen called pathogen- or microbe-associated molecular patterns (PAMPs or MAMPs). A prevailing model of plant-parasite interactions is that successful pathogens secrete virulence effectors that target host proteins integral to basal defense. In response, plants evolved the ability to detect virulence effectors with resistance proteins in a process called effector-triggered immunity (ETI). Recognition can be either direct or indirect. The guard model (or the related decoy model) describes the indirect recognition process, which posits that plant resistance proteins detect virulence effectors by guarding their targets (29,30). These resistance proteins, which are typically NB-LRR proteins like LOV, become activated when the effector interacts with its target.

Based on the dependence for both LOV and TRXh5 and the requirement for victorin binding to TRXh5, it was postulated that LOV activation conforms to the guard model of resistance protein activation. That is, LOV detects victorin binding to TRXh5. If LOV activation conforms to the guard model, it would require an interaction between LOV and TRXh5. This possibility was explored through transient expression analyses in *Nicotiana benthamiana* (27). Expression of only *LOV* or *TRXh5* in *N. benthamiana* does not result in cell death, even after treatment with victorin. However, when *TRXh5* and *LOV* are co-expressed in *N. benthamiana*, together they confer victorin sensitivity (27). These data confirm that LOV activation specifically relies on TRXh5, as endogenous *N. benthamiana* TRXs do not support LOV function and TRXh5 and LOV together are both necessary and sufficient to confer victorin sensitivity. Importantly, they also demonstrated that transient expression in *N. benthamiana* provides a functional system for interaction analyses. Employing *N. benthamiana* as an expression system, it was found that LOV and TRXh5 co-localize at the plasma membrane as evidenced by both fluorescent microscopy of YFP fusions of LOV and TRXh5 and subcellular fractionation (27). In addition, yeast two-hybrid analyses demonstrated that TRXh5 interacts with the CC domain of LOV (27). Finally, using bimolecular fluorescence complementation, LOV and TRXh5 were demonstrated to directly interact *in vivo* at the plant plasma membrane in a victorin-independent manner (27). The simplest interpretation of these data is that LOV and TRXh5 form a complex at the plasma membrane and LOV is activated upon victorin binding to TRXh5 (Figure 1). This scenario

recapitulates the guard hypothesis for resistance protein activation and reinforces the view that LOV is a "typical" resistance protein.

In addition to implicating a LOV/TRXh5 interaction, conformance to the guard model also predicts that TRXh5, as a guard, has a role in biotrophic defense and victorin's interaction with TRXh5 should mimic a biotrophic effector. This was found to be the case (27). A major defense function of TRXh5 is to regulate the redox state of NPR1 (31). NPR1 regulates both local and systemic acquired resistance (SAR). NPR1 normally exists in the cytosol as a thiol-bound oligomer. However, upon pathogen challenge or treatment with salicylic acid, *TRXh5* is upregulated and reduces the NPR1 oligomer to a monomer (31,32). The monomer can then translocate to the nucleus, where it regulates defense gene expression including the defense marker, *PR1* (31). Thus, TRXh5 has a significant role in defense as a regulator of NPR1. Victorin binds covalently to the first active site cysteine of TRXh5 and as such is an effective inhibitor of its enzymatic activity (27). Consequently, in the absence of LOV, victorin should compromise NPR1 reduction and defense conferred by NPR1. Consistent with this, it was found that seedlings treated with victorin exhibited three-fold lower SA-induced *PR1*-expression (the reporter for NPR1 activation) than controls (27), which is equal to or greater than that observed for TRX and thioredoxin reductase mutants (31). It was also found that victorin increases susceptibility to the bacterial pathogen *Pseudomonas syringae* pv. *maculicola* race ES4326 (*Pma*) by five fold (27), an increase equal to that displayed by a NPR1 mutant (31,33). Thus, as implicated by the guard model, victorin acts as a virulence effector by inhibiting TRXh5 and consequently repressing SAR and basal resistance to *Pma*.

Mechanistic studies demonstrate that LOV activation conforms to the guard model of R protein activation. Further, these studies demonstrate that victorin functions as a "classic" biotrophic effector by targeting NPR1-mediated defense and activating a NB-LRR that guards that defense pathway (Figure 1). Collectively, these data reinforce the interpretation that *LOV* is a typical resistance gene.

Functional Analyses of LOV

Structural studies (sequence and mutagenesis) reveal that LOV has the same/similar structural requirements as known resistance proteins such as RPM1 and the RPP8 family members. Furthermore, mechanistic analyses reveal that LOV is activated in a manner consistent with the guard model of resistance protein activation. Thus, both structural and mechanistic studies support that LOV is a "typical" resistance protein that confers disease susceptibility. As a final approach to the characterization of LOV, a functional analysis of LOV was conducted (34).

Because expression of *LOV* and *TRXh5* in *N. benthamiana* is sufficient for conferring victorin sensitivity this expression system facilitated a TRV (tobacco rattle virus)-mediated, high-throughput gene silencing approach to interrogate additional genes necessary for LOV/TRXh5-mediated function (34). Functional analyses employed 1) a directed approach where genes known to impact resistance protein function were analyzed for their effect on LOV; and 2) a random approach where ~ 4000 genes represented in a *N. benthamiana* mixed-elicitor cDNA (cNbME) library (35) were assessed. The first approach revealed that LOV function (victorin sensitivity) requires the chaperone SGT1 which influences LOV protein accumulation, a role for SGT1 shared by many resistance proteins (36). The second approach revealed a requirement for six genes that, when silenced, suppressed LOV1-

mediated, victorin-induced cell death. In addition, these six genes were required for cell death conferred by autoactivation of the known resistance gene, *RPP8* and five of them for cell death conferred by autoactivation of the *PTO* resistance gene. In addition to cell death, four of these genes inhibited actual disease resistance conferred by *PTO* to *Pseudomonas syringae* pv. *tabaci* (34). Collectively, the results support that the victorin response mediated by LOV is a defense response. Thus, this functional study, in addition to the structural and mechanistic studies support the conclusion that LOV functions like a defense associated NB-LRR and that *LOV1* is likely a resistance gene.

Species Distribution of Victorin Sensitivity

The discovery of victorin sensitivity in Arabidopsis, a plant considerably divergent from oats, and the finding that victorin is recognized as a defeated biotrophic effector that targets an important, common plant defense pathway, suggests that many biotrophic pathogens could produce effectors functionally similar to victorin. Consequently, many plant species may have evolved similar resistance specificities. To test this hypothesis, additional plant species were evaluated for victorin sensitivity. Rice and Brachypodium were evaluated because they are more closely related to oats than Arabidopsis and thus may inform identification of *Vb/Pc2* in oats. Also, they are diploids for which extensive genomic tools are available. Barley was also screened because of its close relationship to oats, and because it too is a diploid and its genome is becoming progressively better characterized. Lastly, victorin sensitivity was evaluated in *Phaseolus vulgaris*. This “search” was motivated by a previous unrelated study which could be interpreted to indicate victorin sensitivity in pinto beans (37). In each of these species victorin sensitivity has been identified. Collectively, these studies reveal that sensitivity occurs in diverse species including two dicots, Arabidopsis, the legume, *Phaseolus vulgaris*, and four monocots, rice, Brachypodium, barley and of course oats. The finding that victorin sensitivity exists in a wide variety of plant species indicates that victorin sensitivity is widespread and conceivably could be identified in most plant species if sufficient germplasm was evaluated. This finding is entirely consistent with the hypothesis that victorin mimics a commonly deployed biotrophic effector and evokes a defense response in a wide variety of plants.

Concluding Remarks

Cumulative research has established that victorin is absolutely required for *C. victoriae* virulence and that the oat *Vb* gene confers both victorin sensitivity and susceptibility to *C. victoriae*. Further, genetic and physiological data indicate that *Vb* and the *Pc2* rust resistance gene are one and the same. It has been found that victorin sensitivity in Arabidopsis appears to completely recapitulate the response of oats at all levels including the genetic, physiological, and phenotypic levels. In Arabidopsis, victorin sensitivity and disease susceptibility is conferred by *LOV*, which encodes a NB-LRR protein, a type of protein invariably associated with disease resistance. Further, *LOV* shares the structural requirements of other plant resistance proteins; appears to be under positive selection for resistance function; is activated through a mechanism conforming to the guard model of resistance protein activation; and shares functional requirements for triggering a hypersensitive cell death response and disease resistance. Consequently, results strongly indicate that *LOV* is a resistance gene and that victorin is recognized as an avirulence determinant. Thus, the necrotroph, *C. victoriae*, exploits defense-associated HR cell death. That is, victorin is (or mimics) a defeated virulence effector and *C. victoriae* is expressing victorin precisely because it has been defeated and

triggers R-gene mediated defense. *C. victoriae* is exploiting this response for susceptibility. Examination of Arabidopsis indicates that this “inverted model” of defense is correct and therefore that a likely resistance gene, *LOV*, and by extension, *Pc2* can confer disease susceptibility.

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Figure Captions

Figure 1. Model for Victorin Activation of LOV1. Thioredoxin h5 (TRXh5) regulates the defense activity of NPR1 and is guarded by the NB-LRR protein LOV1. Victorin mimics a biotrophic effector and binds to TRXh5 at the first active site cysteine residue leading to LOV1 activation (indicated by the star) and downstream defense signaling and activation of the hypersensitive cell death (HR) response. Cell death facilitates pathogenesis by the necrotrophic pathogen, *Cochliobolus victoriae*.

Figure 1