

# Plant disease susceptibility conferred by a “resistance” gene

Jennifer M. Lorang, Teresa A. Sweat, and Thomas J. Wolpert\*

\*Department of Botany and Plant Pathology, Center for Genome Research and Biocomputing, Oregon State University, Corvallis, OR 97331

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The molecular nature of many plant disease resistance (*R*) genes is known; the largest class encodes nucleotide-binding site-leucine-rich repeat (NBS-LRR) proteins that are structurally related to proteins involved in innate immunity in animals. Few genes conferring disease susceptibility, on the other hand, have been identified. Recent identification of susceptibility to the fungus *Cochliobolus victoriae* in *Arabidopsis thaliana* has enabled our cloning of *LOV1*, a disease susceptibility gene that, paradoxically, is a member of the NBS-LRR resistance gene family. We found *LOV1* mediates responses associated with defense, but mutations in known defense response pathways do not prevent susceptibility to *C. victoriae*. These findings demonstrate that NBS-LRR genes can condition disease susceptibility and resistance and may have implications for *R* gene deployment.

*Cochliobolus victoriae* | disease susceptibility | nucleotide binding site-leucine-rich repeat | victorin

In the 1940s, a disease epidemic occurred in oats because of wide-spread planting of “Victoria-type” oats, which contain the *Pc-2* gene for resistance to the rust fungus, *Puccinia coronata*. Oats containing *Pc-2* proved to be universally susceptible to a new disease, Victoria blight, caused by the fungus *Cochliobolus victoriae* (1, 2). Pathogenicity of *C. victoriae* depends on the production of a toxin called victorin, and in oats, both toxin sensitivity and Victoria blight disease susceptibility are conferred by the dominant *Vb* gene. Despite extensive efforts, rust resistance (*Pc-2*) and Victoria blight susceptibility (*Vb*) have not been genetically separated and are surmised to share identity (3, 4), thus suggesting an unexpected relationship between plant disease resistance and susceptibility.

In recent years, knowledge of genes regulating plant disease resistance has dramatically increased (5), but characterization of genes conferring disease susceptibility is limited (6–8). The information gap between the nature of resistance and that of susceptibility is likely due to differences in their genetic tractability. Gene-for-gene type resistance (9) is typically triggered by activation of a genetically dominant resistance gene product by a dominant, pathogen-derived, avirulence (*Avr*) gene product. *Avr* proteins often act as virulence determinants in the absence of their *R* protein partners (5), indicating that their primary role is in virulence and that recognition by *R* genes evolved out of this role. The largest class of *R* genes encodes nucleotide-binding site-leucine-rich repeat (NBS-LRR) proteins. The only known function of these proteins in plants is in conditioning disease resistance (10). In animals, structurally related proteins mediate the innate immune response (11, 12). *R* gene-mediated signaling pathways, identified through mutant screens, generally require salicylic acid (SA), jasmonic acid, and/or ethylene (13) and often include activation of hypersensitive cell death (HR).

For the majority of plant diseases, the genetics of susceptibility are less tangible. Host susceptibility is typically defined in the context of a gain or loss of resistance (5, 14, 15) and pathogens often possess multiple virulence factors (called effectors), each contributing incrementally to the disease phenotype. A notable exception is *Os8N3*, a genetically dominant rice gene that is up-regulated by a bacterial type-III effector protein, and that

confers gene-for-gene-specified disease susceptibility (7). Likewise, for Victoria blight of oats and a handful of other diseases caused by necrotrophic pathogens (pathogens that incite cell death during pathogenesis), susceptibility is conditioned in a gene-for-gene manner by a single dominant locus in the host and a single pathogen-derived host-selective toxin (HST) (4).

*C. victoriae* produces the HST, victorin, and the oat gene *Vb* conditions both victorin sensitivity and disease susceptibility. Interestingly, although victorin is causal to disease susceptibility, it rapidly induces resistance-like physiology in oats, including callose deposition, a respiratory burst, lipid peroxidation, ethylene evolution, extracellular alkalization, phytoalexin synthesis,  $K^+$  efflux, and apoptotic-like cell death (4). This reinforces the idea that Victoria blight susceptibility and rust resistance are regulated by the same gene and that the physiology of disease susceptibility can resemble resistance. Victoria-type oats are allohexaploid and not readily amenable to molecular genetic studies. However, identification of victorin sensitivity and Victoria blight susceptibility in *Arabidopsis thaliana* (16) has enabled our investigation of this disease susceptibility pathway. We report similarities between this susceptibility response and disease resistance, including identification of the susceptibility locus, *LOV1*, as a coiled-coil NBS-LRR, *R* gene family member. These findings provide a unique platform for discussion of disease susceptibility and may have implications for deployment of *R* genes.

## Results

***LOV1* Encodes a CC-NBS-LRR Protein with Extensive Similarity to the *RPP8* Resistance Gene Family.** A locus conferring disease susceptibility to *C. victoriae* in *Arabidopsis*, called *LOV1*, was mapped to the interval between *Nga63* and *NCCI* on Chromosome 1 (16). We fine-mapped *LOV1*, using an additional 200 victorin-insensitive *F2* progeny of a cross between a victorin-sensitive line, *LOV1* (ecotype *Cl-0*), and victorin-insensitive *Col-4*. PCR markers were created by identifying small insertions/deletions that are polymorphic between *Ler* and *Col-0* (17), designing primers flanking each insertion/deletion, and testing for polymorphisms between *LOV1* and *Col-4*. Polymorphic markers were subsequently used to map *LOV1* to a 193-kb interval between markers 3571 and 3764 (Fig. 1*A*).

A genomic library was prepared from DNA of *LOV1* (*Cl-0*) in the binary vector pCLD04541 (18), and clones comprising a contig of the 193-kb interval were isolated and introduced into

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The authors declare no conflict of interest.

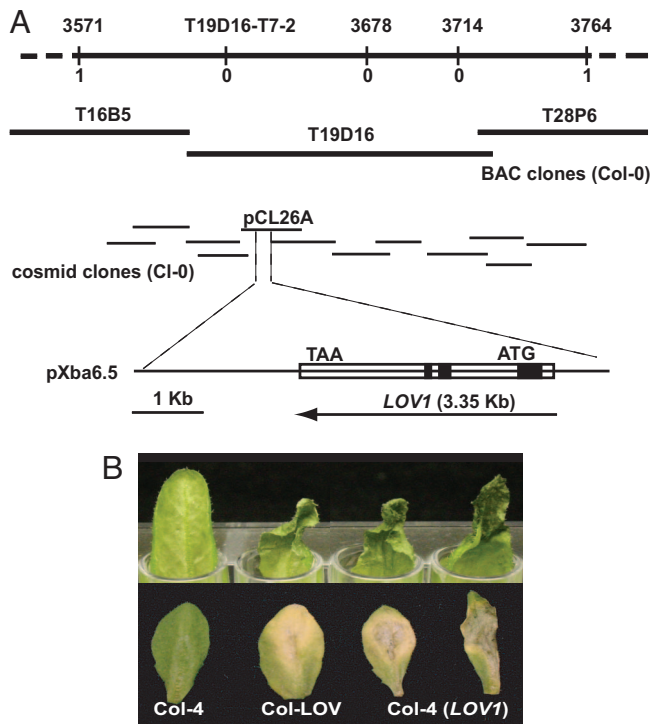
Abbreviations: HR, hypersensitive cell death; HST, host-selective toxin; NBS-LRR, nucleotide-binding site-leucine-rich repeat; SA, salicylic acid.

The sequence reported in this paper has been deposited in the GenBank database (accession no. EF472599).

\*To whom correspondence should be addressed. Email: wolpert@science.oregonstate.edu.

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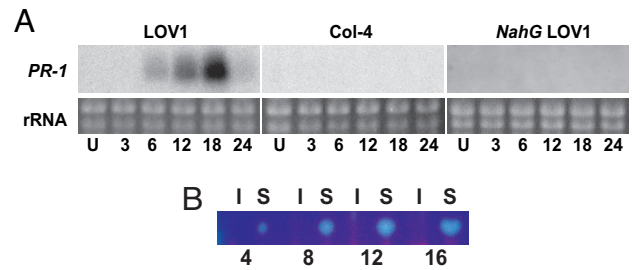
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**Fig. 1.** Isolation of *LOV1*, a gene that confers sensitivity to victorin and susceptibility to *C. victorariae* in *A. thaliana*. (A) Map-based cloning of the *LOV1* locus from *A. thaliana* ecotype Cl-0. SSLP markers created for mapping *LOV1*, designated by their kilobase location on chromosome 1, and CAPS marker T19D16-T7-2 are shown above the line. The number of recombinants found between each marker and *LOV1* is shown below the line. BAC clones spanning the mapped interval were obtained from The *Arabidopsis* Information Resource, and cosmid clones covering the interval were isolated from a Cl-0 genomic library. Clones containing *LOV1* are designated pCL26A and pXba6.5, respectively. The *LOV1* ORF is shown as an open rectangle. Introns are indicated in black. (B) *Arabidopsis* leaves 36 h after treatment with 10 µg/ml victorin (Upper) and 5 days after inoculation with 10 µl of 1 × 10<sup>5</sup>/ml *C. victorariae* spores (Lower). Genotypes include Col-4 (victorin insensitive), Col-LOV (Col-4 near-isogenic for *LOV1*), and Col-4 (*LOV1*) (Col-4 transgenic for *LOV1*).

victorin-insensitive *Arabidopsis* (Col-4) (Fig. 1B). One cosmid clone, pCL26A, and the subclone of this cosmid, pXba6.5, conferred victorin sensitivity and *C. victorariae* susceptibility to Col-4 (Fig. 1A and B). DNA sequencing of clone pXba6.5 revealed a single ORF corresponding to a pseudogene (*At1g10920*) in the annotated genome of *Arabidopsis* ecotype Col-0. Six polymorphisms occur between *At1g10920* (Col-0) and *LOV1* (*LOV1*, Cl-0), including a base pair change eliminating a stop codon and a frameshift insertion in *LOV1* (GenBank accession no. EF472599). Together, these changes result in *LOV1* encoding an ORF for a complete CC-NBS-LRR protein with extensive similarity (86%) and 70% identity to members of the *RPP8* resistance gene family [see supporting information (SI) SI Fig. 5]. Previously identified *RPP8* family members with known function, *RPP8*, *HRT*, and *RCY1*, confer resistance to *Hyaloperonospora (Peronospora) parasitica* Emco5 (19), turnip crinkle virus (20), and cucumber mosaic virus (CMV-Y) (21), respectively. In contrast, *LOV1* confers susceptibility to *C. victorariae* (Fig. 1B).

***LOV1* Conditions Victorin-Dependent Induction of Defense-Associated Proteins.** Because victorin induces resistance-like physiology in oats in a genotype-specific manner, and *LOV1* not only confers genotype-specific victorin sensitivity in *Arabidopsis* but also



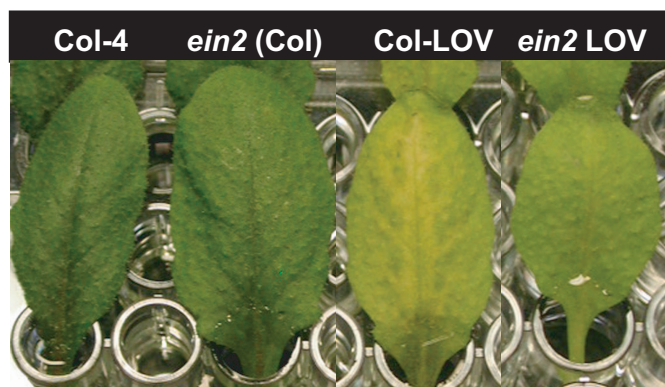
**Fig. 2.** Victorin elicitation of *LOV1*-mediated defense response in *A. thaliana*. Expression of pathogenicity-related protein gene *PR-1* (A) and thin-layer chromatography showing camalexin accumulation (B) in *Arabidopsis* leaves hours (numbers below panels) after infiltration with 30 µg/ml victorin. U, untreated; S, victorin-sensitive *LOV1*; I, victorin-insensitive Col-4.

encodes an R-like protein, we evaluated *Arabidopsis* for *LOV1*-specific expression of defense responses after treatment with victorin. Expression profiles of the resistance-associated gene *PR-1* and production of the phytoalexin, camalexin, are presented in Fig. 2. Victorin rapidly induced *PR-1* expression and camalexin production in a genotype-specific manner in *Arabidopsis* line *LOV1* (Cl-0) but not in Col-4. *PR-2*, *PR-5*, and *PDF1.2*, resistance-associated genes known to be induced by other necrotrophic fungi (22), were not induced in either plant genotype (data not shown). *PR-1* induction is SA-dependent because *PR-1* expression was not induced in *NahG* plants, in which SA is degraded (23).

**Multiple Defense Signaling Pathways Are Dispensable for *C. victorariae* Susceptibility.** We assessed signaling requirements for *LOV1*-conditioned disease susceptibility by examining *LOV1* genotypes having mutations in SA- (*NahG*, *EDS1*, *NDRI*, *NPRI*), jasmonic acid- (*COII*, *JARI*), and ethylene- (*EIN2*) mediated pathways, in the pathway for biosynthesis of the phytoalexin, camalexin (*PAD3*), and in the defense-related gene *DND1*, which is required for cell death during the HR (24). Susceptibility of victorin-sensitive *Arabidopsis* to *C. victorariae* was independent of all of these mutations (SI Table 1). Because disease appeared unaffected by these mutations, we pursued a more sensitive test by evaluating contributions of these signaling pathways to victorin sensitivity.

*LOV1* conditions incomplete dominance to victorin sensitivity. Plants heterozygous for *LOV1* are slightly less sensitive to victorin than are homozygous plants. Subtle effects of signaling pathways on victorin sensitivity, therefore, might be seen by assessing dilute concentrations of toxin on both homozygous and heterozygous *LOV1 Arabidopsis* genotypes. When plants heterozygous for *LOV1* in various mutant backgrounds (SI Table 1) were evaluated with victorin, a slight attenuation of victorin sensitivity was evident in *ein2* mutants at a concentration of 5 µg/ml victorin (Fig. 3), but no consistent alteration of victorin sensitivity occurred in other mutant backgrounds (SI Table 1). This finding indicates ethylene may play a subtle role in victorin-induced disease susceptibility. Ethylene also plays a subtle role in *RPP8* family member, *RCY1*-mediated resistance to cucumber mosaic virus. Cucumber mosaic virus resistance was reduced by 8% in *ein2 Arabidopsis* (20).

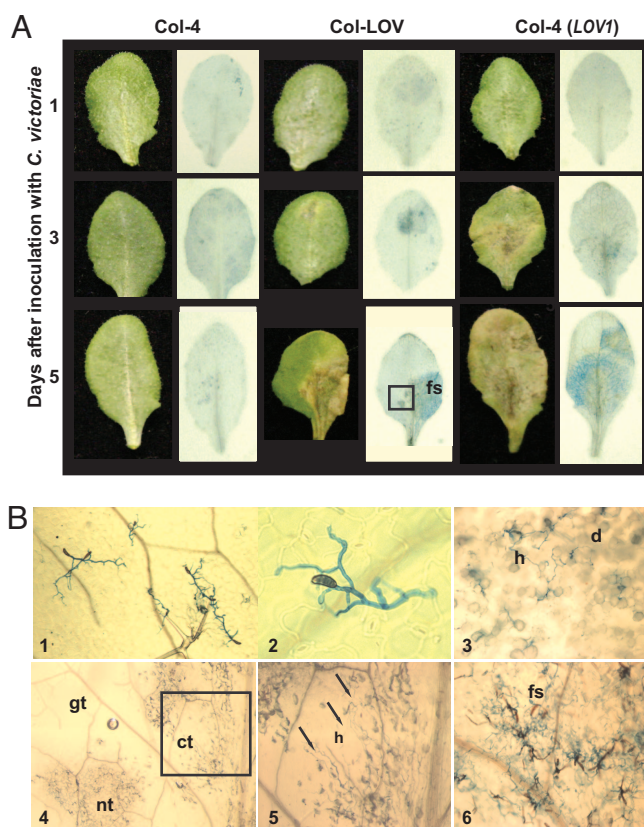
Complete resistance mediated by several *R* genes requires *RAR1*, *SGT1b*, and/or *HSP90* to maintain NBS-LRR steady-state levels (25–30). *RAR1* and *HSP90* are thought to act as cochaperones that positively regulate NBS-LRR levels (26, 28–30). *SGT1b* may play a role in cellular protein degradation and can function antagonistically to *RAR1* in *RPP8*-conditioned disease resistance (25, 28). *HSP90* and *SGT1b* have been shown to physically interact with several *R* proteins (26, 31). We



**Fig. 3.** Effects of *ein2-1* mutation on *LOV1*-mediated victorin sensitivity. *A. thaliana* leaves 36 h after treatment with 5  $\mu$ g/ml victorin.

evaluated *RAR1*, *SGT1b*, and *HSP90* contributions to *LOV1*-mediated victorin sensitivity. Effects of mutations *rar1-1* and *sgt1b* on victorin sensitivity were assessed in a *LOV1* background (SI Table 1). Geldanamycin, an *HSP90*-specific inhibitor, was used to assess the requirement for *HSP90* in victorin sensitivity. Slight attenuation of victorin sensitivity was observed in *rar1* (Ws) plants at 36 h after victorin treatment but was not consistently reproducible. By 48–72 h this phenotype was not discernible. Neither *sgt1b* nor inhibition of *HSP90* with geldanamycin reduced *Arabidopsis* sensitivity to victorin (SI Table 1 and data not shown). *RPP8*-mediated disease resistance also does not require functional *SGT1b* or *HSP90*, and it is only slightly affected by mutation in *RAR1* (28). In summary, the *LOV1*-conditioned disease susceptibility response appears similar to the *RPP8*-conditioned disease resistance response in that known defense response signaling pathways, individually, are not required for either response, and proteins that affect steady-state levels of some R proteins (*RAR1*, *SGT1b*, and *HSP90*) are not required to keep *LOV1* at a presumed threshold level (28).

***C. victoriae* Infection Process of *Arabidopsis*.** During infection of oat and *Arabidopsis*, *C. victoriae* develops appressoria and penetrates tissue of compatible hosts, but in incompatible plant genotypes, fungal penetration stops following appressorium development (16, 32). Analysis of *C. victoriae* infection of oat suggests that the fungus may penetrate host tissue before host cells are dying, implying the fungus does not merely gain nutrients from dead or dying cells (32). We compared symptom development and fungal growth over time in victorin-sensitive and victorin-insensitive *Arabidopsis* to correlate host symptom development with *C. victoriae* growth in host tissue (Fig. 4). Macroscopic observations of disease progress over a 5-day period in victorin-insensitive Col-4, near-isogenic line Col-LOV, and transgenic Col-4 (*LOV1*) are shown in Fig. 4A. Microscopic observations of select trypan-blue-stained leaves in Fig. 4A are shown in Fig. 4B. At 24 h after inoculation, fungal spores had germinated and formed appressoria on leaves of *C. victoriae*-susceptible and resistant *Arabidopsis*, but macroscopic symptoms were not evident (Fig. 4A). Col-4 plants remained symptomless for the duration of the experiment, and fungal hyphae on Col-4 leaves were limited to the leaf surface (Fig. 4A and B). Interestingly, although *C. victoriae* did not penetrate Col-4 tissue, hyphae continued to grow over the leaf surface, repeatedly forming appressoria in an apparent attempt to penetrate tissue (Fig. 4B). By 3 days after inoculation, chlorotic, dying and dead cells were visible on Col-LOV and transgenic Col-4 (*LOV1*) plants [dead and dying cells stain blue-black with trypan blue (19), and fungal hyphae appear bright blue]. Symptom development clearly preceded hyphal invasion of tissue. Comparisons of live and stained tissue



**Fig. 4.** Genotype-specific symptom development and *C. victoriae* ingress in *A. thaliana*. (A) Leaves of *C. victoriae*-resistant (Col-4) and *C. victoriae*-susceptible [Col-LOV and Col-4 (*LOV1*) transgenic] plants days after inoculation with 10  $\mu$ l of  $1 \times 10^5$ /ml *C. victoriae* spores are shown before (Left) and after (Right) staining with trypan blue. Dead and dying cells appear blue-black and fungal hyphae appear bright blue. (B) Select leaves from A, depicted with Nomarski (panels 1–3 and 6) or stereoscope (panels 4 and 5) microscopy; Col-4, 5 days after inoculation, panel 1 (magnification:  $\times 40$ ), and 2 (magnification:  $\times 400$ ); Col-LOV, at 3 days, panel 3 (magnification:  $\times 100$ ); and 5 days after inoculation, panel 4 (magnification:  $\times 25$ ), panel 5 (magnification:  $\times 63$ ), and panel 6 (magnification:  $\times 80$ ). The boxed region of the Col-LOV leaf at 5 days after inoculation in A, contains green, chlorotic, and necrotic tissues and is shown at  $25\times$  magnification (4). A higher magnification of the boxed region in 4 is shown in 5. Arrows point to hyphae in chlorotic tissue. Hyphae are abundant and sporulation is occurring from the same leaf, to the right of the midvein (6). fs, fungal sporulation; h, hyphae; d, dead or dying cells; gt, green tissue; ct, chlorotic tissue; nt, necrotic tissue.

indicate that hyphae were absent from chlorotic tissue and were evident around some, but not all, stained, dead, or dying cells. By 5 days after inoculation, hyphae were abundant and sporulating from dead tissue, and hyphal invasion of new tissue appeared more aggressive. Hyphae extended into some chlorotic areas close to tissue that appeared green. However, healthy green tissue did not appear to contain hyphae (Fig. 4A and B). In summary, *C. victoriae* acts as a necrotroph early in infection. Symptom development and cell death appear to precede fungal proliferation. Further, based on the interaction on the insensitive genotypes, it is likely that cell death is necessary for penetration. Later, when infection is well established in necrotic tissue, expanding hyphae invade tissue more aggressively and enter chlorotic regions in which cells do not appear to be dead.

## Discussion

We mapped and cloned a dominant disease susceptibility gene called *LOV1* from *Arabidopsis* (ecotype Cl-0) (Fig. 1). We found that *LOV1* encodes a CC-NBS-LRR protein, a class of proteins

that, previously, was only known to function in conditioning plant disease resistance. *LOV1* is a member of the *RPP8* disease resistance gene family (SI Fig. 5). However, whereas other functional family members, *RPP8*, *HRT*, and *RCY1*, confer resistance to *H. (Peronospora) parasitica* Emco5 (19), turnip crinkle virus (20), and cucumber mosaic virus (21), respectively, *LOV1* confers susceptibility to *C. victoriae*. We demonstrate that like other NBS-LRR proteins, *LOV1* mediates responses associated with disease resistance. *LOV1* conditions rapid, SA-dependent induction of the pathogenicity-related protein gene, *PR-1*, and production of the phytoalexin, camalexin (Fig. 2). Additionally, victorin-inducible electrolyte leakage and DNA degradation associated with HR-like cell death in sensitive oat tissue (33) have also been shown to occur in a *LOV1*-dependent manner in *Arabidopsis* (T.A.S. and T.J.W., unpublished data). Collectively, these findings strongly suggest that *LOV1*-conditioned disease susceptibility shares features of disease resistance responses. This also raises a conundrum. How does rapidly eliciting “resistance” result in disease susceptibility? How does victorin perception, directly or indirectly, by a CC-NBS-LRR protein overcome resistance when, in fact, it induces resistance-like physiology?

During incompatible interactions of *C. victoriae* with oat or *Arabidopsis* (absence of *Vb* or *LOV1*, respectively), appressoria develop, but host tissue is not penetrated (refs. 32 and 16 and Fig. 4). Because *C. victoriae* produces a “toxin” and has been considered to be a necrotroph, its penetration of tissue may be arrested by basal and/or victorin-induced resistance until victorin-induced cell death occurs, then allowing the fungus to invade the dead and dying tissue. We provide some evidence for this scenario in that early in infection, fungal growth lags behind chlorosis and necrosis. Hyphae align with some but not all dying cells (Fig. 4). However, later in infection, hyphal invasion was evident in chlorotic tissue in which cells did not appear to be dead. In investigations of *C. victoriae* infection of oat, susceptible mesophyll cells were reported to contain hyphae before host cells were visibly affected (32). Separation of pathogenesis and host cell death has also been reported for HST-producing *Alternaria alternata* (4) and is implicated for the closely related, HST-producing pathogen, *C. carbonum*. In this latter case, HC-toxin is not a toxin *per se*, but rather functions as a cytostatic agent (3). Taken together, these findings indicate that the role of host cell death in susceptibility to HST-producing pathogens is not clearly resolved. The precise temporal relationship between fungal invasion, defense response and cell death for these pathogens, including *C. victoriae*, requires further investigation.

*C. victoriae* may not be avoiding resistance (by penetrating after cell death) but, alternatively, might be immune to negative effects of, and actually benefit from, the host resistance response. Several lines of evidence suggest that some fungi are tolerant to aspects of plant disease resistance physiology and actually use it for nutritional gain. For example, susceptibility to *B. cinerea* is decreased in a *dnd1* background but increased in *Arabidopsis* undergoing a *P. syringae*-induced HR response (34). Furthermore, reactive oxygen species (ROS) are known to play a role in resistance to biotrophic and hemibiotrophic pathogens, but in susceptibility to the necrotrophic pathogens, *B. cinerea*, *S. sclerotiorum*, and *Cercospora* species (4). Although oat *Pc-2* evolved to halt infection of *P. coronata*, *C. victoriae* may be exploiting this resistance pathway to gain nutrition during pathogenesis.

As one approach to evaluate the role of defense in the *C. victoriae*–*Arabidopsis* interaction, we conducted analysis of defense-associated signaling mutations in a *LOV1* background. These analyses revealed that *LOV1*-mediated susceptibility, similar to the *RPP8*-conditioned resistance response (35), does not require SA, jasmonic acid, or ethylene-mediated signaling pathways. Also, these signaling pathways did not contribute to resistance to *C. victoriae*, because susceptibility did not appear to

be enhanced in mutant backgrounds (SI Table 1). Nonetheless, SA and ethylene do participate in the *LOV1*-mediated response. *LOV1*-specified, victorin-induced *PR-1* expression is SA-dependent (Fig. 2), and victorin-induced expression of *ATTRX5*, a gene required for *LOV1*-conditioned *C. victoriae* susceptibility is also SA-dependent (36). Furthermore, ethylene involvement has been demonstrated in *Vb*-conditioned susceptibility of oats to *C. victoriae* (33), and a slight attenuation of *LOV1*-mediated victorin sensitivity occurs in *ein2 Arabidopsis* (Fig. 3). SA, ethylene, and camalexin synthesis pathways are also activated during *RPP8*-mediated resistance (37), although they are not required (35). A requirement for (or dispensability of) these commonly activated signaling pathways is clearly pathogen dependent (13). Signal requirements for several *R* gene-mediated pathways remain unknown. Novel pathways have been proposed for *RPP7*- and *RPP8*-mediated resistance (19), and known signaling pathways account for only a portion of the resistance mediated by *RCY1* (21). Given the similarity of *LOV1* to *RPP8* and *RCY1* and the finding that an extensive screen for suppressors of *LOV1* did not reveal genes previously known to be required for defense (36), *LOV1* could also function in an uncharacterized pathway, one that is sufficient for resistance to some pathogens (such as obligate biotrophs, as is the case for *RPP8* and *RCY1*) but readily exploited for susceptibility by other pathogens such as *C. victoriae*. Such opposing functions in host response have been reported for MLO proteins, which are required for compatible interactions of powdery mildew pathogens with barley and *Arabidopsis* but contribute to resistance to necrotrophic and hemibiotrophic pathogens of *Arabidopsis* (8).

The dispensability of defense-associated signaling in victorin sensitivity and disease susceptibility could also indicate that *LOV1* does not function through a defense-related pathway. As an alternative, *LOV1* could be mediating a condition of disease susceptibility analogous to *Os8N3*, a rice susceptibility gene that is activated by a bacterial type III effector protein (7). With few exceptions (6, 8), disease susceptibility has been considered a suppression or absence of resistance (5), but for *Os8N3*, disease susceptibility is dominant, disease resistance is recessive, and susceptibility is conferred by positive action of a virulence effector. However, if the function of *LOV1* and *Vb* are analogous to *Os8N3*, then in the *C. victoriae* interaction with oat, the victorin/*Vb*-mediated response would necessarily be distinct from the *P. coronata* /*Pc-2* resistance response, which contradicts genetic evidence (4). Furthermore, like other NBS-LRR R proteins, activation of *LOV1* is necessary for its function. Missense mutations resulting in amino acid changes in the P-loop domain of *LOV1* at residue 192 or 199 abolish its function (T.A.S. and T.J.W., unpublished data). Given this and the findings that *LOV1* and *Vb/Pc-2* both mediate defense-associated physiology and that *LOV1* is highly related to *RPP8* resistance family members, the possibility that *LOV1* signals in a susceptibility pathway that is unrelated to resistance seems unlikely. Furthermore, the necrotrophic nature of *C. victoriae* pathogenesis early in infection (Fig. 4) suggests that an HR could condition disease susceptibility.

In animal systems, TLR (Toll/interleukin 1 receptor (TIR) domain, LRR motif) proteins are one class of proteins that mediate innate immunity and are structurally related to NBS-LRR proteins (12). Although TLR proteins typically modulate innate immunity, in some instances fungal, bacterial and viral pathogens, by various mechanisms, have exploited their interaction with TLR proteins to increase pathogen virulence/host susceptibility (12). Similarly, *LOV1*, an NBS-LRR R protein family member, is targeted by a pathogen effector, victorin, and this interaction results in disease susceptibility, regardless of the specific mechanism by which this occurs. Furthermore, this phenomenon is probably not unique. An NBS-LRR gene has also been implicated in susceptibility to Milo disease (38).

Pathogen effectors, first identified genetically as avirulence determinants, have since been shown to target defense machinery and play a role in virulence (5). Likewise, *R* genes, first identified as a result of their genetic dominance, could now prove to be targets of pathogen effectors and have roles in susceptibility. Identifying *LOV1* as a gene encoding a NBS-LRR protein strongly suggests that a disease resistance gene and a disease susceptibility gene can share identity and supports the prospect of *Pc-2/Vb* identity in oat. A role for *R* genes in disease susceptibility could have implications for engineering of disease resistance in plants and *R* gene deployment.

## Materials and Methods

**Plant Material, Growth Conditions, and Victorin Sensitivity Assays.** Seeds of *A. thaliana* Col-4, Ws, and mutant lines, *jar1-1* (39), *npr1-1* (40), *ein2-1* (41), *pad3* (42), and *NahG* (23), all in Col-0, were obtained from the Ohio State University *Arabidopsis* Biological Resource Center (Columbus, OH). *LOV1* (16) and Col-LOV (36) were generated in our laboratory. Other seeds were obtained from the following individuals: Sainsbury Laboratory [*eds1-1* Ws (43)], B. Staskawicz (University of California, Berkeley, CA) [*ndr1-1* (44)], B. Kunkel (Washington University, St. Louis, MO) [*coi1-35* (45)], A. Bent (University of Wisconsin, Madison, WI) [*dnd1* (24)], and J. Chang [*rar1-1* (25)]. Seeds were placed in 0.1% agar for 5 days at 4°C, pipetted onto soil, and grown at 22°C under a long-day photoperiod (16 h light, 8 h dark). Victorin sensitivity assays were conducted as described in ref. 16, by using 1–20 µg/ml victorin C, which was purified as described in ref. 46.

**Fine-Mapping and Cloning of *LOV1*.** Initial mapping of *LOV1* to the Nga63 and NCCI interval of Chromosome 1 was described in ref. 16. For fine-mapping, an additional 200 victorin-insensitive, F<sub>2</sub> progeny of a *LOV1* × Col-4 cross were subjected to analysis with PCR. DNA for PCR was purified from leaf tissue according to Edwards *et al.* (47).

A genomic library was prepared in the binary vector pCLD04541 (18). DNA was isolated from *LOV1* with a CTAB method (48) and further purified by ultracentrifugation in cesium chloride. DNA was partially digested with *Sau3A* and ligated into the *Bam*HI site of pCLD04541. Ligations were packaged with Gigapack III XL extracts and transformed into XL1-Blue MR cells (Stratagene, La Jolla, CA). The resultant genomic library was screened (48) with <sup>32</sup>P-labeled DNA of BAC clone T19D16 and selected regions of BAC clones T16B5 and T28P6, which were obtained from ABRC. Positive clones from this screen were end-sequenced, arranged into a contig, and transformed into *Agrobacterium tumefaciens* strain GV3101 by triparental mating (49). Electroporation does not work well with pCLD04541.

Col-4 plants were transformed by using the floral dip method (50). Seed from dipped plants were plated on nutrient agar (16) supplemented with 100 µg/ml kanamycin and 100 µg/ml cefotaxime. Transgenic seedlings were transplanted to soil after 1 week. Transgenic plants were assayed for victorin sensitivity as described. A 6.5-kb *Xba*I fragment containing *LOV1* was subcloned from pCL26A into pCB302 (51) and electroporated into *A. tumefaciens* strain GV3101. Transgenic plants were made as described above, except putative transgenic seed were planted directly in soil wet with 0.02% glufosinate-ammonium.

**Isolation of *LOV1* Signal Transduction Mutants.** F<sub>2</sub> and F<sub>3</sub> progeny from crosses of *LOV1* and signal transduction mutant lines (*NahG*, *npr1-1*, *ndr1-1*, *ein2*, *coi1-35*, *jar1*, *pad3*, *dnd1*, respectively) were screened for both victorin sensitivity (as described above) and for PCR markers linked to respective loci of interest. PCR markers for following the segregation of above loci were marker 3571 for *LOV1* (see fine-mapping above), gene-specific

primers for *NDRI-1* (forward, AATCTACTACGACGATGTC-CAC; reverse, GTAACCGATGGCAACTTTCAC) and *NahG* (forward, CAGAAGGTATCGCCCAATTC; reverse, ACCT-TCCAGCACATGGCTAC) or markers linked to each locus selected from sequence information available at The *Arabidopsis* Information Resource (www.arabidopsis.org). The presence of several mutations could also be confirmed by visible phenotypes, such as lack of *PR-1* expression in *npr1-1* and *NahG*, dwarfism of *dnd1*, leaky male-sterility of *coi1-35*, and triple-mutant response of *ein2-1* grown on MS agar with 0.5 mM ACC. *eds1-1* and *rar1* are in ecotype Ws, which is toxin sensitive and could be tested directly for victorin sensitivity.

Approximately 100 F<sub>2</sub> plants for each cross (above) were screened for victorin sensitivity and by PCR. Individual plants homozygous for or heterozygous for *LOV1*, and also homozygous for each mutant allele, were allowed to self-fertilize and also tested in the F<sub>3</sub> generation for victorin sensitivity and presence of the appropriate PCR markers.

***C. victorariae* Susceptibility Assays.** *C. victorariae* spores were prepared as described in ref. 16. Initially, for Col-0, *LOV1*, *ein2-1* (Col-0), *ein2LOV1*, *NahG* (Col-0), *NahGLOV1*, *npr1-1* (Col-0), and *npr1LOV1*, *C. victorariae* (10<sup>6</sup> spores per ml) *C. victorariae* spores were sprayed onto 32 3- to 4-week-old plants with an aspirator until runoff. Plants were then incubated at 100% humidity in a growth chamber at 22°C under a long-day photoperiod (16 h light, 8 h dark) until symptoms appeared. After finding that no plant lacking the *LOV1* gene had any disease lesions, and all plants having the *LOV1* gene (*LOV1*, *ein2LOV1*, *NahGLOV1*, and *npr1LOV1*) appeared equally susceptible, we used a simpler detached leaf assay.

Six detached leaves of approximately the same age (third through sixth true leaves) from each plant genotype were placed in a sealed Petri dish lined with moist filter paper. For time-course experiments shown in Fig. 4, leaves were not detached but remained on plants which were covered to maintain humidity. Ten microliters of *C. victorariae* spores, washed as described and resuspended to a concentration of 10<sup>5</sup> spores per ml, were pipetted onto the center of each leaf. Leaves were observed daily for up to 10 days for symptom development and signs of fungal growth. Again, leaves from plant genotypes lacking *LOV1* showed no symptom development. Most leaves of the *LOV1* genotype showed chlorosis, and then necrosis and visible hyphae, which eventually sporulated. The infection varied somewhat from leaf to leaf depending on leaf age and shape, which affected fungal distribution and wetness and consequently, the area of initial infection (e.g., leaves that curled up from the surface were less wet in spots and moisture pooled in other spots). Because of this variability, and because the only obvious differences in disease development occurred between *LOV1*- and *lov1*-containing genotypes, we simply scored plants as susceptible or resistant. An exception to this was that Col-4 transgenic for *LOV1* typically appeared more sensitive to victorin and slightly more susceptible to *C. victorariae*, likely due to gene copy number. Also, the Ws and Ws-0 ecotypes were somewhat less sensitive to victorin and less susceptible to *C. victorariae* (SI Table 1).

The *rar1-1* mutation was reported to be in Ws-0 (25). However, because the *rar1-1* plants looked like the Ws ecotype, we checked Ws, Ws-0, and *rar1-1* for the sequence of *LOV1* and evaluated polymorphisms for seven SSLP markers dispersed throughout the genome. The *rar1-1* plants had the same *LOV1* sequence and SSLP profile as Ws, whereas Ws-0 plants had a slightly different *LOV1* sequence and were polymorphic for 4 SSLP markers in comparison to *rar1-1* plants. For this reason we used both Ws and Ws-0 as controls for our *rar1-1* assays and obtained equivalent results with each. Results for Ws are presented.

Visualization of fungi was according to Lorang *et al.* (16) as

modified from Keogh *et al.* (52). Specimens were visualized with stereoscope and Nomarski optics microscopy.

**Geldanamycin Treatment.** Sensitivity to victorin after treatment with geldanamycin was assayed with the detached leaf assay described above, except that test leaves were subjected to a 2-h pretreatment with 10  $\mu$ M geldanamycin (EMD Biosciences, Inc., La Jolla, CA) in a final concentration of 0.2% DMSO. Control leaves were pretreated with 0.2% DMSO only. To detect subtle effects of geldanamycin on victorin sensitivity, multiple victorin concentrations of 0.5, 5, and 10  $\mu$ g/ml were evaluated. In LOV1 plants with or without geldanamycin treatment, victorin-elicited leaf symptoms were obvious within 24 h but were less pronounced in the 0.5  $\mu$ g/ml toxin treatment. Control leaves treated with geldanamycin only showed phytotoxic affects of treatment (leaf yellowing) at 60–72 h after treatment. This was not a problem, because at this time victorin treated plants had already displayed clear symptoms.

**Northern Analyses and Camalexin Extraction.** Northern analyses were according to Sweat and Wolpert (36). Plasmids containing

cDNA clones of *PR-1*, *PR-2*, and *PR-5* were obtained from Syngenta Biotechnology (Research Triangle Park, NC). The cDNA inserts were excised by using EcoRI/XhoI for *PR-1* and *PR-2* and BamHI/KpnI for *PR-5* and gel-purified for use as probes. A gene-specific probe for *PDF1.2* was PCR-amplified from genomic DNA by using the primers 5'-GCAATGGTGG-AAGCACAGAA-3' and 5'-CTCATAGAGTGACAGAGACT-3'. Camalexin was extracted and analyzed similar to the way described in ref. 42. After incubation for the indicated times, leaf disks (7 mm) were prepared from detached leaves, using a no. 3 cork borer. Three-leaf disks were combined for each repetition at each time point.

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