UC Berkeley

UC Berkeley Previously Published Works

Title

Spray-induced gene silencing to identify powdery mildew gene targets and processes for powdery mildew control

Permalink

https://escholarship.org/uc/item/8b27n539

Journal

Molecular Plant Pathology, 24(9)

ISSN

1464-6722

Authors

McRae, Amanda G

Taneja, Jyoti

Yee, Kathleen

et al.

Publication Date

2023-09-01

DOI

10.1111/mpp.13361

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Received: 13 September 2022 | Revised: 9 May 2023 | Accepted: 11 May 2023

DOI: 10.1111/mpp.13361

Molecular Plant Pathology WILEY

ORIGINAL ARTICLE

Spray-induced gene silencing to identify powdery mildew gene targets and processes for powdery mildew control

Amanda G. McRae[1] | Jyoti Taneja[1] | Kathleen Yee[1] | Xinyi Shi[1] | Sajeet Haridas[2] | Kurt LaButti[2] | Vasanth Singan[2] | Igor V. Grigoriev[1,2] | Mary C. Wildermuth[1]

[1]Department of Plant and Microbial Biology, University of California, Berkeley, California, USA

[2]US Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, Berkeley, California, USA

Correspondence
Mary C. Wildermuth, Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA.
Email: mwildermuth@berkeley.edu

Funding information
American Vineyard Foundation, Grant/Award Number: 2138 and 2366; National Institute of Food and Agriculture, Grant/Award Number: 1016994; National Science Foundation, Grant/Award Number: MCB-1617020 and PFI-TT-1919244; U.S. Department of Energy, Grant/Award Number: DE-AC02-05CH11231

Abstract

Spray-induced gene silencing (SIGS) is an emerging tool for crop pest protection. It utilizes exogenously applied double-stranded RNA to specifically reduce pest target gene expression using endogenous RNA interference machinery. In this study, SIGS methods were developed and optimized for powdery mildew fungi, which are widespread obligate biotrophic fungi that infect agricultural crops, using the known azole-fungicide target *cytochrome P450* 51 (CYP51) in the *Golovinomyces orontii–Arabidopsis thaliana* pathosystem. Additional screening resulted in the identification of conserved gene targets and processes important to powdery mildew proliferation: *apoptosis-antagonizing transcription factor* in essential cellular metabolism and stress response; lipid catabolism genes *lipase a*, *lipase 1*, and *acetyl-CoA oxidase* in energy production; and genes involved in manipulation of the plant host via abscisic acid metabolism (*9-cis-epoxycarotenoid dioxygenase*, *xanthoxin dehydrogenase*, and a putative *abscisic acid G-protein coupled receptor*) and secretion of the effector protein, *effector candidate 2*. Powdery mildew is the dominant disease impacting grapes and extensive powdery mildew resistance to applied fungicides has been reported. We therefore developed SIGS for the *Erysiphe necator–Vitis vinifera* system and tested six successful targets identified using the *G. orontii–A. thaliana* system. For all targets tested, a similar reduction in powdery mildew disease was observed between systems. This indicates screening of broadly conserved targets in the *G. orontii–A. thaliana* pathosystem identifies targets and processes for the successful control of other powdery mildew fungi. The efficacy of SIGS on powdery mildew fungi makes SIGS an exciting prospect for commercial powdery mildew control.

KEYWORDS
Arabidopsis thaliana, dsRNA, *Erysiphe necator*, *Golovinomyces orontii*, grapevine, powdery mildew, spray-induced gene silencing

Amanda G. McRae and Jyoti Taneja contributed equally to this work.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2023 The Authors. *Molecular Plant Pathology* published by British Society for Plant Pathology and John Wiley & Sons Ltd.

1 | INTRODUCTION

Powdery mildew (PM) fungi are widespread obligate biotrophic pathogens that infect a variety of agriculturally important crops including barley, wheat, cucurbits, grapevine, and ornamentals such as roses (Glawe, 2008). PM fungi are the causal agents of powdery mildew disease, so named because the fungal mycelial mat and asexual reproductive structures appear as white powdery spots on the surface of plant organs such as leaves. In brief, the PM life cycle includes spore germination, development of an appressorial germ tube, penetration of the plant cell wall, development of the haustorium (the fungal feeding structure) in the penetrated epidermal cell, and subsequent surface hyphal expansion coupled with the formation of secondary haustoria. Asexual reproductive structures, conidiophores, containing new spores, start to develop 5–15 days after germination depending on environmental conditions, thus propagating the infection. Although sexual reproduction can occur, it is rare (Hacquard et al., 2013; Wicker et al., 2013).

The PM fungus *Erysiphe necator* is the causative agent of powdery mildew disease of grapes and the dominant pathogen impacting grapes in California and worldwide (Gadoury et al., 2012). Powdery mildew control accounts for 74% of pesticides used by Californian grape growers (Sambucci et al., 2014). A combination of sulphur (S) and synthetic fungicides is used throughout the growing season to control PM fungal infection. However, new PM controls are required due to the increasing prevalence of PM fungal resistance to synthetic fungicides (Frenkel et al., 2015; Kunova et al., 2021; Miles et al., 2021; Vielba-Fernández et al., 2020), increasing restrictions on systemic fungicide use, particularly in the European Union, and increasing human health and environmental concerns associated with both systemic fungicides (Bastos et al., 2021; Calvert et al., 2008) and S (Hinckley & Matson, 2011; Raanan et al., 2017).

One new method being developed to combat agricultural plant pathogens is RNA interference (RNAi). This approach uses conserved eukaryotic host and/or pathogen RNAi machinery to silence the targeted pathogen gene by specifically degrading the targeted pathogen messenger RNA, preventing its subsequent translation to protein (Baulcombe, 2004; Majumdar et al., 2017; Zotti et al., 2018). Long double-stranded RNA (dsRNA) specific to the pathogen gene target transcript is processed to multiple small interfering RNAs (siRNAs) of 21–28 bp by the endogenous RNAi machinery (Meister & Tuschl, 2004). The siRNA is then incorporated into the RNA-induced silencing complex (RISC) where it guides sequence-specific cleavage of the targeted gene transcript. Initially, RNAi strategies against plant pathogens involved stable introduction of the dsRNA into the host plant, requiring the development of transgenic crops. The resulting host-induced gene silencing (HIGS) was shown to be effective in reducing powdery mildew (Pliego et al., 2013) and other fungal diseases including Fusarium head blight (Machado et al., 2018), wheat stripe rust (Zhu et al., 2017), and grey mould (Xiong et al., 2019). However, despite years of advancement, there are few RNAi-based plant products targeting pests in the marketplace. This is due to plant transformation limitations, the timeline to product development, regulatory burdens, and consumer resistance to transgenic crops (Bramlett et al., 2020). The few crops developed, assessed, and approved for human consumption include insect-resistant SmartStax maize (Darlington et al., 2022; Head et al., 2017) and virus-resistant papaya (Gonsalves, 2006) and plum (Scorza et al., 2013).

Topical application of dsRNA or siRNA, referred to as spray-induced gene silencing (SIGS), offers the possibility of a rapidly developed, nontransgenic, and specific approach for limiting crop loss due to pests. Furthermore, the dsRNAs are rapidly degraded in the environment (Bachman et al., 2020) and can be designed to be highly specific to the targeted pest. SIGS was first found to be effective against the necrotrophic fungus *Botrytis cinerea* (Wang et al., 2016) and the hemibiotrophic fungus *Fusarium graminearum* (Koch et al., 2016), both ascomycetes. SIGS was subsequently shown to work against other fungal phytopathogens including the obligate biotrophs *Phakopsora pachyrhizi* (Hu et al., 2020), a basidiomycete, and *Hyalaperonospora arabidopsidis*, an oomycete (Bilir et al., 2019). However, SIGS is not universally effective against fungi, with efficacy apparently dictated by the efficiency of pathogen RNA uptake (Qiao et al., 2021).

In this study we used the PM fungus *Golovinomyces orontii* MGH1 (Gor) that infects curcubits and crucifers including the resource-rich model species *Arabidopsis thaliana* (Micali et al., 2008). We established that it can take up dsRNA and that topical application of dsRNA can effectively limit Gor growth and asexual reproduction on *Arabidopsis*. The SIGS pipeline was developed and optimized using the known azole-fungicide target CYP51 (Frenkel et al., 2015). CYP51 is required for the synthesis of sterol, a component of fungal cell membranes, and SIGS against *CYP51* successfully reduced growth of *F. graminearum* (Koch et al., 2016) and *B. cinerea* (Nerva et al., 2020). Using our optimized SIGS pipeline, the efficacy of SIGS against individual Gor gene targets in reducing PM proliferation on *Arabidopsis* was assessed. The ease of growing and maintaining *A. thaliana* coupled with its rapid growth cycle (6–8 weeks, seed-to-seed) enables rapid screening of broadly conserved PM fungal targets. By screening multiple PM fungal targets annotated as being associated with a given functional process, we ascertained the relative importance of a given process to PM growth and asexual reproduction, enabling prioritization for further detailed functional investigations. This is critical as PM fungi cannot be cultured and thus it has been difficult to interrogate gene function. To determine whether PM fungal gene targets identified as important using the Gor–*A. thaliana* system are also important to an agriculturally relevant system, we developed and optimized the SIGS pipeline for *E. necator* C-strain (En)–*Vitis vinifera*. Similar efficacy was seen in reducing En growth and reproduction on grapevine thereby paving the way for topical RNAi-based control of powdery mildew fungi on agricultural and ornamental horticultural species.

2 | RESULTS

2.1 | Gor spores can take up environmental RNA

To determine if Gor can take up RNA directly from its environment, germinated conidia were incubated with in vitro–transcribed, fluorescein-labelled RNA, similar to Wang et al. (2016). Gor uptake of RNA was rapid and observed within 1.5 h of application, demonstrating that a PM fungus can take up RNA independent of the host plant. Fluorescein uptake was observed in ungerminated and germinated conidia, with RNase treatment decreasing background fluorescein signal (Figure 1).

2.2 | SIGS against *GorCYP51* significantly reduces powdery mildew growth and asexual reproduction on *A. thaliana*

To assess the efficacy of SIGS in reducing Gor growth and asexual reproduction on *A. thaliana*, we developed assays using whole plants and detached leaves. Our rationale for the use of two assays was to discover if one is more sensitive or rapid than the other. In addition, we wanted to ascertain whether detached-leaf assays give similar results to whole-plant assays, as transport and other whole-plant processes do not occur in detached leaves. The timing of progression of the Gor life cycle on *A. thaliana* is highly reproducible using our procedures, and spore counting (normalized to leaf fresh weight) was employed as an endpoint assessment that integrates earlier impacts on Gor growth and development.

Two 21 bp siRNAs, siRNA-1 and siRNA-2, and two long dsRNAs, dsRNA-1 (199 bp) and dsRNA-2 (228 bp), targeting *GorCYP51* were designed and tested (Figure 2a and Table S1). In whole-plant and detached-leaf experiments using two treatments of 40 mg of *CYP51* dsRNA-1, spore production decreased by an average of 46% and 30%, respectively, compared to control treatments (Figure 2b). A reduction in visible powdery mildew disease was also observed for *CYP51* dsRNA-1 sprayed plants compared to control water-sprayed plants (Figure 2c). Surprisingly, dsRNA-2 did not reproducibly limit Gor spore production despite significant overlap with dsRNA-1. However, treatment with siRNA-1, which resides within dsRNA-2, decreased spore production by 35% and 40% in whole plant and detached leaves, respectively. siRNA-2, which targets an entirely different region of *CYP51* than the other RNAs, did not significantly reduce spore production (Figure 2b).

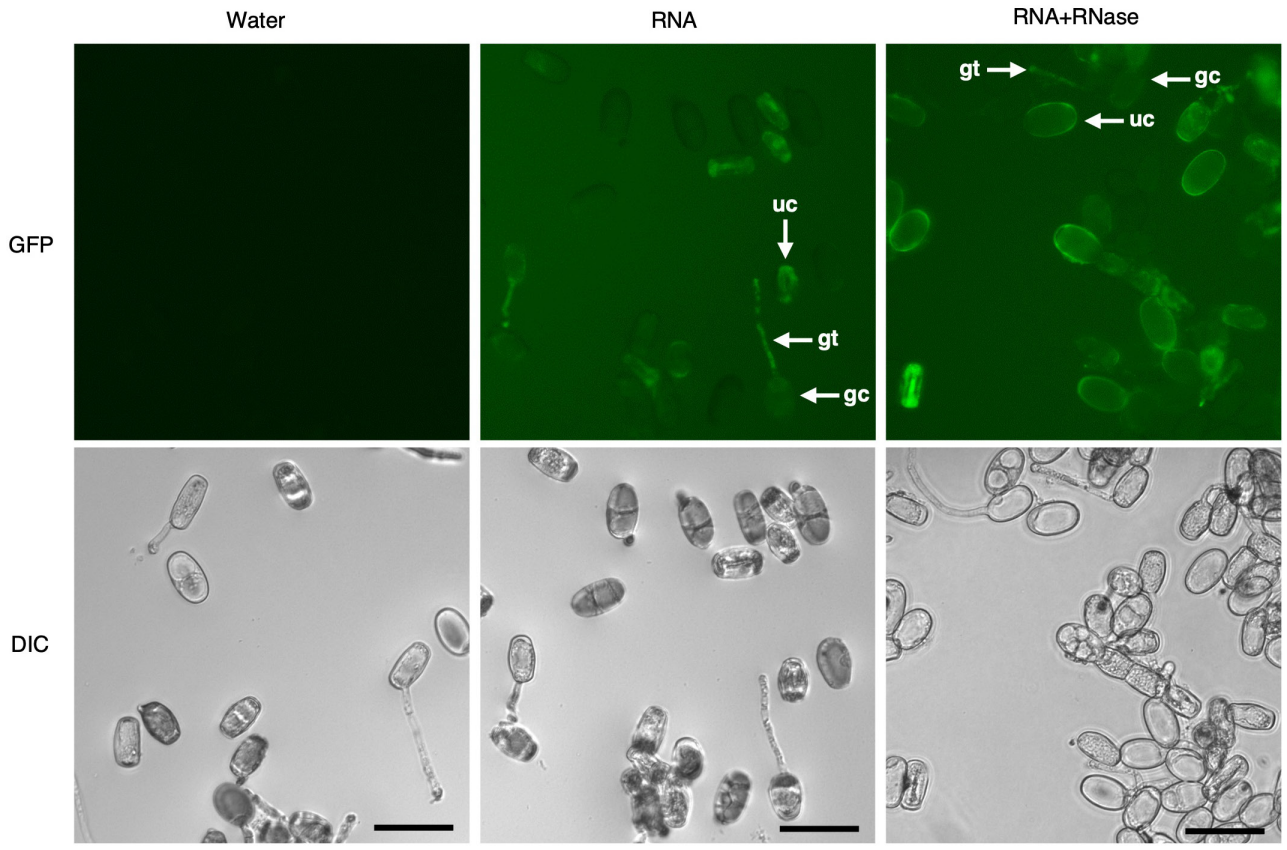

FIGURE 1 *Golovinomyces orontii* takes up extracellular RNA. Germinated spores were incubated with water, RNA containing fluorescein-conjugated UTPs (fRNA) or fRNA treated with RNase before imaging. Representative images are displayed; an independent experiment gave similar results. DIC, differential interference contrast; GFP, green fluorescent protein filter set; uc, ungerminated conidium; gc, germinated conidium; gt, germ tube. Scale bar = 50 μm.

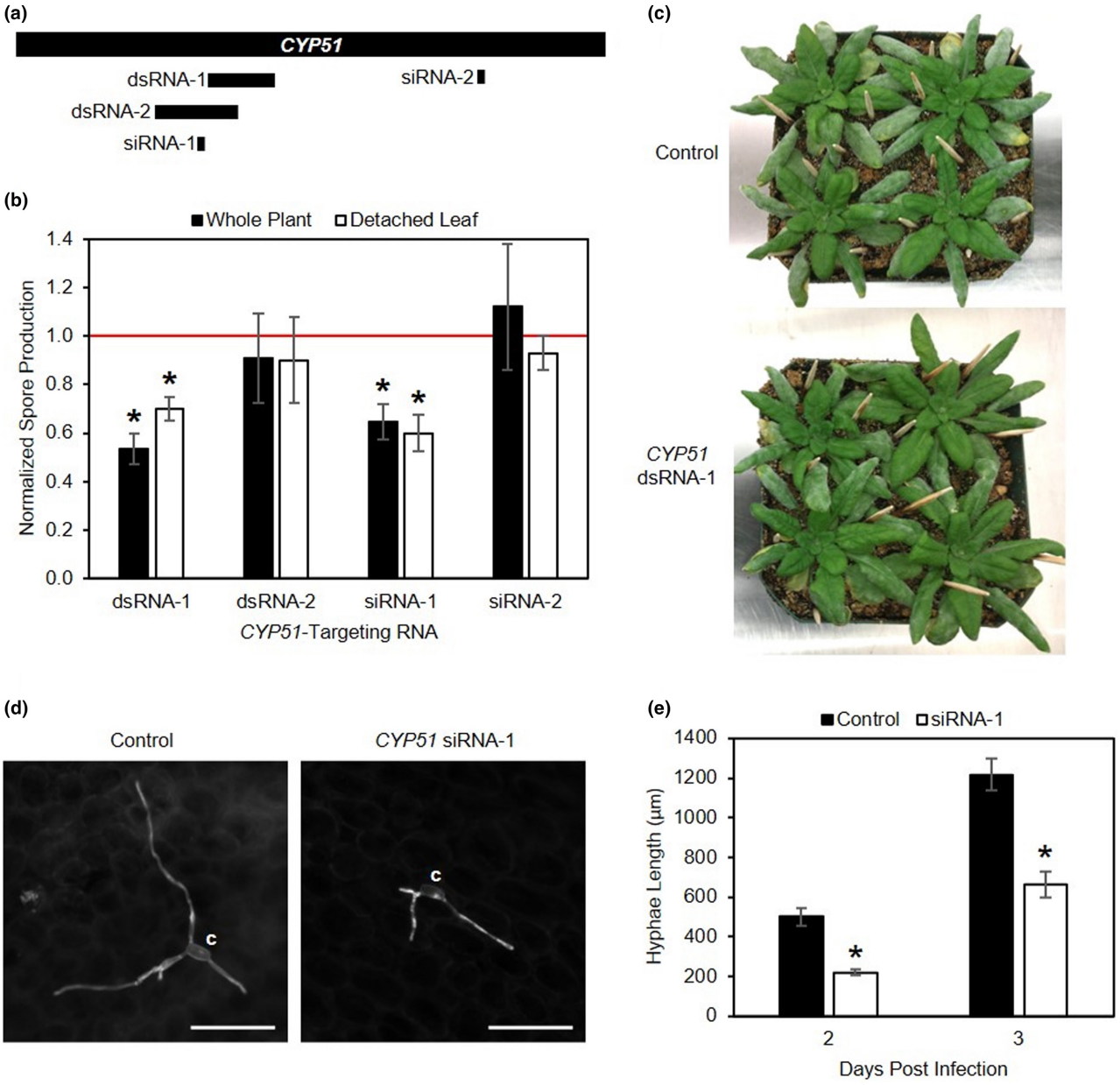

FIGURE 2 Spray-induced gene silencing targeting *Golovinomyces orontii CYP51* reduces spore production and hyphal length. (a) Diagram of *CYP51* transcript (1578 bp) and designed inhibitory RNAs. siRNAs were synthesized. siRNA-1:5′-GUACGUGCCCAUAAUUCAAAA/UUGAAUUAUGGGCACGUACGA-3′. siRNA-2: 5′-CGGAUGUACUAGUCGUGAUCC/AUCACGACUAGUACAUCCGGG-3′. See Table S1 for long dsRNA primers. (b) Mean spore production/g fresh weight normalized to control parallel samples, $n \geq 3$ (c) Powdery mildew on representative control and parallel *CYP51* dsRNA-1 sprayed *Arabidopsis thaliana* plants, at 9 days postinoculation. Toothpicks indicate leaves sampled for quantitative analysis. (d) Representative images of calcofluor white-stained *G. orontii* on control and siRNA-1 treated leaves. c, conidium. Scale bar = 100 µm. (e) Mean hyphae length per colony from detached leaf experiments, $n \geq 35$. Error bars ± *SEM*. *$p < 0.05$ by Student's *t* test (unpaired, two-sided) compared to control. Similar results observed in independent experiments.

As an additional PM fungal growth assessment, we chose to examine whether we could resolve the impact of SIGS on earlier development of the Gor colony: hyphae length at 2 and 3 dpi. Treatment with *CYP51* siRNA-1 did not alter hyphal branching architecture (Figure 2d), but did reduce the average total hyphae length of *CYP51* siRNA-1 colonies on *Arabidopsis* detached leaves by 56% and 45% at 2 and 3 days postinoculation (dpi), respectively, compared to control samples (Figure 2e).

2.3 | Expression patterns of Gor gene targets during fungal infection of *A. thaliana*

For subsequent SIGS analysis, we prioritized PM fungal genes within three broad functional categories, (1) Essential Cellular Function and Stress Response, (2) Energy Production, and (3) Manipulation of the Plant Host, based on conservation among PM species, functional annotation, and metabolic pathway analysis. In addition, RNA-Seq was

performed to examine the expression of Gor genes over the time course of fungal infection of *A. thaliana* at 0 (inoculation), 6 (spore germination), 12 (appressorial development and plant cell wall penetration), 24 (haustorium), 72 (continued colony expansion—hyphal growth and formation of secondary haustoria), and 120 (asexual reproduction) h postinoculation (hpi), as the pattern of expression can provide insights into the functional role of the protein. As shown in Figure 3, Category 1 gene targets, *CYP51* required for fungal cell membrane component synthesis and the cell fate/stress response regulator *apoptosis-antagonizing transcription factor* (*AATF*), exhibited similar low levels of expression throughout PM development including asexual development. Category 2 targets involved in glycogen metabolism, *glycogen debranching enzyme 1* (*GDB1*), *glycogen phosphatase 1* (*GPH1*), and *glucose-induced degradation 9* (*GID9*), displayed low to moderate expression over the time course of infection with the highest levels of expression evident before the haustoria formed at 24 hpi (Figure S1). Similarly, Category 2 lipid metabolism gene targets *lipase 1* (*LIP1*), *lipase A* (*LIPA*), *acyl-coenzyme A oxidase* (*ACX*), and *diglyceride acyltransferase* (*DGAT*) exhibited low to moderate expression with slightly elevated expression prior to haustoria formation (Figures 3 and S1). Category 3 Gor gene targets predicted to function in manipulation of the plant host via abscisic acid (ABA) metabolism (*9-cis-epoxycarotenoid dioxygenase* [*NCED*], the xanthoxin dehydrogenase *abscisic acid 2* [*ABA2*], and the putative *abscisic acid G-protein coupled receptor* [*ABAR*]) displayed low to moderate elevated early expression that levelled off once the haustoria had formed. Expression of the known PM secreted protein *effector candidate 2* (*EC2*) in Category 3 was at levels one to two orders of magnitude higher than the other genes targeted in this study and remained highly expressed throughout Gor growth and development on the leaf (Figure 3).

2.4 | SIGS of Gor genes identifies processes that contribute to Gor proliferation on *A. thaliana*

Long dsRNA against the additional 12 selected Gor gene targets were designed and SIGS using the *A. thaliana* whole-plant assays ascertained their impact on spore production. For those gene targets for which SIGS reduced average spore production in the whole-plant assay, we also assessed efficacy in reducing PM spore production using the detached-leaf assay for comparison of screening methods. SIGS against eight of 12 new targets tested significantly reduced PM proliferation (Figure 4). Within Energy Production (Category 2), SIGS against each of the three Gor targets known or predicted to function in lipid catabolism (*LIP1*, *LIPA*, *ACX*) reduced PM spore production by at least 50% using either the whole-plant or detached-leaf assay, while SIGS against the gene target involved in storage lipid biosynthesis, *DGAT*, did not. Surprisingly, SIGS against each of the three targets (*GDB1*, *GPH1*, *GID9*) involved in Energy Production via glycogen metabolism was not effective in reducing spore production. For Manipulation of the Plant Host (Category 3), SIGS against each of the three gene targets predicted to function in ABA metabolism (*NCED*, *ABA2*, *ABAR*) reduced Gor proliferation by at least 50% in either the whole-plant or detached-leaf assays. In addition, despite its extremely high level of expression, SIGS against the effector protein *EC2* reduced spore production by 60% (whole-plant assay) and 40% (detached-leaf assay). In Essential Cellular Function and Stress Response (Category 1), *AATF* showed reduced mean spore production when targeted by SIGS using both whole-plant and detached-leaf assays, but this difference was only statistically significant (with a 25% reduction) when assessed using the detached-leaf assay. Both assay methods resulted in a similar range of spore reduction for a given target, indicating that either could be used in screening. While the detached-leaf assay could reduce variability, it is not more rapid and did not uniformly present a more marked impact on spore production than the whole-plant assay (Figure 4). For example, three (*CYP51*, *ACX*, and *EC2*) of the nine targets assessed using both assays showed enhanced reduction in the whole-plant assay compared to the detached-leaf assay.

2.5 | SIGS methods and gene targets translate to the En–*V. vinifera* pathosystem

We next sought to test whether SIGS gene targets identified using the Gor–*Arabidopsis* system are effective in limiting En proliferation in grapevine, a commercially relevant system. First, procedures were developed and optimized for the SIGS En–grapevine system with *CYP51* as the gene target, as we did for the Gor–*Arabidopsis* system. Whole-plant and leaf-disc assays were developed, with the same timing of dsRNA sprays (0 and 2 dpi) and with dosages similar to those used in the Gor–*Arabidopsis* system; 20 mg of RNA was employed in En–grapevine whole-plant assays and 40 mg in leaf-disc assays. New long dsRNAs were designed for En orthologues of Gor gene targets as there is sequence divergence between Gor and En transcripts (Figure S2). SIGS against *EnCYP51* reduced spore production by 62% and 45% in grapevine whole-plant and leaf-disc assays, respectively (Figure 5a). In addition, infected grape leaves exhibited less visible powdery mildew disease (coverage and density) when sprayed with dsRNA targeting *EnCYP51* than with water alone (Figure 5b). We then tested SIGS against *En AATF*, *LIP1*, *LIPA*, *NCED*, and *EC2* in the En–grapevine system using either the whole-plant or leaf-disc assay. SIGS using dsRNA against these En gene targets significantly reduced En spore production by 53%–64% (Figure 5a).

In summary, six diverse PM fungal gene targets for which SIGS effectively limited spore production of Gor on *Arabidopsis* were tested in the SIGS En–*V. vinifera* system and all six showed a significant reduction in En spore production on grapevine. They represent targets with known or predicted functional roles within our prioritized three broad functional categories, Category 1—Essential Cellular Function and Stress Response (*CYP51*, *AATF*), Category 2—Energy Production (*LIP1*, *LIPA*) associated with lipid catabolism, and Category 3—Manipulation of the Plant Host, via ABA hormone metabolism (*NCED*) or a secreted effector protein (*EC2*). This shows that that the rapid Gor–*Arabidopsis* system can be used to identify

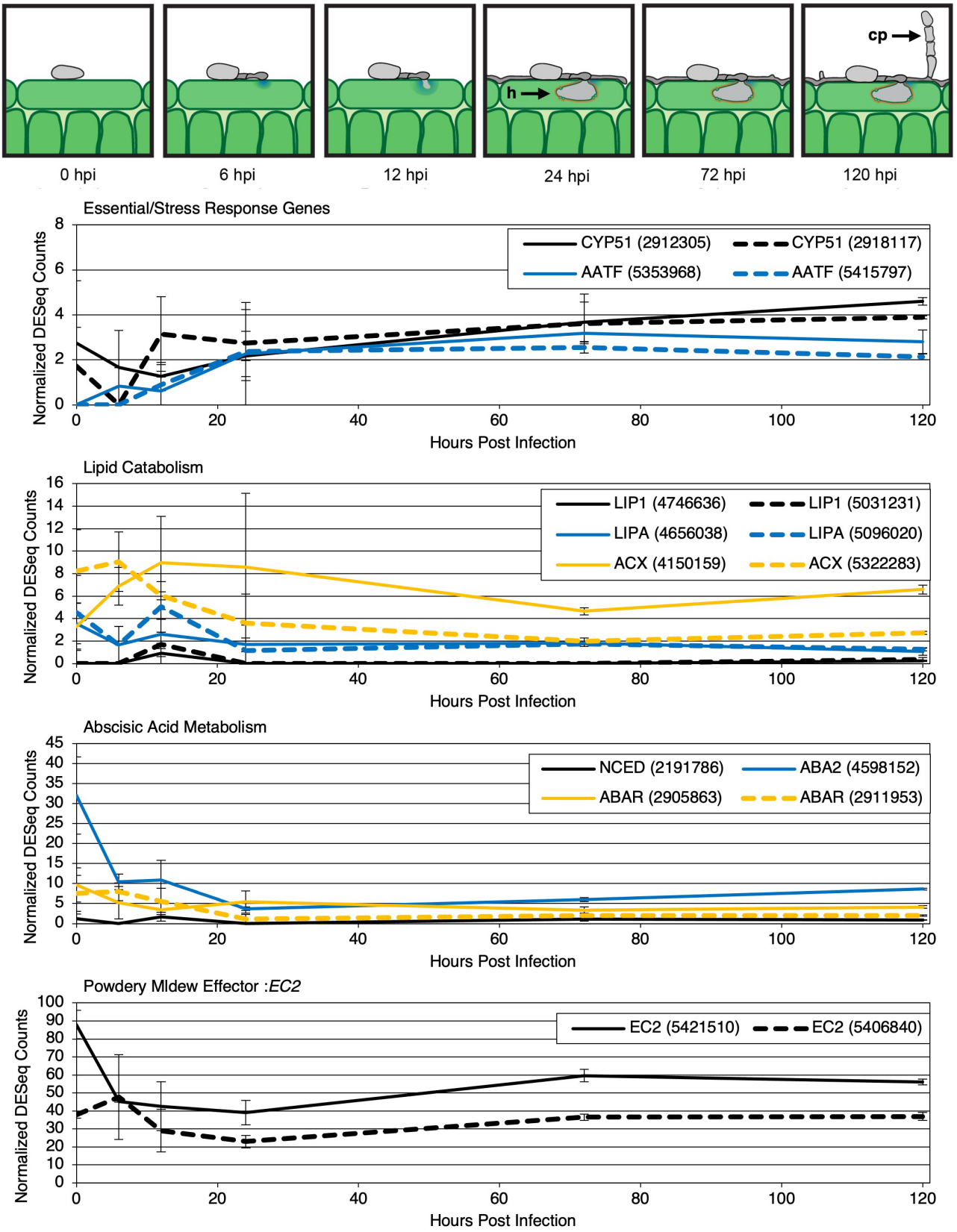

FIGURE 3 Time course of *Golovinomyces orontii* (Gor) development on *Arabidopsis* with *G. orontii* gene expression shown for spray-induced gene silencing targets by function. Developmental timeline: inoculation (0), spore germination (6), appressorium development and cell wall penetration (12), haustorial maturation (24), colony expansion (72), and asexual reproduction (120) h postinoculation (hpi). Mean DESeq2 normalized gene expression (±*SEM*) from triplicate samples. Most Gor gene targets have two copies due to recent duplication. h, haustoria; cp, conidiophore, asexual reproductive structure with new conidia.

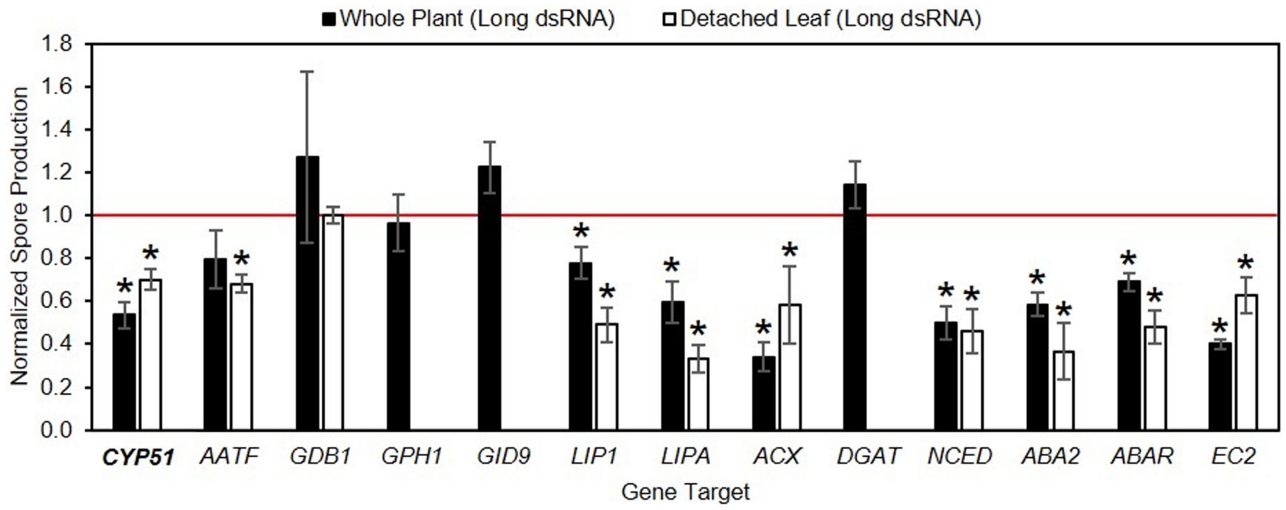

FIGURE 4 *Golovinomyces orontii* spore production on *Arabidopsis* is impacted by long dsRNA targeting diverse genes. Mean normalized spore production per gram fresh weight ± *SEM* displayed from $n \geq 3$. *$p < 0.05$ by Student's *t* test (unpaired, two-tailed) compared to control. Independent experiments gave similar results.

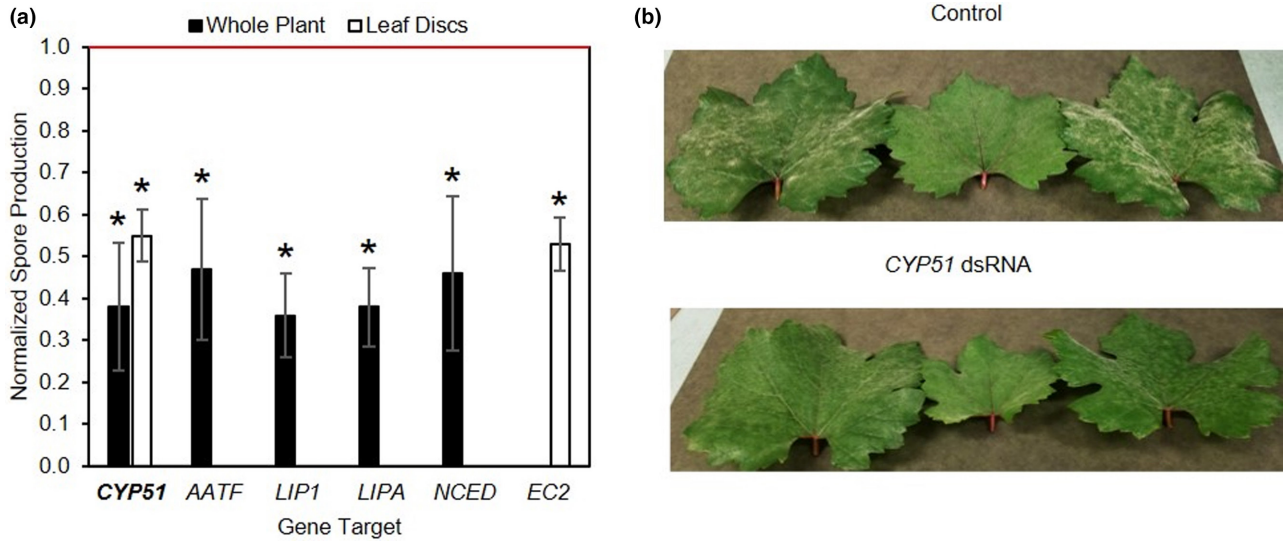

FIGURE 5 Long dsRNA targeting *Erysiphe necator* (En) genes reduces spore production on grapevine. (a) Mean normalized spore production per gram fresh weight ± *SEM*, $n \geq 3$. Note $n = 2$ for *AATF*. *$p < 0.05$ by Student's *t* test (unpaired, two-tailed) compared to control. (b) Representative grapevine leaves at 16 days postinoculation with En treated with *CYP51* dsRNA or water control.

successful SIGS PM fungi targets to be applied to the control of other PMs.

3 | DISCUSSION

Herein, we develop a pipeline to silence PM fungi genes and determine their contribution to PM proliferation (Figure 6). Screening PM fungal gene targets in *Arabidopsis* is rapid and provides insight into genes important to diverse PM species, as c.75% of genes in PM fungi are conserved (Wu et al., 2018). The effective PM gene targets identified by screening using the Gor–*Arabidopsis* system also contributed to En proliferation in grapevine. This suggests conserved effective PM fungal gene targets identified using the Gor–*Arabidopsis* system could be used to silence the orthologous genes in other powdery mildews of importance to agriculture and ornamental horticulture.

Because PM fungi are obligate biotrophs that cannot be cultured, it has been difficult to interrogate gene function. Recently, Ruiz-Jiménez et al. (2021) showed SIGS against the melon PM fungus, *Podosphaera xanthii*, can be used to identify conserved proteins with no annotated functional domain that are important to PM fungal proliferation; SIGS against three such targets dramatically reduced PM coverage on melon leaves. We showed SIGS can rapidly identify genes and processes that contribute to PM growth and reproduction. Although we chiefly employed an endpoint assay of spore production in these analyses, earlier growth stage-specific impacts can also be assessed. One such assessment, hyphal growth, is shown in

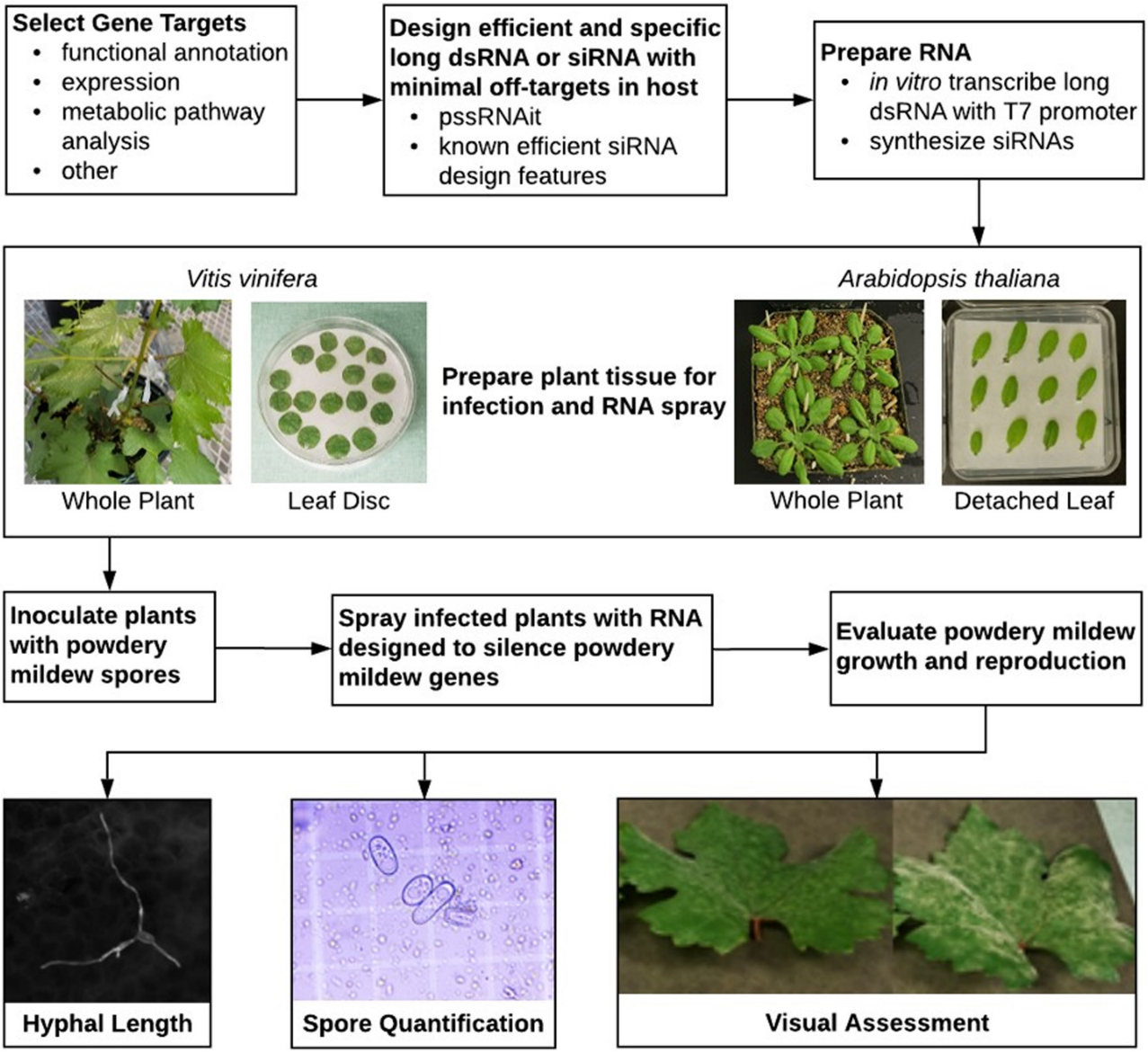

FIGURE 6 Flowchart of spray-induced gene silencing methodology.

Figure 2. Examination of the pattern of expression for genes of interest can provide indicators of potential phase-specific functions. For example, CYP51 is required for the synthesis of an essential fungal cell wall sterol and thus is anticipated to be required throughout infection and growth. Indeed, its expression is constant (Figure 3), and SIGS targeting *CYP51* shows reductions in hyphal growth (assessed at 2–3 dpi) and in the endpoint assay, spore production (assessed at 8–10 dpi), by similar amounts (Figure 2).

Using SIGS, we probed the importance of 13 genes in total and identified nine targets that significantly reduced Gor spore production on *Arabidopsis* (Table S1 and Figure 4). These nine effective PM fungal targets include genes in the three broad functional categories tested: (1) Essential Cellular Function and Stress Response, (2) Energy Production, and (3) Manipulation of the Plant Host. Furthermore, the efficacy of SIGS is independent of the level of target gene expression or the pattern of expression (Figures 3 and 4). To explore functional processes of importance to PM fungal proliferation, we examined multiple distinct gene targets associated with a chosen functional process. Within Category 2—Energy Production, three targets associated with glycogen metabolism and three with lipid catabolism were interrogated. Similarly, three distinct genes predicted to be involved in ABA metabolism (Category 3—Manipulation of the Plant Host) were assessed. The nine gene targets with demonstrated importance to PM growth and reproduction include the three lipid catabolism genes (*LIP1*, *LIPA*, and *ACX*) and the three ABA metabolism genes (*NCED*, *ABA2*, and *ABAR*), while all three glycogen metabolism targets showed no SIGS phenotype (Figure 4). This highlights the utility of screening multiple genes predicted to function in the same process. If SIGS against one of three targets associated with a given functional process does not impact spore production, it would suggest further dsRNAs against that target be tested as not all long dsRNAs or siRNAs selected using

current guidelines are equally effective in silencing a given target. For example, SIGS against *GorCYP51* effectively reduced fungal spore production using dsRNA-1 but not with the partially overlapping dsRNA-2 (Figure 2). Moreover, siRNA-1, which resides within dsRNA-2, limited spore production. Together, these results suggest inefficient dsRNA-2 uptake and/or processing of dsRNA-2 to siRNAs may be responsible. The two additional successful SIGS gene targets of the nine are the cell fate/stress response regulator *AATF* (Category 1) and the secreted effector *EC2* (Category 3).

Of these nine targets, SIGS against the En orthologues of six of these targets was assessed. New dsRNAs were designed against the En orthologues due to differences in transcript sequence compared to Gor orthologues (Figure S2). We favoured using dsRNAs against the same region of the gene transcript when possible, but only the En dsRNAs for *NCED* and *EC2* overlapped with Gor dsRNAs for that gene transcript (Figure S2). *EnCYP51* and *AATF* dsRNAs partially overlap the transcript region covered by the Gor dsRNAs and *EnLIP1* and *LIPA* dsRNAs did not overlap with the sequence area covered by the Gor dsRNAs against these targets. Despite these differences, a similar range of spore reduction was observed using the En–grapevine system. Furthermore, there was enhanced reduction per target for En–grapevine compared to the parallel assay in Gor–*Arabidopsis* (Figures 4 and 5). This may be due to enhanced dsRNA uptake in the En–grapevine system, reduced gene copy number in En compared to Gor (e.g., of *LIP1*, *LIPA*, and *AATF*), or other factors.

Once a phenotype has been observed with SIGS against a given target, further optimization of the applied RNA sequence may further increase efficacy (Chandra et al., 2019; Kweon et al., 2022). The efficiency of dsRNA-mediated silencing depends on several factors including uptake, processing to siRNAs, accessibility of the target region of the gene, and structural features of the siRNA resulting in efficient loading of the siRNA antisense strand into the RISC complex (Fakhr et al., 2016; Reynolds et al., 2004). Understanding features of gene hotspots for effective RNA silencing is an active area of investigation and, as discovered, these features will further inform our RNA design. In the meantime, using current best practice RNAi design, we demonstrate how assessing SIGS against multiple targets known or predicted to act in the same functional process can identify processes to be prioritized for additional effort. Below, we further discuss the successful SIGS targets of PM fungi and their predicted functions in the PM fungi–host interaction.

3.1 | Essential cellular function and stress response

Genes involved in essential cellular functions, such as the sterol demethylase *CYP51*, are expected to be effective SIGS targets as they have been for chemical fungicides. In addition, genes that moderate cellular stress can be essential to growth and reproduction of the PM species on its host. Although PM fungi evade activating plant defence and limit those defences that do occur, they are exposed to damaging compounds such as reactive oxygen species and specialized metabolites produced at the infection site.

3.1.1 | *CYP51*

SIGS against *CYP51* reduced PM proliferation on *Arabidopsis* (Figure 4), with >60% mean reduction of En spore production on grapevine (Figure 5). This complements previous reports that SIGS against the essential fungal gene *CYP51* successfully limited growth of the fungal hemibiotrophic pathogen *F. graminearum* (Koch et al., 2016) and the necrotrophic pathogen *B. cinerea* (Nerva et al., 2020).

3.1.2 | *Apoptosis antagonizing transcription factor*

AATF (also known as Che-1) is a eukaryotic cell-fate transcriptional regulator known to suppress apoptosis, promote proliferation (and tumours), mediate the DNA damage response, and integrate cellular stress response (Iezzi & Fanciulli, 2015; Kaiser et al., 2020). Most studies on AATF have been done in animal systems, and AATF function in fungi has not been explicitly explored. In fungi, regulated cell death is a factor in fungal development, response to external compounds/stress, and heterokaryon incompatibility (Gonçalves et al., 2017). Although inhibition of plant cell death is critical to PM growth and development, little is known about the role of regulated cell death in obligate biotrophic fungi, including PM fungi. *GorAATF*, like *GorCYP51*, exhibited low-level expression throughout development including asexual reproduction on the leaf (Figure 3). SIGS against *GorAATF* reduced spore production on *Arabidopsis*, with an even greater impact (55% reduction) against *EnAATF* on grapevine (Figures 4 and 5). MycoCosm analyses (Grigoriev et al., 2014) found AATF orthologues with the same domain structure to be present in all high-quality PM fungi genomes and a small subset of other Leotiomycetes.

3.2 | Energy production

Energy production is critical to PM fungi as it is for all organisms. During early colonization, the PM fungus derives its energy from the breakdown of energy-rich compounds in the spore. For example, PM fungus spores are replete with glycogen and triacylglycerols (TAGs) that are mobilized and degraded during germination and appressorial development (Both et al., 2005). Once the fungal feeding structure (haustorium) has developed, PM fungi rely on plant resources as they have lost the ability to synthesize costly compounds readily obtained from the host plant (Spanu, 2012). Among PM fungal gene targets tested in the Energy Production category, genes involved in lipid catabolism emerged as an important contributor to proliferation. The three PM fungal gene targets *LIP1*, *LIPA*, and *ACX* predicted to function in lipid catabolism exhibited low level expression throughout the infection process, with *LIP1* and *LIPA* exhibiting slightly elevated expression at 12 hpi, before the haustorium had fully formed (Figure 3). SIGS against each of these Gor targets reduced spore production by >50% (Figure 4), with >60% reduction of spore production for the tested En targets (*LIP1* and *LIPA*), providing direct experimental evidence of their importance to PM growth and development (Figures 4 and 5).

3.2.1 | *Lipase 1*

LIP1 contains a secretion signal and a carboxylesterase family protein domain; it is predicted to catalyse the hydrolysis of TAGs into diacylglycerols and a carboxylate. *Blumeria graminis* f. sp. *tritici* Lip1 localizes to the surface of conidia, germ tubes, and/or hyphae, and the enzyme has broad lipid specificity with the capacity to hydrolyse leaf epicuticular waxes (Feng et al., 2009). This function of Lip1 has been associated with promoting PM early colonization events via enhanced adhesion to the leaf surface and/or enhanced rate of appressorial germ tube formation (Feng et al., 2009); however, loss of function has not been explored. *LIP1* mutants of *B. cinerea* and *F. graminearum* show no defects in colonization or growth (Subramoni et al., 2010). This may be attributed to the plethora of *LIP1*-like genes in these fungi, compared with obligate plant biotrophs (Feng et al., 2009), minimizing the impact of single *LIP1* mutants. Alternatively, the function of Lip1 may be more critical for obligate biotrophic PM fungi. For example, Lip1 activity may release specific cues from the plant host surface that promote PM development (Feng et al., 2009) or provide energy sources for PM growth and reproduction. Here, using SIGS against *LIP1* in the Gor–*Arabidopsis* and En–grapevine systems, we directly show the important role that Lip1 plays in PM growth and development with >50% and >60% reduction in spore production in each system, respectively (Figures 4 and 5).

3.2.2 | *Lipase A*

LIPA contains a secretion signal and a class 3 lipase domain (PF01764; Mistry et al., 2021); as such, it is predicted to function as an extracellular TAG lipase. Orthologues of LipA are present in both plant and animal fungal pathogens, and we found *LIPA* to be present in all PM fungi and Leotiomycetes analysed including the plant necrotrophs *B. cinerea* and *S. sclerotiorum*. FGL2 (fg01240;ABW74155.1), the *F. graminearum LIPA* orthologue, has lipolytic activity and plays a critical role in virulence on both maize cobs and wheat heads (Nguyen, 2008; Nguyen et al., 2010). We found SIGS against *LIPA* resulted in a dramatic reduction in spore production for both Gor (67%) and En (62%) (Figures 4 and 5). Operating in the extracellular space, Gor LipA could act on plant TAGs to release free fatty acids for fungal acquisition. In addition, the released free fatty acids could function as signalling molecules.

3.2.3 | *Acetyl-CoA oxidase*

SIGS against *GorACX*, peroxisomal ACX, was also highly effective, reducing spore production by 66% in whole-plant assays (Figure 4). ACX participates as a rate-limiting enzyme in fatty acid β-oxidation and α-linolenic acid metabolism, therefore in PM fungi ACX could be involved in degradation of long-chain free fatty acids for energy generation or the production of oxylipins as fungal reproductive signals or as plant hormone jasmonic acid (ant)agonists (Poirier et al., 2006).

3.3 | Manipulation of the plant host

Many plant pathogens including PM fungi manipulate plant functions such as cell fate, phytohormone metabolism, and defence through the secretion of effector proteins that enter the plant cell (Lo Presti et al., 2015; Weßling et al., 2014). Furthermore, plant pathogens can directly manipulate plant hormone responses by making a plant hormone or hormone (ant)agonist (Ronald & Joe, 2018). Herein we used SIGS to explore a potential role for PM manipulation of host ABA metabolism and assessed whether the highly expressed PM effector *EC2* is a suitable SIGs target.

3.3.1 | *NCED* and *ABA2*

The plant hormone ABA plays a critical role in diverse plant processes including seed development, dormancy, and germination; regulation of growth and development; stomatal closure; and response to abiotic and biotic stressors (Jia et al., 2022). Fungal production of ABA appears to be limited to plant-associated fungi, acting as a virulence factor to mediate ABA-dependent plant responses (Takino et al., 2019). Fungi and plants employ distinct ABA biosynthetic routes (Jia et al., 2022; Takino et al., 2019). However, the annotated Gor genome contains two plant ABA biosynthetic enzymes, NCED and ABA2, and reciprocal protein BLAST analysis found the Gor proteins to be the best protein BLAST hits of the *Arabidopsis* plant proteins and vice versa. By contrast, no Gor orthologue of the fungal terpene synthase (e.g., BcABA3 in *Botrytis cinerea*) that catalyses the first step of fungal ABA synthesis was identified. NCED (EC 1.1.3.11.51), the rate-limiting enzyme in plant ABA synthesis, cleaves 9-*cis* violaxanthin and 9-*cis*-neoxanthin to xanthoxin. ABA2 (EC 1.1.1. 288), a xanthoxin dehydrogenase, then converts xanthoxin to abscisic aldehyde. The specificity of the enzyme functions and their sequential activities suggest fungal acquisition of these genes from the plant, as does the absence of *NCED* in PM fungi of monocots (Wisecaver & Rokas, 2015). However, it should be noted that *GorNCED* resides in a gene cluster involved in fungal carotenoid metabolism and fungi are capable of making a diverse array of carotenoid-derived products with functions in signalling, development, and environmental response (Avalos & Carmen Limón, 2015).

3.3.2 | *ABAR*

While Gor does not contain a plant PYR/RCAR ABA receptor (Ma et al., 2009; Park et al., 2009) homologue, a putative plant ABA G-protein coupled receptor (ABAR) (Pandey et al., 2009) homologue was identified in Gor. GorABAR is a nine-transmembrane domain protein with an N-terminal GPHR domain (PF12537) and an ABA GPCR domain (PF12430) (Mistry et al., 2021). The Gor and *Arabidopsis* ABAR proteins are the reciprocal best protein BLAST hits of each other. Furthermore, all PM fungi contain a highly conserved putative ABAR, while other Leotiomyctes analysed did not.

While the PYR/RCAR ABA receptors account for dominant ABA-associated plant responses, the *Arabidopsis* ABA GPCRs can bind ABA in vitro (Pandey et al., 2009) and thus could potentially function as an ABA or ABA intermediate receptor in fungi.

The expression patterns of Gor *NCED*, *ABA2*, and *ABAR* were fairly similar, with peaks at 0 and/or 12 hpi and some expression throughout (Figure 3). *ABA2* exhibited the highest level of expression and a more dramatic peak of expression (at 0 hpi). SIGS against each of these Gor gene targets led to a substantial decrease in spore production of 63% to 52% (Figure 4), with a similar impact when *EnNCED* was silenced (Figure 5). These findings could be consistent with a known role for ABA—impacting fungal appressorium formation and penetration. For the hemibiotrophic rice pathogen *Magnaporthe oryzae*, exogenous application of ABA promotes fungal spore germination and appressoria formation (Spence et al., 2015). Furthermore, a *M. oryzae* ABA biosynthetic mutant exhibits reduced appressoria formation and lesion formation on rice. PM fungal infection alters the expression of ABA biosynthetic and responsive genes in the host plant (Chandran et al., 2010; Hayes et al., 2010). This could be associated with ABA promotion of the plant source-to-sink transition associated with PM fungal infection, alteration of plant defences and/or localized drought response. A recently identified PM fungal effector targets the plant ABA biosynthetic pathway (Li et al., 2020), confirming the importance of its regulation. While the role of host ABA in PM infection and growth needs to be further resolved, our findings introduce additional complexity to the interaction as the PM fungus itself may be able to synthesize ABA intermediates to manipulate host function, or for its own, yet undetermined, purposes.

3.3.3 | *EC2*

The effector EC2 is a widely conserved and highly expressed PM fungal effector first described in *B. graminis* f. sp. *hordei* as BEC2 (CSEP0214; Schmidt et al., 2014). EC2 contains a cysteine-rich fungal extracellular membrane (CFEM) domain that is unique in fungi and contains eight conserved cysteine residues. Fungal CFEM-containing proteins have been shown to play roles in fungal pathogenesis and development (Kou et al., 2017; Zhu et al., 2017). Our analysis found PM fungi EC2 effectors form their own cluster within fungal CFEM-containing protein domain architectures.

Previous studies of EC2 suggest its involvement in early colonization and particularly penetration success. Transient transformation of the cucurbit PM fungus, *Podosphaera xanthii*, with *PxaEC2-GFP* localized it to growing hyphal tips of the fungus (Martínez-Cruz et al., 2018). This location could be consistent with a role in enhancing penetration events to form both primary and secondary haustoria as the colony expands. Overexpression of *EC2* did not alter penetration rates or spore production by an adapted PM fungus but did increase penetration success of a nonadapted PM fungus (Schmidt et al., 2014). This suggests sufficient EC2 protein is present in compatible adapted interactions. Previous reports did not examine the impact of reducing *EC2* expression on PM growth and reproduction, as we do here.

Consistent with studies with other powdery mildew species (e.g., Fonseca et al., 2019; Jones et al., 2014; Schmidt et al., 2014), we found *GorEC2* is one of the most highly expressed genes (Figure 3). Furthermore, we showed SIGS against *GorEC2* and *EnEC2* effectively reduced spore production in the Gor–*Arabidopsis* and En–grapevine systems (Figures 4 and 5). Further analysis is needed to define the specific phase(s) of infection impacted by *EC2* reduction. However, our finding that *GorEC2* expression remains very high throughout development (Figure 3), coupled with its importance in the growth and reproduction of the adapted PMs Gor and En on their respective hosts (shown via SIGS) is consistent with a proposed role for EC2 in enhancing penetration events to form both primary and secondary haustoria as the colony continues to expand. Importantly, our results show that PM fungal effectors, even those that are very highly expressed, can be targeted via SIGS to reduce PM growth and development.

3.4 | Application of SIGS against powdery mildews in agriculture and ornamental horticulture

Here, we translated our findings using the Gor–*Arabidopsis* system to the commercially important study of En infection of grapevine where new PM fungal control methods are greatly needed. We identified nine Gor targets that resulted in strongly reduced PM fungal proliferation on plants (Figure 4). Six of these were also tested in the En–grapevine system and SIGS against all six targets dramatically reduced En spore production (Figure 5). This suggests effective conserved SIGs PM fungal targets identified in one system can be adapted to control PM fungi of other economically important agricultural and ornamental plants. Optimization of SIGS to control PM in agricultural settings could include further refinement of the applied dsRNA sequence against a given target, modification of the dsRNA, the formulation and/or delivery methods (Cagliari et al., 2019). In addition, multiplexing dsRNAs targeting genes within a functional process/pathway or from differing processes/pathways has the potential to further increase efficacy as recently shown for dsRNAs targeting the agricultural pest whitefly (Jain et al., 2022). The benefits of topical RNAi in agriculture include its sequence specificity (and minimal off-target effects), rapid biodegradability in the environment, and little to no environmental or human health impacts (Fletcher et al., 2020).

4 | EXPERIMENTAL PROCEDURES

4.1 | RNA fluorescence labelling and uptake

Fluorescein-labelled 300 base β-actin-mouse was synthesized using MAXIscript T7 Transcription Kit (Invitrogen) and Fluorescein RNA Labeling Mix (Roche). To induce germination, 12 dpi spores were

tapped onto a glass slide, placed in a Petri dish containing water, covered in foil for 1 h, unwrapped, and left overnight in ambient light. Spores were blotted onto 1% agar pad on glass slide before applying 20 μL of water or water plus fluorescein-labelled RNA (800 ng). Spores were incubated with RNA for 90 min before imaging with a Zeiss Axio Imager fluorescence microscope. For RNase treated samples, 1 μL each of benzonase, nuclease, and RNase A was added 30 min before microscopy.

4.2 | Plant growth and spore inoculation

A. thaliana ecotype Col-0 were grown in growth chambers at 22°C, 70% relative humidity, and a 12 h photoperiod with 150 μmol m^{-2} s^{-1}. photosynthetically active radiation. At 4–4.5 weeks plants were inoculated by settling tower (Reuber et al., 1998) with Gor at 14:30 ± 1.5 h. For whole plant assays, *Arabidopsis* was grown in 4-inch pots (c.10 cm; four plants per pot). On the day of spore inoculation and dsRNA treatment, three or four mature, fully expanded, and exposed leaves per plant were marked with toothpicks for later harvesting at 8–10 dpi. Visual inspection to qualitatively assess extent of powdery mildew disease for the marked leaves used the criterion in Reuber et al. (1998), which includes leaf coverage and density. Detached leaf assay plants were grown in plant trays (16.6 × 12.4 × 5.8 cm insert boxes, 12 plants per box). Before infection, mature, fully expanded leaves (similar to above) were plucked and petioles inserted into ½× Murashige and Skoog salts/0.8% agar overlaid with Whatman no. 1.0 filter paper, with 12–15 leaves per plate. A low to moderate inoculum of 10–14 dpi Gor conidia (one or two half-covered leaves) was used for hyphal assays and a heavy dose (four or five fully infected leaves) for spore counting.

En was obtained from Andrew Walker, UC Davis, and maintained on detached leaves of Chardonnay and Carignan grapevine varieties. Grapevine hard cuttings were from Foundation Plant Services, UC Davis, and grown at UC Berkeley Oxford Tract Greenhouse. Cuttings were rooted on a mist bench, transferred to 2-gallon (c.9 L) pots with supersoil, and fertilized with Osmocote. Grapevine plants were sprayed weekly with sulphur to keep them mildew-free. For whole-plant and leaf-disc assays, leaves from the third or fourth node of 4-month-old potted Chardonnay plants were used for optimal resolution of differences in PM proliferation as ontogenic resistance to powdery mildew is associated with older leaves (Gadoury et al., 2012). For whole-plant assays, three leaves from the nodes above were marked and sprayed with c.60,000 En spores/mL. For leaf-disc assays, 12–15 10-mm leaf discs were cut out (four discs per leaf) avoiding the midvein and edges, placed on 0.8% agar plates, and inoculated (heavy dose) by settling tower.

4.3 | siRNA and long dsRNA design and synthesis

pssRNAit (https://www.zhaolab.org/pssRNAit) was used to design the siRNAs and dsRNAs, and limit off-target gene silencing in the host *A. thaliana* or *V. vinifera*. Publicly available annotated genomes for *G. orontii* MGH1 (https://mycocosm.jgi.doe.gov/Golor4/Golor4.home.html) and *E. necator* C-strain (Jones et al., 2014; https://mycocosm.jgi.doe.gov/Erynec1/Erynec1.home.html) were used to obtain target gene sequences. As the tested orthologous genes in Gor and En vary in transcript identity between 59% and 71%, and the efficacy of siRNAs (either synthesized or derived from dsRNAs) is dramatically reduced by mismatches, new dsRNAs were designed for the orthologous En gene targets using pssRNAit, prioritizing the same region of the transcript (e.g., middle third) as the successful dsRNA against the Gor gene when possible (Figure S2).

siRNAs were designed as 21-base sense and antisense strands that anneal over 19 bases with two-base overhangs at the 3′ ends. Designed long dsRNAs ranged in length from 199 to 344 bp. For long dsRNA synthesis, templates were amplified from cDNAs obtained from Gor-infected *Arabidopsis* leaves or En-infected grapevine leaves, using gene-specific primers incorporating T7-polymerase recognition sequence (5′-TAATACGACTCACTATAGGGGG-3′) at the 5′ end (Table S1). dsRNAs were in vitro synthesized using the HiScribe T7 High Yield RNA Synthesis Kit following manufacturer's instructions (New England Biolabs). dsRNA was purified using the Monarch RNA Cleanup Kit (New England Biolabs) and reannealed by heating to 94°C and slowly cooling to room temperature over 50 min. siRNAs (21 bp) were synthesized by Horizon Discovery (Lafayette, Colorado). Target genes and primer sequences are provided in Table S1.

4.4 | SIGS protocol

The SIGS protocol and pipeline was optimized using long dsRNA against *CYP51*. The spraying method, amount of RNA, number of RNA applications, timing of RNA application, RNA purification method, and PM proliferation assays were optimized to improve efficiency and reproducibility of results. Plants or detached leaves or leaf discs were infected with Gor or En as described above and sprayed with nuclease-free water (control) or RNA dissolved in nuclease-free water using a gravity-feed airbrush (pressure of 5 psi, c.34.5 kPa). For spore assays, two RNA treatments were performed, day 0 (after inoculation) and 2 dpi, and 40 μg of RNA per treatment was employed excepting the grapevine whole-plant assays for which 20 μg of RNA was sprayed per treatment. For hyphal length assays, only the first RNA treatment at day 0 was performed, as leaves were harvested starting at 2 dpi.

4.5 | Powdery mildew quantification

4.5.1 | Hyphal length measurements

The select infected leaves were fixed, cleared in 3:1 (vol/vol) ethanol:acetic acid, and stained with calcofluor white (Sigma-Aldrich) to visualize and quantify fungal growth. Cleared leaves were washed with water for 5 min, stained with 6 μg/mL calcofluor white solution

for 15 min, rinsed with water for 10 min, and mounted on slides in 50% glycerol. Fungal colonies were imaged using a 10× objective of Zeiss Axio Imager fluorescence microscope, Qimaging QiClick and Micropublisher cameras, and Sutter Instruments Lambda LS Light Source with DAPI Long Pass filter set. Hyphal lengths were measured using Fiji image analysis software ImageJ (https://imagej.net/Fiji/Downloads).

4.5.2 | Spore quantification

The 12–15 mature, fully expanded *A. thaliana* leaves were harvested (from four or five plants) per replicate at 8–10 dpi for whole-plant or detached-leaf assays. For grapevine whole-plant assays, three leaves from nodes 3 or 4 were collected at c.16 dpi per replicate. For grapevine leaf-disc assays 12–15 10-mm leaf discs were collected at 14–21 dpi per replicate. Tissues were collected in 50-mL Falcon tubes, weighed, vortexed in 15 mL of 0.01% Tween 80, filtered through 40 μm mesh, and centrifuged. Spore pellets were resuspended to 200–1200 μL with water. For each sample, nine 1 × 1 mm fields of a Neubauer-improved haemocytometer were counted. RNA-treated spore count/g fresh weight (FW) were normalized to the parallel water-treated control spore count/g FW.

4.6 | Gor target gene selection for SIGS analysis

Gor target gene selection included analysis of conservation among PM species, functional annotation, metabolic pathway analysis, and pattern of expression (see RNA-Seq). Examination of multiple distinct targets proposed to function in the same process was included when possible.

MycoCosm (Grigoriev et al., 2014) MCL clustering analysis of each Gor protein was used to assess prevalence in published PM fungal genomes *B. graminis* f. sp. *hordei* DH14 (Frantzeskakis et al., 2018), *B. graminis* f. sp. *tritici* 99644 (Müller et al., 2019), En (Jones et al., 2014), *Golovinomyces cichoracearum* UMSG1 and UMSG3 (Wu et al., 2018), and early diverging *Parauncinula polyspora* (Frantzeskakis et al., 2019), and in other published Leotiomycete genomes, including those that colonize plants such as *B. cinerea* (Amselem et al., 2011; Staats & van Kan, 2012), *Sclerotinia sclerotiorum* (Amselem et al., 2011), *Cadosphora* sp. SDE1049 (Knapp et al., 2018), and *Oidiodendron maius* (Kohler et al., 2015; Martino et al., 2018). Domain structure analysis including similarity by E-value and annotated enzymatic reactions by EC number were also considered. BLASTP was performed for proteins absent by MCL analysis. For those Gor proteins with homology to *Arabidopsis* proteins, reciprocal BLASTP analysis of *A. thaliana* and Gor proteins determined best hits based on E-values with coverage and percentage identity. Annotated protein domains (e.g., Mistry et al., 2021) and EC numbers for the top *A. thaliana* and Gor protein hits were also examined.

Functional annotation and metabolic pathway analysis employed MycoCosm (Grigoriev et al., 2014) with examination of annotated protein domains (Mistry et al., 2021) and EC numbers and associated metabolic pathways via KEGG (Kanehisa & Goto, 2000).

4.7 | RNA-Seq

RNA-Seq data for Gor gene expression analysis were downloaded from DOE JGI Data Portal DOI: 10.46936/10.25585/60001036, project ID 1290666 (JGI CSP 1657 to M. Wildermuth). The RNA-Seq data were generated for Gor infection of *A. thaliana* Col-0 at 0, 6, 12, 24, 72, and 120 hpi. Mature, fully expanded leaves were harvested at each time point, with three independent biological replicates per time point. In brief, leaf samples were ground using two 3-mm stainless-steel balls for 1 min at 25 Hz/s and immediately frozen in liquid nitrogen. RNA was isolated using Qiagen RNeasy Plant Mini Kit according to the manufacturer's protocol and treated with on-column RNase-free DNase set as recommended. Library preparation and sequencing using the Illumina platform was performed by JGI. RNA read counts were normalized using DESeq2 package v. 1.36 in R Studio v. 3.3.0 (Love et al., 2014).

ACKNOWLEDGEMENTS

This research was supported by awards to M.C.W. from the American Vineyard Foundation 2138 and 2366, the National Science Foundation MCB-1617020 and PFI-TT-1919244, and the USDA National Institute of Food and Agriculture, Hatch project 1016994. The work on *G. orontii* MGH1 genome sequencing, assembly, and annotation and RNA-Seq time course data (proposal: 10.46936/10.25585/60001036) conducted by the US Department of Energy Joint Genome Institute (https://ror.org/04xm1d337), a DOE Office of Science User Facility, is supported by the Office of Science of the US Department of Energy operated under contract DE-AC02-05CH11231. We thank Kerrie Berry and Chris Daum and their teams for coordination of JGI data productions. For their assistance we thank J. Jaenisch (UC Berkeley), Figure 3 diagram; S. Upadhyaya (UC Berkeley), DESeq2 analysis; A. Walker and S. Riaz (UC Davis), grapevine system setup; and K. Ryan and H. Xue (UC Berkeley) for manuscript review.

CONFLICT OF INTEREST STATEMENT

A.G.M., J.T., and M.C.W. are co-inventors on INHIBITORY RNA FOR THE CONTROL OF PHYTOPATHOGENS, PCT/US2022/025330, 19 April 2022; priority date 19 April 2021.

DATA AVAILABILITY STATEMENT

G. orontii MGH1 (Golor4) genome is available for download at https://mycocosm.jgi.doe.gov/Golor4/Golor4.home.html from DOE JGI Data Portal, JGI CSP 1657, project ID 1056001 to M. Wildermuth. RNA-Seq time course data for *G. orontii* MGH1 infection of 4-week-old *A. thaliana* is available for download from DOE JGI Data Portal, DOI: 10.46936/10.25585/60001036, JGI CSP

1657, project ID 1290666 to M. Wildermuth. Methods and results herein are included in INHIBITORY RNA FOR THE CONTROL OF PHYTOPATHOGENS, PCT/US2022/025330 filed on 19 April 2022; US application no. 63/176,795 filed 19 April 2021.

ORCID

Amanda G. McRae https://orcid.org/0000-0003-0641-0548
Jyoti Taneja https://orcid.org/0000-0002-5386-0683
Sajeet Haridas https://orcid.org/0000-0002-0229-0975
Kurt LaButti https://orcid.org/0000-0002-5838-1972
Vasanth Singan https://orcid.org/0000-0002-9983-5707
Igor V. Grigoriev https://orcid.org/0000-0002-3136-8903
Mary C. Wildermuth https://orcid.org/0000-0003-4927-4144

REFERENCES

Amselem, J., Cuomo, C.A., van Kan, J.A., Viaud, M., Benito, E.P., Couloux, A. et al. (2011) Genomic analysis of the necrotrophic fungal pathogens *Sclerotinia sclerotiorum* and *Botrytis cinerea*. *PLoS Genetics*, 7, e1002230.

Avalos, J. & Carmen Limón, M. (2015) Biological roles of fungal carotenoids. *Current Genetics*, 61, 309–324.

Bachman, P., Fischer, J., Song, Z., Urbanczyk-Wochniak, E. & Watson, G. (2020) Environmental fate and dissipation of applied dsRNA in soil, aquatic systems, and plants. *Frontiers in Plant Science*, 11, 21.

Bastos, R.W., Rossato, L., Goldman, G.H. & Santos, D.A. (2021) Fungicide effects on human fungal pathogens: cross-resistance to medical drugs and beyond. *PLoS Pathogens*, 17, e1010073.

Baulcombe, D. (2004) RNA silencing in plants. *Nature*, 431, 356–363.

Bilir, Ö., Telli, O., Norman, C., Budak, H., Hong, Y. & Tör, M. (2019) Small RNA inhibits infection by downy mildew pathogen *Hyaloperonospora arabidopsidis*. *Molecular Plant Pathology*, 20, 1523–1534.

Both, M., Csukai, M., Stumpf, M.P. & Spanu, P.D. (2005) Gene expression profiles of *Blumeria graminis* indicate dynamic changes to primary metabolism during development of an obligate biotrophic pathogen. *The Plant Cell*, 17, 2107–2122.

Bramlett, M., Plaetinck, G. & Maienfisch, P. (2020) RNA-based biocontrols—a new paradigm in crop protection. *Engineering*, 6, 522–527.

Cagliari, D., Dias, N.P., Galdeano, D.M., Dos Santos, E.Á., Smagghe, G. & Zotti, M.J. (2019) Management of pest insects and plant diseases by non-transformative RNAi. *Frontiers in Plant Science*, 10, 1319.

Calvert, G.M., Karnik, J., Mehler, L., Beckman, J., Morrissey, B., Sievert, J. et al. (2008) Acute pesticide poisoning among agricultural workers in the United States, 1998–2005. *American Journal of Industrial Medicine*, 51, 883–898.

Chandra, G.S., Asokan, R., Manamohan, M. & Kumar, N.K. (2019) Enhancing RNAi by using concatemerized double-stranded RNA. *Pest Management Science*, 75, 506–514.

Chandran, D., Inada, N., Hather, G., Kleindt, C.K. & Wildermuth, M.C. (2010) Laser microdissection of *Arabidopsis* cells at the powdery mildew infection site reveals site-specific processes and regulators. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 460–465.

Darlington, M., Reinders, J.D., Sethi, A., Lu, A.L., Ramaseshadri, P., Fischer, J.R. et al. (2022) RNAi for western corn rootworm management: lessons learned, challenges, and future directions. *Insects*, 13, 57.

Fakhr, E., Zare, F. & Teimoori-Toolabi, L. (2016) Precise and efficient siRNA design: a key point in competent gene silencing. *Cancer Gene Therapy*, 23, 73–82.

Feng, J., Wang, F., Liu, G., Greenshields, D., Shen, W., Kaminskyj, S. et al. (2009) Analysis of a *Blumeria graminis*-secreted lipase reveals the importance of host epicuticular wax components for fungal adhesion and development. *Molecular Plant-Microbe Interactions*, 22, 1601–1610.

Fletcher, S.J., Reeves, P.T., Hoang, B.T. & Mitter, N. (2020) A perspective on RNAi-based biopesticides. *Frontiers in Plant Science*, 12, 51.

Fonseca, N.R., Caballero, J.I., Kim, M.S., Stewart, J.E., Guimaraes, L.M.S., Alfenas, A.C. et al. (2019) Transcriptome analysis of a powdery mildew pathogen (*Podosphaera pannosa*) infecting *Eucalyptus urophylla*: de novo assembly expression profiling and secretome prediction. *Forest Pathology*, 49, 508.

Frantzeskakis, L., Kracher, B., Kusch, S., Yoshikawa-Maekawa, M., Bauer, S., Pedersen, C. et al. (2018) Signatures of host specialization and a recent transposable element burst in the dynamic one-speed genome of the fungal barley powdery mildew pathogen. *BMC Genomics*, 19, 381.

Frantzeskakis, L., Németh, M.Z., Barsoum, M., Kusch, S., Kiss, L., Takamatsu, S. et al. (2019) The *Parauncinula polyspora* draft genome provides insights into patterns of gene erosion and genome expansion in powdery mildew fungi. *mBio*, 10, e01692-19.

Frenkel, O., Cadle-Davidson, L., Wilcox, W.F. & Milgroom, M.G. (2015) Mechanisms of resistance to an azole fungicide in the grapevine powdery mildew fungus, *Erysiphe necator*. *Phytopathology*, 105, 370–377.

Gadoury, D.M., Cadle-Davidson, L., Wilcox, W.F., Dry, I.B., Seem, R.C. & Milgroom, M.G. (2012) Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. *Molecular Plant Pathology*, 13, 1–16.

Glawe, D.A. (2008) The powdery mildews: a review of the world's most familiar (yet poorly known) plant pathogens. *Annual Review Phytopathology*, 46, 27–51.

Gonçalves, A.P., Heller, J., Daskalov, A., Videira, A. & Glass, N.L. (2017) Regulated forms of cell death in fungi. *Frontiers in Microbiology*, 8, 1837.

Gonsalves, D. (2006) Transgenic papaya: development, release, impact and challenges. *Advances in Virus Research*, 67, 317–354.

Grigoriev, I.V., Nikitin, R., Haridas, S., Kuo, A., Ohm, R., Otillar, R. et al. (2014) MycoCosm portal: gearing up for 1000 fungal genomes. *Nucleic Acids Research*, 42, 699–704.

Hacquard, S., Kracher, B., Maekawa, T., Vernaldi, S., Schulze-Lefert, P., Loren, V. et al. (2013) Mosaic genome structure of the barley powdery mildew pathogen and conservation of transcriptional programs in divergent hosts. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 2219–2228.

Hayes, M.A., Feechan, A. & Dry, I.B. (2010) Involvement of abscisic acid in the coordinated regulation of a stress-inducible hexose transporter (VvHT5) and a cell wall invertase in grapevine in response to biotrophic fungal infection. *Plant Physiology*, 153, 211–221.

Head, G.P., Carroll, M.W., Evans, S.P., Rule, D.M., Willse, A.R., Clark, T.L. et al. (2017) Evaluation of SmartStax and SmartStax PRO maize against western corn rootworm and northern corn rootworm: efficacy and resistance management. *Pest Management Science*, 73, 1883–1899.

Hinckley, E.L. & Matson, P.A. (2011) Transformations, transport, and potential unintended consequences of high sulfur inputs to Napa Valley vineyards. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 14005–14010.

Hu, D., Chen, Z.Y., Zhang, C. & Ganiger, M. (2020) Reduction of *Phakopsora pachyrhizi* infection on soybean through host- and spray-induced gene silencing. *Molecular Plant Pathology*, 21, 794–807.

Iezzi, S. & Fanciulli, M. (2015) Discovering Che-1/AATF: a new attractive target for cancer therapy. *Frontiers in Genetics*, 6, 141.

Jain, R.G., Fletcher, S.J., Manzie, N., Robinson, K.E., Li, P., Lu, E. et al. (2022) Foliar application of clay-delivered RNA interference for whitefly control. *Nature Plants*, 8, 535–548.

Jia, K.P., Mi, J., Ali, S., Ohyanagi, H., Moreno, J.C., Ablazov, A. et al. (2022) An alternative, zeaxanthin epoxidase-independent abscisic acid biosynthetic pathway in plants. *Molecular Plant*, 15, 151–166.

Jones, L., Riaz, S., Morales-Cruz, A., Amrine, K.C., McGuire, B., Gubler, W.D. et al. (2014) Adaptive genomic structural variation in the grape powdery mildew pathogen, *Erysiphe necator*. *BMC Genomics*, 15, 1081.

Kaiser, R., Erber, J., Höpker, K., Fabretti, F. & Müller, R.U. (2020) AATF/Che-1—an RNA binding protein at the nexus of DNA damage response and ribosome biogenesis. *Frontiers in Oncology*, 10, 919.

Kanehisa, M. & Goto, S. (2000) KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research*, 28, 27–30.

Knapp, D.G., Németh, J.B., Barry, K., Hainaut, M., Henrissat, B., Johnson, J. et al. (2018) Comparative genomics provides insights into the lifestyle and reveals functional heterogeneity of dark septate endophytic fungi. *Scientific Reports*, 8, 6321.

Koch, A., Biedenkopf, D., Furch, A., Weber, L., Rossbach, O., Abdellatef, E. et al. (2016) An RNAi-based control of *Fusarium graminearum* infections through spraying of long dsRNAs involves a plant passage and is controlled by the fungal silencing machinery. *PLoS Pathogens*, 12, e1005901.

Kohler, A., Kuo, A., Nagy, L.G., Morin, E., Barry, K.W., Buscot, F. et al. (2015) Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists. *Nature Genetics*, 47, 410–415.

Kou, Y., Tan, Y.H., Ramanujam, R. & Naqvi, N.I. (2017) Structure–function analyses of the Pth11 receptor reveal an important role for CFEM motif and redox regulation in rice blast. *New Phytologist*, 214, 330–342.

Kunova, A., Pizzatti, C., Saracchi, M., Pasquali, M. & Cortesi, P. (2021) Grapevine powdery mildew: fungicides for its management and advances in molecular detection of markers associated with resistance. *Microorganisms*, 9, 1541.

Kweon, Y., Fang, M., Shin, S.Y., Lee, D., Kim, K.H. & Shin, C. (2022) Sequence optimization and multiple gene-targeting improve the inhibitory efficacy of exogenous double-stranded RNA against pepper mottle virus in *Nicotiana benthamiana*. *Applied Biological Chemistry*, 65, 87.

Li, X., Liu, Y., He, Q., Li, S., Liu, W., Lin, C. et al. (2020) A candidate secreted effector protein of rubber tree powdery mildew fungus contributes to infection by regulating plant ABA biosynthesis. *Frontiers in Microbiology*, 11, 387.

Lo Presti, L., Lanver, D., Schweizer, G., Tanaka, S., Liang, L., Tollot, M. et al. (2015) Fungal effectors and plant susceptibility. *Annual Review of Plant Biology*, 66, 513–545.

Love, M.I., Huber, W. & Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15, 550.

Ma, Y., Szostkiewicz, I., Korte, A., Moes, D., Yang, Y., Christmann, A. et al. (2009) Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science*, 324, 1064–1068.

Machado, A.K., Brown, N.A., Urban, M., Kanyuka, K. & Hammond-Kosack, K.E. (2018) RNAi as an emerging approach to control Fusarium head blight disease and mycotoxin contamination in cereals. *Pest Management Science*, 74, 790–799.

Majumdar, R., Rajasekaran, K. & Cary, J.W. (2017) RNA interference (RNAi) as a potential tool for control of mycotoxin contamination in crop plants: concepts and considerations. *Frontiers in Plant Science*, 8, 200.

Martínez-Cruz, J., Romero, D., De Vicente, A. & Pérez-García, A. (2018) Transformation by growth onto agro-infiltrated tissues (TGAT), a simple and efficient alternative for transient transformation of the cucurbit powdery mildew pathogen *Podosphaera xanthii*. *Molecular Plant Pathology*, 19, 2502–2515.

Martino, E., Morin, E., Grelet, G.A., Kuo, A., Kohler, A., Daghino, S. et al. (2018) Comparative genomics and transcriptomics depict ericoid mycorrhizal fungi as versatile saprotrophs and plant mutualists. *New Phytologist*, 217, 1213–1229.

Meister, G. & Tuschl, T. (2004) Mechanisms of gene silencing by double-stranded RNA. *Nature*, 431, 343–349.

Micali, C., Göllner, K., Humphry, M., Consonni, C. & Panstruga, R. (2008) The powdery mildew disease of *Arabidopsis*: a paradigm for the interaction between plants and biotrophic fungi. *The Arabidopsis Book*, 6, e0115.

Miles, T.D., Neill, T.M., Colle, M., Warneke, B., Robinson, G., Stergiopoulos, I. et al. (2021) Allele-specific detection methods for QoI fungicide-resistant *Erysiphe necator* in vineyards. *Plant Disease*, 105, 175–182.

Mistry, J., Chuguransky, S., Williams, L., Qureshi, M., Salazar, G.A., Sonnhammer, E. et al. (2021) Pfam: the protein families database in 2021. *Nucleic Acids Research*, 49, D412–D419.

Müller, M.C., Praz, C.R., Sotiropoulos, A.G., Menardo, F., Kunz, L., Schudel, S. et al. (2019) A chromosome-scale genome assembly reveals a highly dynamic effector repertoire of wheat powdery mildew. *New Phytologist*, 221, 2176–2189.

Nerva, L., Sandrini, M., Gambino, G. & Chitarra, W. (2020) Double-stranded RNAs (dsRNAs) as a sustainable tool against gray mold (*Botrytis cinerea*) in grapevine: effectiveness of different application methods in an open-air environment. *Biomolecules*, 10, 200.

Nguyen, L.N., Dao, T.T., Živković, T., Fehrholz, M., Schäfer, W. & Salomon, S. (2010) Enzymatic properties and expression patterns of five extracellular lipases of *Fusarium graminearum in vitro*. *Enzyme and Microbial Technology*, 46, 479–486.

Nguyen, N.L. (2008) *Importance of secreted lipases for virulence of the phytopathogenic fungus Fusarium graminearum*. PhD thesis. Hamburg: University of Hamburg.

Pandey, S., Nelson, D.C. & Assmann, S.M. (2009) Two novel GPCR-type G proteins are abscisic acid receptors in *Arabidopsis*. *Cell*, 136, 136–148.

Park, S.Y., Fung, P., Nishimura, N., Jensen, D.R., Fujii, H., Zhao, Y. et al. (2009) Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science*, 324, 1068–1071.

Pliego, C., Nowara, D., Bonciani, G., Gheorghe, D.M., Xu, R., Surana, P. et al. (2013) Host-induced gene silencing in barley powdery mildew reveals a class of ribonuclease-like effectors. *Molecular Plant-Microbe Interactions*, 26, 633–642.

Poirier, Y., Antonenkov, V.D., Glumoff, T. & Hiltunen, J.K. (2006) Peroxisomal beta-oxidation—a metabolic pathway with multiple functions. *Biochimica et Biophysica Acta*, 1763, 1413–1426.

Qiao, L., Lan, C., Capriotti, L., Ah-Fong, A., Nino Sanchez, J., Hamby, R. et al. (2021) Spray-induced gene silencing for disease control is dependent on the efficiency of pathogen RNA uptake. *Plant Biotechnology Journal*, 19, 1756–1768.

Raanan, R., Gunier, R.B., Balmes, J.R., Beltran, A.J., Harley, K.G., Bradman, A. et al. (2017) Elemental sulfur use and associations with pediatric lung function and respiratory symptoms in an agricultural community (California, USA). *Environmental Health Perspectives*, 125, 087007.

Reuber, T.L., Plotnikova, J.M., Dewdney, J., Rogers, E.E., Wood, W. & Ausubel, F.M. (1998) Correlation of defense gene induction defects with powdery mildew susceptibility in *Arabidopsis* enhanced disease susceptibility mutants. *The Plant Journal*, 16, 473–485.

Reynolds, A., Leake, D., Boese, Q., Scaringe, S., Marshall, W.S. & Khvorova, A. (2004) Rational siRNA design for RNA interference. *Nature Biotechnology*, 22, 326–330.

Ronald, P. & Joe, A. (2018) Molecular mimicry modulates plant host responses to pathogens. *Annals of Botany*, 121, 17–23.

Ruiz-Jiménez, L., Polonio, Á., Vielba-Fernández, A., Pérez-García, A. & Fernández-Ortuño, D. (2021) Gene mining for conserved, non-annotated proteins of *Podosphaera xanthii* identifies novel target candidates for controlling powdery mildews by spray-induced gene silencing. *Journal of Fungi*, 7, 735.

Sambucci, O., Alston, J.M. & Fuller, K.B. (2014) The costs of powdery mildew management in grapes and the value of resistant varieties: evidence from California. In: *Robert Mondavi Institute Center for Wine Economics, CWE Working Paper Number 1402*, pp. 1–51.

Schmidt, S.M., Kuhn, H., Micali, C., Liller, C., Kwaaitaal, M. & Panstruga, R. (2014) Interaction of a *Blumeria graminis* f. sp. *hordei* effector candidate with a barley ARF-GAP suggests that host vesicle trafficking is a fungal pathogenicity target. *Molecular Plant Pathology*, 15, 535–549.

Scorza, R., Callahan, A., Dardick, C., Ravelonandro, M., Polak, J., Malinowski, T. et al. (2013) Genetic engineering of *Plum pox virus* resistance: 'HoneySweet' plum—from concept to product. *Plant Cell, Tissue and Organ Culture*, 115, 1–12.

Spanu, P.D. (2012) The genomics of obligate (and nonobligate) biotrophs. *Annual Review of Phytopathology*, 50, 91–109.

Spence, C.A., Lakshmanan, V., Donofrio, N. & Bais, H.P. (2015) Crucial roles of abscisic acid biogenesis in virulence of rice blast fungus *Magnaporthe oryzae*. *Frontiers in Plant Science*, 6, 1082.

Staats, M. & van Kan, J.A. (2012) Genome update of *Botrytis cinerea* strains B05.10 and T4. *Eukaryotic Cell*, 11, 1413–1414.

Subramoni, S., Suárez-Moreno, Z.R. & Venturi, V. (2010) Lipases as pathogenicity factors of plant pathogens. In: Timmis, K.N. (Ed.) *Handbook of hydrocarbon and lipid microbiology*. Berlin: Springer, pp. 3269–3277.

Takino, J., Kozaki, T., Ozaki, T., Liu, C., Minami, A. & Oikawa, H. (2019) Elucidation of biosynthetic pathway of a plant hormone abscisic acid in phytopathogenic fungi. *Bioscience, Biotechnology, and Biochemistry*, 83, 1642–1649.

Vielba-Fernández, A., Polonio, Á., Ruiz-Jiménez, L., de Vicente, A., Pérez-García, A. & Fernández-Ortuño, D. (2020) Fungicide resistance in powdery mildew fungi. *Microorganisms*, 8, 1431.

Wang, M., Weiberg, A., Lin, F.M., Thomma, B.P., Huang, H.D. & Jin, H. (2016) Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection. *Nature Plants*, 2(16), 151.

Weßling, R., Epple, P., Altmann, S., He, Y., Yang, L., Henz, S.R. et al. (2014) Convergent targeting of a common host protein-network by pathogen effectors from three kingdoms of life. *Cell Host & Microbe*, 16, 364–375.

Wicker, T., Oberhaensli, S., Parlange, F., Buchmann, J.P., Shatalina, M., Roffler, S. et al. (2013) The wheat powdery mildew genome shows the unique evolution of an obligate biotroph. *Nature Genetics*, 45, 1092–1096.

Wisecaver, J.H. & Rokas, A. (2015) Fungal metabolic gene clusters–caravans traveling across genomes and environments. *Frontiers in Microbiology*, 6, 161.

Wu, Y., Ma, X., Pan, Z., Kale, S.D., Song, Y., King, H. et al. (2018) Comparative genome analyses reveal sequence features reflecting distinct modes of host-adaptation between dicot and monocot powdery mildew. *BMC Genomics*, 19, 705.

Xiong, F., Liu, M., Zhuo, F., Yin, H., Deng, K., Feng, S. et al. (2019) Host-induced gene silencing of *BcTOR* in *Botrytis cinerea* enhances plant resistance to grey mould. *Molecular Plant Pathology*, 20, 1722–1739.

Zhu, W., Wei, W., Wu, Y., Zhou, Y., Peng, F., Zhang, S. et al. (2017) BcCFEM1, a CFEM domain-containing protein with putative GPI-anchored site, is involved in pathogenicity, conidial production, and stress tolerance in *Botrytis cinerea*. *Frontiers in Microbiology*, 8, 1807.

Zotti, M., Dos Santos, E.A., Cagliari, D., Christiaens, O., Taning, C. & Smagghe, G. (2018) RNA interference technology in crop protection against arthropod pests, pathogens and nematodes. *Pest Management Science*, 74, 1239–1250.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: McRae, A.G., Taneja, J., Yee, K., Shi, X., Haridas, S., LaButti, K. et al. (2023) Spray-induced gene silencing to identify powdery mildew gene targets and processes for powdery mildew control. *Molecular Plant Pathology*, 24, 1168–1183. Available from: https://doi.org/10.1111/mpp.13361