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February 16, 2023

Dear NSF Staff:

This letter is declare that the PI of this proposal, Prof. John M. McDowell, served as a Rotating Program Officer in Integrative Organismal Systems from Sept. 16, 2021 until Sept. 15, 2022.

Therefore, per the PAPPG Chapter II.1.f (page II-7, 23-1) we designate a co-PI on this proposal as the substitute negotiator on Prof. McDowell's behalf. This individual is Sherif Sherif, associate professor at Virginia Tech.

Thank you for enacting this request.

Sincerely,

for

Trudy M. Riley

Associate Vice President for Research and Innovation, Sponsored Programs
Virginia Polytechnic Institute and State University

COVER SHEET FOR PROPOSAL TO THE NATIONAL SCIENCE FOUNDATION

PROGRAM ANNOUNCEMENT/SOLICITATION NO./ DUE DATE
NSF 21-546 02/16/2023

☐ Special Exception to Deadline Date Policy

FOR NSF USE ONLY
NSF PROPOSAL NUMBER
2319757

FOR CONSIDERATION BY NSF ORGANIZATION UNIT(S) (Indicate the most specific unit known, i.e. program, division, etc.)
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546001805

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TITLE OF PROPOSED PROJECT
EDGE FGT: RNAi-based tools to unlock functional genomics of obligate oomycete plant pathogens

SHOW LETTER OF INTENT ID IF APPLICABLE

REQUESTED AMOUNT	PROPOSED DURATION (1-60 MONTHS)	REQUESTED STARTING DATE	SHOW RELATED PRELIMINARY PROPOSAL NO. IF APPLICABLE
$ 400,000	36 months	08/20/2023	

THIS PROPOSAL INCLUDES ANY OF THE ITEMS LISTED BELOW

☒ TYPE OF PROPOSAL **Research**
☒ COLLABORATIVE STATUS **Collaborative from one organization**
☐ BEGINNING INVESTIGATOR
☐ DISCLOSURE OF LOBBYING ACTIVITIES
☐ PROPRIETARY & PRIVILEGED INFORMATION
☐ HISTORIC PLACES
☐ LIVE VERTEBRATE ANIMALS IACUC App. Date
PHS AnimalWelfareAssurance Number

☐ HUMAN SUBJECTS Human Subjects Assurance Number
Exemption Subsection or IRB App. Date
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☐ POTENTIAL LIFE SCIENCES DUAL USE RESEARCH OF CONCERN
☐ OFF-CAMPUS OR OFF-SITE RESEARCH

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CO-PI/PD				
CO-PI/PD				

CERTIFICATION PAGE

Certification for Authorized Organizational Representative(or Equivalent)

By electronically signing and submitting this proposal, the Authorized Organizational Representative(AOR) is:(1)certifying that statements made here in are true and complete to the best of the individual's knowledge; and(2)agreeing to accept the obligation to comply with NSF award terms and conditions if an award is made as a result of this proposal. Further, the proposer is hereby providing certifications regarding conflict of interest, flood hazard insurance, responsible and ethical conduct of research, organizational support,and safe and inclusive working environments for off-campus or off-site research, as set forth in the NSF Proposal & Award Policies & Procedures Guide(PAPPG).Willful provision of false information in this application and its supporting documents or in reports required under an ensuing award is a criminal offense(U.S.Code,Title 18,Section §1001).

Certification Regarding Conflict of Interest

The AOR is required to complete certifications stating that the organization has implemented and is enforcing a written policy on conflicts of interest (COI), consistent with the provisions of PAPPG Chapter IX.A; and that, to the best of the individual's knowledge, all financial disclosures required by the conflict of interest policy were made; and that conflicts of interest, if any, were, or prior to the organization's expenditure of any funds under the award, will be, satisfactorily managed, reduced or eliminated in accordance with the organization's conflict of interest policy. Conflicts that cannot be satisfactorily managed, reduced or eliminated and research that proceeds without the imposition of conditions or restrictions when a conflict of interest exists, must be disclosed to NSF via use of the Notifications and Requests module with Research.gov

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Two sections of the National Flood Insurance Act of 1968 (42 USC §4012a and §4106) bar Federal agencies from giving financial assistance for acquisition or construction purposes in any area identified by the Federal Emergency Management Agency (FEMA) as having special flood hazards unless the:

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Certification Regarding Responsible and Ethical Conduct of Research (RECR)

(This Certification applies to proposals submitted prior to July 31, 2023, and is not applicable to proposals for conferences, symposia, and workshops.)

By electronically signing the Certification Pages, the Authorized Organizational Representative is certifying that, in accordance with the NSF Proposal & Award Policies & Procedures Guide, Chapter IX.B., the institution has a plan in place to provide appropriate training and oversight in the responsible and ethical conduct of research to undergraduates, graduate students and postdoctoral researchers who will be supported by NSF to conduct research. The AOR shall require that the language of this certification be included in any award documents for all subawards at all tiers.

Certification Regarding Responsible and Ethical Conduct of Research (RECR)

(This Certification applies to proposals submitted on or after July 31, 2023, and is not applicable to proposals for conferences, symposia, and workshops.)

By electronically signing the Certification Pages, the Authorized Organizational Representative is certifying that, in accordance with the NSF Proposal & Award Policies and Procedures Guide, Chapter IX.B., the institution has a plan in place to provide appropriate training and oversight in the responsible and ethical conduct of research to undergraduate students, graduate students, postdoctoral researchers, faculty, and other senior personnel who will be supported by NSF to conduct research. As required by Section 7009 of the America Creating Opportunities to Meaningfully Promote Excellence in Technology, Education, and Science (COMPETES) Act (42 USC 1862o–1), as amended, the training addresses mentor training and mentorship.The AOR shall require that the language of this certification be included in any award documents for all subawards at all tiers.

Certification Regarding Organizational Support

By electronically signing the Certification Pages, the Authorized Organizational Representative (or equivalent) is certifying that there is organizational support for the proposal as required by Section 526 of the America COMPETES Reauthorization Act of 2010. This support extends to the portion of the proposal developed to satisfy the Broader Impacts Review Criterion as well as the Intellectual Merit Review Criterion, and any additional review criteria specified in the solicitation. Organizational support will be made available, as described in the proposal, in order to address the broader impacts and intellectual merit activities to be undertaken.

Certification Regarding Dual Use Research of Concern

By electronically signing the certification pages, the Authorized Organizational Representative is certifying that the organization will be or is in compliance with all aspects of the United States Government Policy for Institutional Oversight of Life Sciences Dual Use Research of Concern.

Certification Requirement Specified in the William M.(Mac)Thornberry National Defense Authorization Act for Fiscal Year 2021, Section 223(a)(1) (42 USC 6605(a)(1))

By electronically signing the Certification Pages, the Authorized Organizational Representative is certifying that each individual employed by the organization and identified on the proposal as senior personnel has been made aware of the certification requirements identified in the William M.(Mac) Thornberry National Defense Authorization Act for Fiscal Year 2021, Section 223(a)(1) (42 USC 6605(a)(1)).

Certification Regarding Safe and Inclusive Working Environments for Off-Campus or Off-Site Research

(This certification applies only to proposals in which data/information/samples are being collected off-campus or off-site, such as fieldwork and research activities on vessels and aircraft.)

By electronically signing the Certification Pages, the Authorized Organizational Representative is certifying that, in accordance with the NSF Proposal & Award Policies and Procedures Guide, Chapter II.E.9, the organization has a plan in place **for this proposal** regarding safe and inclusive working environments.

AUTHORIZED ORGANIZATIONAL REPRESENTATIVE	SIGNATURE	DATE
NAME **Jane Lee**	**Electronic Signature**	**Feb 16 2023 02:56 PM**

TELEPHONE NUMBER	EMAIL ADDRESS	FAX NUMBER
540-231-6653	**janelee@vt.edu**	

Project Summary

Overview

This proposal addresses two problems: First, downy mildew pathogens of plants are currently inaccessible to functional genomics approaches, despite their interesting obligate life strategies, their importance as models for plant-pathogen interactions, and their impact as crop parasites. This is because of their obligate lifestyle, which precludes conventional genetic transformation. Second, the success of downy mildews as crop pathogens is because they can easily evolve resistance to fungicides and overcome genetic resistance (i.e., immune responses) in their plant hosts. RNA-based biofungicides show promise for disease control, but end-users and consumers lack awareness of this new approach.

Given these problems, the goals of this proposal are to (1) develop new tools for functional genomics of diverse downy mildew species; (2) disseminate these tools and promote their application for disease control in agricultural settings; (3) engage diverse audiences to build awareness of RNA biofungicides and (4) leverage the distinctive aspects of this project for the benefit of our trainees.

Our approach builds on two recent breakthroughs from the PIs of this project. First, RNA interference (RNAi) can be triggered against specific downy mildew genes simply by mixing short, synthetic, double-stranded RNAs (SS-dsRNAs) in suspensions of downy mildew spores. Treated spores can then be analyzed in isolation or inoculated onto plants to assess effects on interaction with the plant host. This approach is surprisingly simple but also prohibitively expensive for large-scale functional genomics and for applications in the field, due to costs of dsRNA synthesis. Moreover, this approach needs optimization (e.g., protection of dsRNA from environmental degradation) and generalization to downy mildew species that capture the diversity of this group's biology and impacts on crop production. The second breakthrough is the development of a process for one-step production and encapsulation of double-stranded RNA in anucleate "mini-cells" of *E. coli*. The resultant minicell-encapsulated dsRNAs (ME-dsRNAs) are protected from environmental degradation, can be shelved for long periods of time, and provide effective resistance to fungal pathogens when applied as a spray to plants, including harvested fruit. This protection is an example of dsRNA-based "Spray-Induced Gene Silencing" (SIGS) which has shown promise as a tool for research and control of diseases caused by viruses, fungi, nematodes and insects. However, neither ME-dsRNAs nor SIGS in general have been tested on downy mildews.

Therefore, the specific aims of this proposal are to (1) Develop low-cost procedures for production of "naked" and ME-dsRNA in *E. coli*, and test the RNAi efficacy of these formulations compared to SS-dsRNA; (2) Develop protocols for SIGS against downy mildews, based on treatment of leaves and seeds and guided by results from spore treatments in Aim 1; (3) Test the protocols from Aims 1 and 2 for efficacy against diverse downy mildews of crops, and optimize as necessary.

Intellectual merit

Downy mildew pathogens comprise a large group of oomycete species that parasitize plants in natural ecosystems and cause important crop diseases. Because downy mildews cannot be cultured apart from their hosts, reverse genetic and functional genomic studies have been impossible until now. This proposal builds upon the above breakthroughs to enable low-cost, high throughput functional genomic screens. Aims 1 and 2 are designed to provide complementary functional genomics tools, while Aim 3 generalizes these tools for broad applicability to diverse downy mildews and potential utility against other oomycete species. This will open up previously inaccessible avenues of fundamental research on genotype-phenotype relationships that will dramatically enhance understanding of downy mildew-plant interactions, at scales ranging from molecular to evolutionary.

Broader Impacts

In addition to enabling functional genomics, all three aims are designed to lay the groundwork for new applications of SIGS for control of downy mildew and other crop diseases. The project will also disseminate our protocols to the research community and will engage growers and the general public to understand the potential strengths and limitations of SIGS for plant disease control, framed in the topical context of "control of plant diseases using RNA vaccines". The project will provide an eclectic training experience for postdoctoral scholars and undergraduate researchers. Broadening participation, in every dimension, will be central to our outreach and training efforts.

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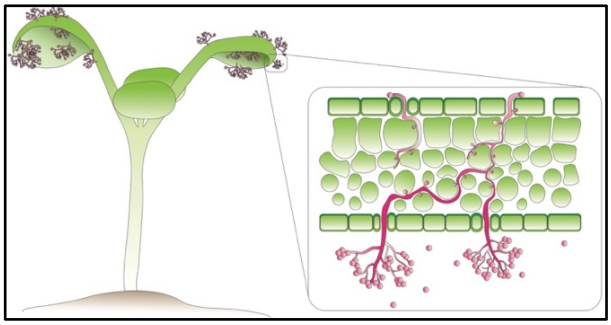
For font size and page formatting specifications, see PAPPG section II.B.2.

*Proposers may select any numbering mechanism for the proposal. The entire proposal however, must be paginated. Complete both columns only if the proposal is numbered consecutively.

Background

Introduction to downy mildews: Downy mildews comprise over 700 species that have been documented as plant pathogens (Tör et al., 2023). Downy mildews infect aerial parts of their plant hosts and are generally specific to one host species (Lucas et al., 1995). The life cycle of downy mildews is typified by production of fruiting bodies called conidiophores that lend a downy appearance to the leaves from which they are produced. Downy mildews belong to the oomycetes, a large group of filamentous microbes that resemble fungi but reside within the Chromalveolata (Beakes et al., 2012; Thines, 2014). Therefore, plant-pathogenic oomycetes and fungi have evolved convergently to inhabit plant hosts. Oomycetes and fungi share many morphological similarities (e.g., filamentous vegetative structures, assimilative growth, reproduction via spores) but are separated by many differences (e.g., diploid vegetative nuclei, cell walls that are comprised primarily of cellulose rather than chitin) (Latijnhouwers et al., 2003).

Most of the oomycetes that parasitize plants reside within the downy mildew group or within the closely-related *Phytophthora* genus (Agrios, 2005; Thines, 2014). Indeed, recent genome-based phylogenies predict place downy mildews within two, independently evolved clades in the *Phytophthora* genus (Tör et al., 2023). Many of the experiments in this proposal employ *Hyaloperonospora arabidopsidis* (hereafter *Hpa*), which is a frequently-occurring pathogen in natural populations of the model plant *Arabidopsis thaliana* (McDowell, 2014). Because *Hpa* is the only naturally-occurring oomycete pathogen of Arabidopsis, it has been adopted as a reference organism for genomic and molecular aspects of plant-downy mildew interactions (John et al., 2019). Other species relevant to this proposal cause destructive diseases of pea (*Peronospora viciae*, hereafter *Pvp*), grape (*Plasmopara viticola, Pvc*), lettuce (*Bremia lactucae, Bl*), and cucumber (*Pseudoperonospora cubensis, Pcub*) (Tör et al., 2023).



Life cycle of *Hpa* on Arabidopsis. Spores are dispersed by wind or rain to a host plant. The spore germinates and produces an infection hypha that typically penetrates between epidermal cells. Filamentous hyphae grow in the intracellular spaces in the mesophyll, branch frequently, and establish feeding structures called haustoria. Hyphal tips emerge from stomata and differentiate into spore-bearing conidiophores. The full disease cycle is 5-7 days.

Downy mildews as exemplars of obligate biotrophy: Downy mildews share two notable life strategies: First, downy mildews are very host-specific: each downy mildew species typically infects only one host plant species (Lucas et al., 1995). Second, downy mildews are obligate biotrophs, meaning that they extract nutrients exclusively from living plant cells and cannot grow apart from their hosts (Holub and Beynon, 1996; Coates and Beynon, 2010). Analysis of downy mildew genomes, in comparison with genomes of non-obligate relatives in *Phytophthora* and other oomycete genera, has revealed several putative signatures of obligate biotrophy (Baxter et al., 2010b). These include a reduced arsenal of genes encoding proteins that support virulence but can be recognized by host plants (“evolution for stealth”), deployment of secreted virulence proteins (called “effectors”) that enter plant cells to suppress immune responses, and gene losses in metabolic pathways that hint at metabolic dependencies on plants (McDowell, 2011). Similar signatures are apparent in fungi that have independently evolved to obligate biotrophy, suggesting that such signatures represent rules of life for obligate plant pathogens (Baxter et al., 2010a).

Functional genomics of downy mildews has been stifled by their obligate lifestyle: In contrast to the progress achieved with descriptive genomics of downy mildews, functional genomic approaches have been inhibited by the inability to culture downy mildews on synthetic media (Tör et al., 2023). This precludes genetic transformation based-tools that have been so useful for genetic and functional genomic analyses of other microbes. Many attempts have been made by the PIs and others to transform plant-grown material, but only transient transformation has been achieved (John et al., 2019; Tör et al., 2023). Alternative approaches have been developed to assay secreted effector function *in planta* including

delivery of predicted effectors from bacteria to plant cells via bacterial secretion [6, 12], and creation of stably transformed plants expressing effector genes under control of plant promoters [3]. These methods have been used by the PIs and others for useful insights into effector protein function inside plant cells. However, these methods strip the effector away from the pathogen where the expression level of a gene may not be comparable to that in the native background. Moreover, single-gene assays do not accurately capture gene function in the native milieu. Finally, these approaches are only applicable to secreted effector proteins that operate inside host cells (Tör et al., 2023). Thus, >95 % of downy mildew gene space is currently inaccessible to reverse genetics and functional genomics. Our approach breaks this barrier by applying double-stranded RNAs (dsRNA) to trigger RNA interference (RNAi) and its attendant silencing of gene activity (Bilir et al., 2019).

Overview of RNAi and its role in plant-pathogen interactions: RNA interference (RNAi) is a post-transcriptional gene regulation mechanism that is conserved across eukaryotes. Dicer proteins process double-stranded RNA (dsRNA) molecules into small-interfering RNAs (siRNAs) that bind to and initiate the degradation of target mRNAs through the cellular machinery of the gene silencing complex (i.e., RISC). siRNAs can also interfere with mRNA translation and induce modification of chromatin structure. The RNAi mechanisms are fully functional in plants, which have the capacity for amplification and transmission of RNAi throughout the plant. The same holds true for plant-pathogenic fungi and oomycetes. Moreover, plants and these pathogens have recently been shown to exchange small RNAs (Cai et al., 2021a). Initially, this was thought to occur by uptake of secretory vesicles that contain siRNAs, but recent findings suggest alternative potential mechanisms (He et al., 2021a; Karimi et al., 2022). Irrespective of the mechanism(s), this “cross-kingdom RNA transport” is sufficient to trigger gene silencing, such that pathogens deploy mobile RNAs to interfere with host gene expression, and vice-versa. This is a relatively new and fast-moving research topic within the plant biotic interaction field, with huge promise to reveal important molecular strategies for pathogen virulence and plant resistance (Huang et al., 2019).

RNAi as a tool for experimental manipulation of plant-microbe interactions: Plant pathogens’ capacity to take up RNA from the environment inspired development of sRNA-based tools to experimentally knock down gene expression (Wang et al., 2017). This approach was first attempted through “host-induced gene silencing” (HIGS) in which plants are transformed with transgenes that produce double-stranded RNAs to target pathogen genes (Koch and Wassenegger, 2021; Karimi and Innes, 2022). RNAs from these transgenes are predicted to move from plant cells to pathogen cells, within which RNAi is induced and amplified, resulting in silencing of the targeted pathogen gene. This approach has been successful in some cases. One example is provided by transgenic lettuce plants that were engineered to express inverted repeats of fragments of two genes from the lettuce downy mildew pathogen *Bremia lactucae:* One targeted gene encodes Cellulose Synthase (this gene is also a major target of the experiments in this proposal). The second targeted gene was HAM34 (Highly Abundant Message #34) (Govindarajulu et al., 2015). Both of the cognate lettuce dsRNA transgenes effectively reduced pathogen growth to 3-10% of the biomass compared to control samples, as measured by qPCR. In infected leaves, mRNA levels of the targeted pathogen genes were reduced to <1% of levels in controls (Govindarajulu et al., 2015).

These results illustrate the potential of sRNA-based strategies for manipulating gene expression in downy mildews. However, this result has not been broadly generalized to other downy mildews. Moreover, HIGS-based approaches against fungi have met with inconsistent success, for reasons that aren’t well understood (Karimi and Innes, 2022). Another drawback of HIGS is its dependence on transgenes: genetic transformation and tissue culture methods are laborious, expensive, and accessible for a limited number of plant species. From a translational perspective, the use of transgenes in agricultural applications adds huge expense in deregulation and still faces uncertainty (at best) from the general public (Taning et al., 2020; Rank and Koch, 2021; Taning et al., 2021).

SIGS as a high-throughput, non-transgenic alternative to HIGS: A major focus of this proposal is SIGS: spray-induced gene silencing based on exogenous application of double-stranded RNA (Cai et al., 2021b). In its simplest form, synthetic dsRNA is sprayed onto a plant organ, followed by artificial inoculation of the pathogen (lab setting) or natural infections (agricultural settings). SIGS is a promising alterative to HIGS because it is non-transgenic and potentially friendly to the environment ((Parker et al.,

2019; Bachman et al., 2020). SIGS can be tailored specifically to the disease problems at hand, with low risk of off-target effects, via the well-understood rules of Watson-Crick base pairing. Accordingly, dsRNAs can be re-designed to account for divergence in target sequences and can be multiplexed to target multiple genes/pathways in the organism of interest and/or to target multiple pathogens from a single formulation. In these respects, SIGS is reminiscent of RNA-based vaccines against viral diseases of humans (this analogy is leveraged in our outreach activities, Broader Impacts). SIGS is also potentially applicable to long-lived species in the field (e.g., trees) for which transgenic approaches are impractical. SIGS also has utility for field-based trials to address fundamental research questions; for example, testing importance of particular effectors for pathogen virulence under real-world field conditions.

From a societal perspective, there are indications that consumers could be more accepting of SIGS compared to transgenes used for pest control (Shew et al., 2017). Considerable private sector interest exists for developing SIGS products with application to agricultural diseases (Mat Jalaluddin et al., 2019).

Many of the initial trials of SIGS have shown promising results under controlled environments (see (Koch et al., 2016; Wang et al., 2016; Cai et al., 2018; He et al., 2021b) for examples), including against the non downy mildew oomycete *Phytophthora infestans* (Kostov et al., 2022). One important insight from these studies is that gene silencing can be triggered when pathogens take up exogenous dsRNA directly from the surface of the infected plant organ (Wang et al., 2016). Additionally, studies suggests that sprayed dsRNA can be taken up by plant cells, which can then activate the canonical pathways for systemic RNAi (Bilir et al., 2022). The resulting siRNAs can then be transmitted to the pathogen to induce silencing. However, the mechanistic bases of SIGS have not been fully elaborated. Moreover, studies on SIGS have also highlighted potential bottlenecks that require optimization for SIGS to reach its full potential (Wang and Dean, 2020; Rank and Koch, 2021). For example, dsRNA is vulnerable to degradation by environmental factors (UV, pH, heat, microbiome); therefore, simple formulations of dsRNA in buffer display limited efficacy and longevity (Christiaens et al., 2018). Moreover, pathogens vary in their capacity to take up exogenous dsRNA. This has not been tested for downy mildew (see Aim 1b) but closely-related *Phytophthora* species have displayed limited uptake of naked dsRNA in buffer (Qiao et al., 2021).

These limiters are being address by developing dsRNA formulations designed to enhance uptake and provide protection, for example chitin fragments, carbon dots, and clay nanosheets (Christiaens et al., 2018; Kostov et al., 2022; Nino-Sanchez et al., 2022). These compounds have shown potential in studies against fungi and/or insects, but none have yet been tested against oomycetes. In this context, a distinctive aspect of this proposal is that we will test a new system for one-step production and protective encapsulation of dsRNA (Islam et al., 2021), described in the next section.

Preliminary Data

Activation of RNAi via application of dsRNA to *Hpa* spores

Recently, the Tör group discovered that application of single- or double-stranded RNA to spores can silence the *Hpa-CesA3* gene and inhibit sporulation of *Hpa*. These results have been published (Bilir et al., 2019) and are summarized below with reference to key figures from the publication (Figs. 1-4). We also summarize unpublished results (Figs. 5-6), including evidence that SS-dsRNAs are effective against pea and lettuce downy mildew and when applied as a seed treatment.

Hpa-CesA3 antisense sRNA inhibits infection and germination. We targeted the cellulose synthase gene (*CesA3*, *HpaG810051*) and we designed 25-nt sense and antisense sRNA oligos from the 5' region of the gene that does not have any sequence similarity with other genes in the *Hpa* genome. The sense or antisense sRNAs were mixed with *Hpa* spores at 5, 10 and 20μM concentrations and seven-day old *Arabidopsis* seedlings were drop-inoculated. At 7 days post-inoculation (dpi), *Hpa* sporulation was visibly reduced on plants inoculated with spore suspensions containing 5 and 10 μM antisense sRNA. No sporulation was observed with spore suspensions containing 20 μM antisense sRNA (Fig. 1). There was no inhibition of sporulation with sense sRNA at same concentrations (Bilir et al., 2019).

Trypan Blue staining of leaves at 7 dpi was used to examine the extent of *Hpa* development in Arabidopsis cotyledons (Fig. 2). Although normal pathogen development was observed from control spores, there were no hyphae or pathogen spores on the cotyledons inoculated with the spore

suspension containing 20μM sRNA, demonstrating that suppression of *CesA3* interferes with the earliest stage of *Hpa* infection.

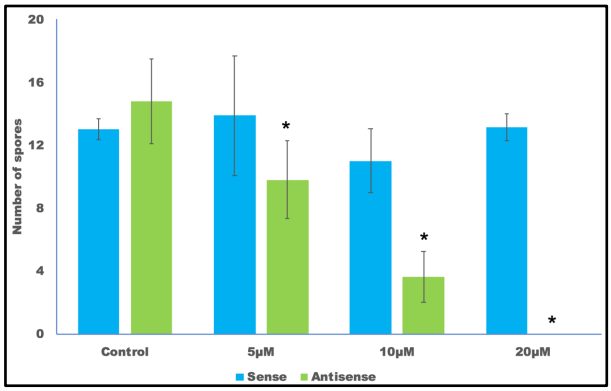


Fig. 1. Antisense, but not sense, sRNA inhibits sporulation. *Arabidopsis* seedlings were drop inoculated with *Hpa*- spores containing sense or antisense sRNA and inoculated into seedlings. After 7 days, spores from 10 seedlings were collected in 250 μL H_2O water and counted. The average and standard error of three replicates are shown.

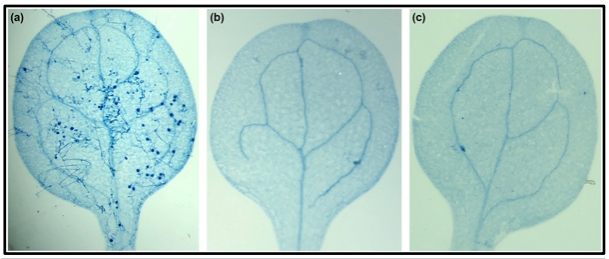


Fig. 2. Antisense, but not sense, sRNA inhibits sporulation. Pathogen inhibition at the infection stage. Arabidopsis seedlings were drop inoculated with Hpa-Emoy2 spores containing 0 or 20 μM antisense sRNA and seedlings were stained with trypan blue at 7 dpi. While there was normal infection with sporulation and oospore development was observed in the control (a), no infection or hyphal development were observed in seedlings inoculated with 20 μM antisense sRNA (b) and (c).

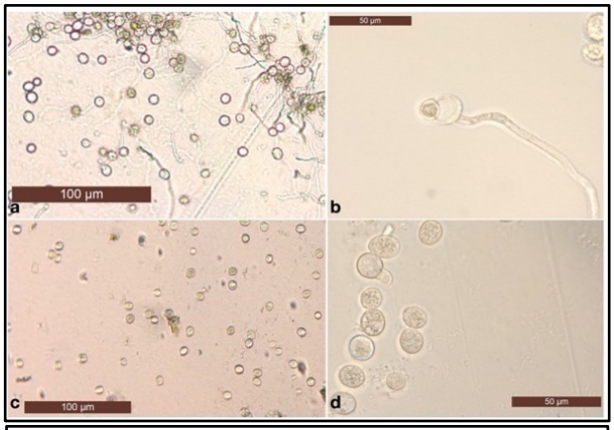


Fig. 3. Germination is inhibited by antisense sRNA targeting the *Hpa-CesA3* gene. *Hpa*-Emoy2 spores containing 0 or 20 μM antisense sRNA were placed on cellophane strips on MS medium and spore germination was examined using a Leica DM5500B light microscope after 48 h. Controls (a) and (b) produced long germination tubes, while spores incubated with 20 μM antisense sRNA (c) did not germinate or the germination tube was arrested (d) within the given time period.

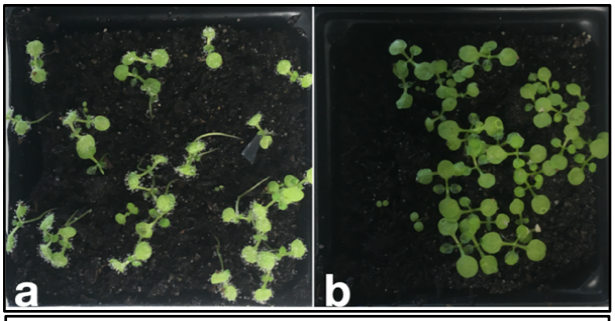


Fig. 4. Application of 1μM SS-dsRNA targeting *Hpa-Tubulin* gene inhibits sporulation. *Hpa* spores were treated with a) control RNA or b) 1μM SS-dsRNA and inoculated onto *Arabidopsis* seedlings. Images taken 7 dpi demonstrate abundant sporulation (a) compared to no sporulation (b)

To directly determine the effect of CesA3 suppression on spore germination, we examined spores mixed with or without antisense sRNA on cellophane strips under a light microscope (Fig. 3). After 48h, untreated control spores were bright and produced germ tubes at various lengths. Contrastingly, spores treated with antisense sRNA became dark brown and germination tubes were mainly absent or, in rare cases, were arrested at 48h. Similar experiments with sense sRNA did not show any inhibition of the germination. The data in Fig. 3 can be quantified as percent germination of treated sample compared to control, and this will be a key assay for the planned experiments. Further investigations with sRNAs revealed that capping of sRNA is essential for suppression of germination and sporulation (Bilir et al., 2019). In addition, we showed the length of sRNA has an impact on the effect of

sRNA. For example, 25-30nt antisense sRNAs worked very well while 24nt did not show silencing (Bilir et al., 2019). We also found ds-sRNA works much better than antisense ss-sRNA (Fig. 4 and (Bilir et al., 2019)). We repeated these experiments with 3 different synthesized sRNAs from Sigma and *in vitro* transcribed sRNAs and obtained similar results (Bilir et al., 2019).

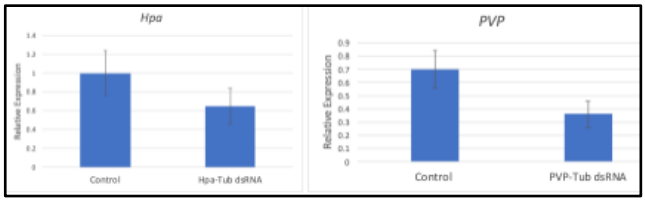


Fig. 5. SS-dsRNA treatment reduces expression of tubulin genes in *Hpa* and *Pvp*.

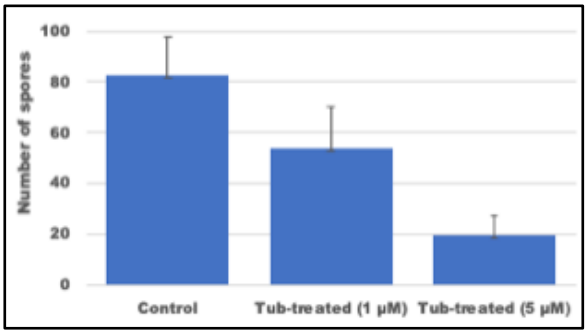


Fig. 6. *Hpa* sporulation is reduced on Arabidopsis seedlings germinated from seeds imbibed with SS-dsRNA targeting tubulin.

SS-dsRNA can also silence other Hpa genes as well as orthologues in Bremia lactucae (Bl) and Peronospora vicia f.sp. pisi (Pvp): To gain information on the properties of sRNA-mediated gene silencing, optimise and test in different downy mildew (DM) pathogens including *Hpa*, *Pvp and Bl,* we targeted tubulin-encoding genes in these DM pathogens and identified the conserved region. We then designed 30-nt long siRNAs against the gene. As a negative control, a siRNA targeting the *Hpa* effector gene *Hpa-HAC1* was used. An *in vitro* spore germination assay was optimised for *Hpa*, *Pvp* and *Bl* was used to test the synthesised siRNAs. All *Hpa, PVP* and *Bl* spore germinations was totally inhibited by siRNAs targeting *Tubulin* gene, similar to results obtained with *Hpa-CesA3* (data not shown). However, siRNA targeting *Hpa-HAC1* did not inhibit spore germination in either of the pathogen. In addition, infection assays showed 1µM ds-siRNA inhibited sporulation on Arabidopsis (Fig. 5). qRT-PCR results supported the germination and infection assays (Fig. 6). Several additional genes have been silenced with this approach with a variety of phenotypes that include reduced or enhanced sporulation and reduced or enhanced virulence (data not shown). However, the degree of silencing varies and mRNA reduction is sometimes modest (e.g., Fig. 5), suggesting a need for additional optimization (Aim 1).

We have also carried out seed imbibement studies with Arabidopsis and pea seeds using naked SS-dsRNA targeting *Tubulin* and challenged seedlings with DM pathogens. There was a clear reduction of *Hpa* sporulation with Arabidopsis (Fig.6), indicating that seed treatments with siRNA could be exploited to protects plants against DM if dsRNA is delivered in a protected manner. We then used the same approach with pea seeds. Although *Tubulin* SS-dsRNA inhibited *Pvp* sporulation, it also reduced plant development (data not shown) presumably due to off-target effect on plant tubulin genes that are highly conserved, suggesting a better siRNA design with a protected delivery method is required (Aim 1).

E. coli mini-cells protect dsRNAs from degradation and enhance their efficacy to trigger RNAi

AgroSpheres has developed a novel bioencapsulation technology that enables the stable delivery of high-payloads of biologicals to agricultural settings. At the core of their technology is the minicell, a spherical anucleate cell that is produced during bacterial fermentation. The minicells do not contain bacterial chromosomal DNA and thus, the EPA and FDA have concluded that the minicell is non-GMO [23]. Co-PI Sherif's group has collaborated with AgroSpheres to develop their technology to demonstrate high-yield production and encapsulation of dsRNA for RNAi applications. These results have been published (Islam et al., 2021) and are summarized below with reference to key figures from the publication (Figs. 7, 9). We also summarize unpublished data (Fig. 8), including evidence that minicells provide dramatically enhanced protection against environmental stressors that degrade dsRNA (Fig. 8)

The Sherif group has created *E. coli* strains with transgenes that express dsRNAs. The minicell-encapsulated dsRNA is protected against environmental stresses such as RNAses (Fig. 7), temperature, and UV (Fig. 8). The dsRNA minicells can be dried down via lyophilization or other methods, stored on the shelf for 24+ months, reconstituted in water and applied to spore suspensions or to plant material.

The mode of action for encapsulated dsRNA has been validated for the minicell-RNAi platform by assessing verifying the knock-down effects on target genes. For example, ME-dsRNAs targeting DICER-like proteins (*DCL1* and *DCL2*) genes of *B. cinerea* can selectively knock down the target genes and cause significant fungal growth inhibition (Islam et al., 2021). In contrast to naked-dsRNA, topical application of ME-dsRNAs prevented disease progression in strawberries for 12 days under greenhouse conditions. Minicell-encapsulated dsRNA (ME-dsRNA) is shielded from RNase degradation and stabilized on strawberry surfaces, enabling the persistence of dsRNA in field- like conditions (Fig. 9). Furthermore, the species-specificity of the ME-dsRNA has been demonstrated by testing the prototype that targets *B. cinerea* against non-target fun\gal species. Minicell-RNAi samples designed to target and interfere with vital *chitin synthase* (*CHS3a, CHS3b1, CHS3b2*) and *dicer* genes (*DCL1, DCL2)* of *B. cinerea* had no inhibitory activity against *Alterneria alternata* or *Penicillium expansum* (Islam et al., 2021). Finally, the technology has even shown efficacy in the field, as it was able to effectively protect grapes in the field against natural infection of gray mold (unpublished).

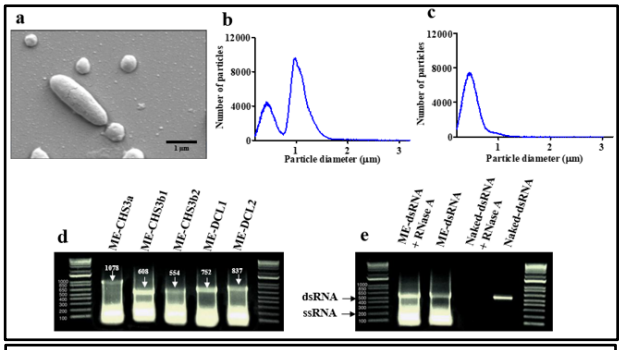


Fig. 7. Characterization, purification, and stability of minicell-encapsulated dsRNAs (ME-dsRNAs). (a) Scanning electron microscopy of bacterial parental and minicells. (b and c) Purification of minicells. In (b) the first peak of the multisizer data represents the minicells and second peak is for *E. coli* cells; In (c); following purification of minicells the *E. Coli* peak disappeared. (d). The sequence length of dsRNAs molecules encapsulated into minicells. (e) Treatment of ME- and naked-dsRNA (CHS3b2) with RNase A.

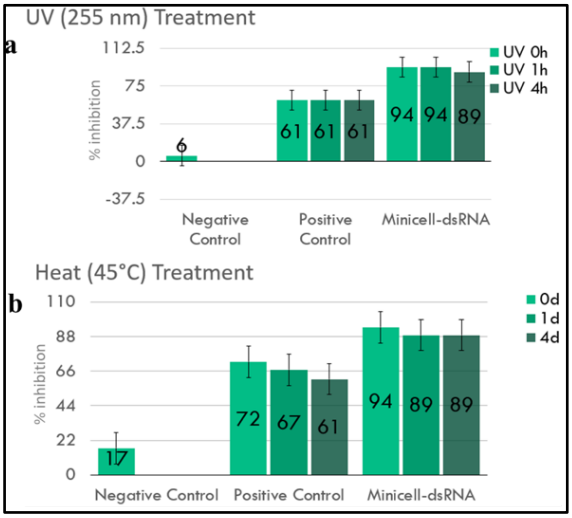


Fig. 8. Minicells stabilize dsRNAs against environmental stressors. ME-dsRNA samples were exposed to (a) UV at 1500mW/cm2, 1.5X exposure compared to typical exposure on a sunny day; (b) 45ºC temperature. The X axis depicts % inhibition of DBM following treatment, compared to an untreated control sample. The Positive Control was from a commercially available sample, formulated for dsRNA stability.

Altogether, these results elucidate the potential of ME-dsRNAs to enable the application of RNAi at low cost and high-throughput for functional genomic screens, comparable to and likely higher in efficacy to naked dsRNAs. Moreover, ME-dsRNAs offer a platform that can readily be translated to large-scale production and deployed as non-plant-incorporated protectant to control a variety of pests and pathogens under field conditions. In other words, this is a viable, scalable, cost-effective solution for commercializing topical RNAi pesticides. This proposal would provide the first data for efficacy of ME-dsRNAs against downy mildews, with broader implications for other applications as summarized in the next section.

Research Community Impact

Downy mildew pathosystems exemplify many important fundamental questions at the nexus of molecular, organismal, and evolutionary aspects of plant-microbial interactions: What is the basis of microbial specificity for particular hosts, and how do these specificities co-evolve along with host immune responses? How do microbes reprogram host cells to suppress immune responses and provide essential nutrients? How is the physiology and metabolism of obligate microbial pathogens intertwined with their hosts, and how are these traits distinct from closely-related, non-obligate species? The plant-biotic interactions research community recognizes the considerable potential of downy mildews to address

these questions, as evidenced by the completion of genome assemblies from 11 downy mildew species and extensive resequencing in some cases (Tör et al., 2023). As noted above, significant insights have been provided from comparative analyses of these assemblies (McDowell, 2011). However, the downy mildew research community's capacity to move beyond descriptive genomics to functional understanding of genomic mechanisms underlying complex multigenic traits has been completely stymied by the obligate lifestyle and the resultant inability to genetically transform these organisms. This proposal builds upon co-PI Tör 's barrier-breaking discovery that induction of RNAi in downy mildews is as simple as adding dsRNA to a spore suspension (Bilir et al., 2019). This observation motivates the experiments in this proposal that are designed to optimize this approach and and provide the downy mildew research community with tools to precisely interrogate genotype-phenotype relationships with unprecedented breath.

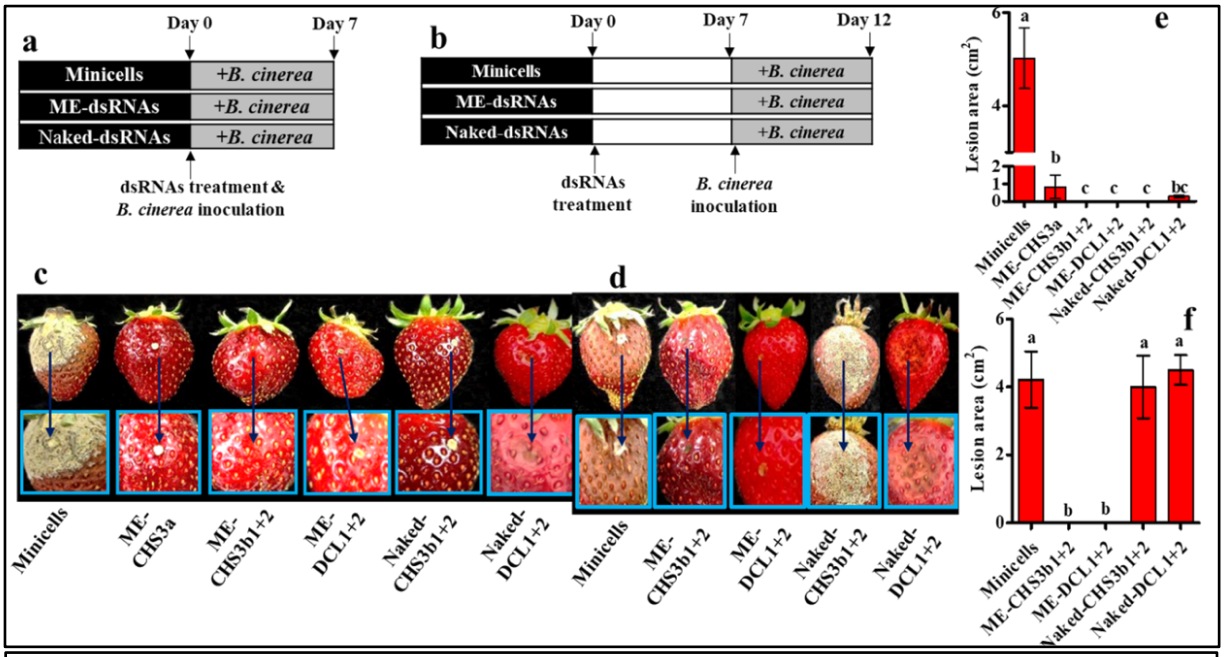


Fig. 9. The effectiveness of topically applied ME-dsRNAs and naked-dsRNAs in inhibiting grey mould diseases under greenhouse conditions. (a) Schematic representation of the time points of ME-dsRNAs and naked-dsRNA applications on strawberry fruit 1 h prior to inoculation, and (b), 7 days prior to *Botrytis fuckeliana* inoculation and the sampling time of the strawberry fruits from the strawberry plants for testing disease severity. (c) and (e). Images showing ME-dsRNAs and naked-dsRNA sprayed onto fruits 1 h prior to *B. fuckeliana* inoculations reducing grey mould disease symptoms and lesion diameter when compared with the application of empty minicells. (d) and (f). Images showing ME-dsRNA sustained protections against grey mould disease compared to naked-dsRNAs and the disease lesion diameter on treated fruits at 12 days post-treatment of ME-dsRNA. Data represent the mean ± SEM. Bars labelled with the different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

One such tool, comprising an important rationale for this proposal, is based on optimization of the *E. coli*-based system for one-step production and encapsulation of dsRNA. This system was pioneered by co-PI Sherif with very promising results against fungal pathogens in laboratory and field settings (e.g., Fig. 9). Although success with fungi does not guarantee success with oomycetes, due to their distinctive biology, successful application of this system to downy mildews would substantially decrease the cost and increase the throughput of sRNA-based functional genomics pipelines. Importantly, our project is designed to produce protocols that are simple, inexpensive, and applicable to most if not all downy mildew species. Looking beyond the downy mildew research community, we anticipate adoption of these protocols for functional genomics of other oomycetes, most importantly for *Phytophthora* species for

which homologous recombination is currently impossible, gene editing technologies are laborious and inconsistently successful, and uptake of naked dsRNA is not robust. Looking even further afield, our proposed application of the *E. coli* platform to SIGS could have broad applicability. In addition to pathogen studies, SIGS is envisioned as a potentially facile tool for control of plant gene expression. However, such efforts have met with inconsistent success, due in large part to inefficient uptake of dsRNA by plant cells. Based on the success of *E. coli* system in SIGS of strawberry and grape, we anticipate that the SIGS protocols from this project will motivate efforts to use SIGS on a range of organisms that extend well beyond the downy mildews. This work also has considerable translational impacts, which are discussed further in the context of Broader Impacts (p. 13-14).

Collaborative relationships

This collaborative proposal brings together a team of three PIs with distinctive, synergistic expertise and a track record of productive collaboration. The PI, John McDowell, has worked on Arabidopsis-*Hpa* interactions since 1995. He co-led the initiative to sequence the first downy mildew genome (*Hpa*) and led the subsequent comparative analysis that resulted in the insights summarized in Background (Baxter et al., 2010b). His current research explores mechanisms through which oomycete pathogens manipulate plant cells to suppress immunity and obtain nutrients. Co-PI Mahmut Tör has also been a long-standing leader in the Arabidopsis-*Hpa* pathosystem. Of relevance to this proposal, he pioneered the sRNA-based approach that this proposal leverages (Bilir et al., 2019). Moreover, he has unique expertise in applying this approach to pea and lettuce downy mildew. Co-PI Sherif has unique expertise in developing the *E. coli* mini-cell system for SIGS (Islam et al., 2021), and was recently funded to investigate the environmental fate of minicell-encapsulated dsRNAs. Tör and McDowell recently published three papers together (Telli et al., 2020; Bilir et al., 2022; Tör et al., 2023) and currently collaborate on a BBSRC-funded grant described below. Tör, McDowell and Sherif have been planning this proposal since summer of 2022 and also collaborated on a Planning Grant proposal to the BBSRC, currently under review.

Experimental Plan

Aim 1-Optimize *in vitro* protocols for triggering RNAi in spores of diverse downy mildew species

Goal and Overview: Our preliminary data demonstrate that sRNA-based silencing can be triggered in three downy mildew species (*Hpa, Pvp* and *Bl*) by application of short, chemically synthesized ds RNAs (SS-dsRNAs, Figs. 1-6) with high-throughput but variable efficiency and high cost, due to the expense of SS-dsRNAs. The goal of this aim is to develop protocols for robust, cost-effective of RNAi via application of dsRNA to suspensions of spores, and to optimize these protocols for applicability to a range of downy mildew species. We will begin by testing whether naked, E. coli-transcribed RNAs (NECT-dsRNAs) are as efficient as SS-dsRNAs, and we will optimize a variety of parameters that could influence the efficacy of gene silencing. We will then compare the efficacy of treatments with naked RNA to the *E. coli*-based system for mini-cell encapsulation of dsRNA (ME-dsRNA). As demonstrated in Preliminary Data (Figs. 7-9), the ME-dsRNA system protects dsRNA against RNAses and environmental stresses and has been applied against fungal plant pathogens with promising results. However, this system has not been tested against downy mildew species. After we have developed protocols that are optimized for *Hpa* and *Pvp*, we will test these protocols on spores from three downy mildew species that have distinctive biological attributes and cause important crop diseases. Ultimately, the experiments in this aim will facilitate functional genomic screens for a broad diversity of downy mildew species; inform the experiments in Aims 2 and 3; and will provide important proof-of-concept data to inform development of RNA fungicides.

Aim 1a: Compare silencing efficacy of SS-dsRNAs to NECT-dsRNAs and optimize length, dose, and target region of NECT-dsRNAs.

Rationale: SS-dsRNAs are sufficient to induce gene silencing in downy mildews, but they are prohibitively expensive for large scale functional genomic screens. NECT-dsRNAs can be produced inexpensively in an *E. coli*-based system (see below) and might be more efficient in triggering RNAi. We will compare the efficiency of silencing *CesA3* using the spore germination phenotype, with key results confirmed by RT-qPCR of *CesA3* transcript abundance. We will also compare NECT-dsRNA to SS-dsRNA on a second, non-essential gene (T814784, see below), using a variety of assays for virulence and gene expression during infection as readouts. The results from these experiments will be compared with assays for uptake of fluorescently labelled dsRNAs (Aim 1b) for insights into the limiting steps for induction of RNAi.

Pathogen-host combinations: All experiments described below will be performed in parallel on two pathosystems: *Hpa* + Arabidopsis and *Pvp* + Pea. As shown in Preliminary Data, SS-dsRNAs efficiently triggers RNAi in *Hpa* and *Pvp*, providing a sound baseline for the comparative experiments in this Aim. Parallel experimentation on two pathogens will provide an immediate indication of whether a single, broadly-applicable workflow for diverse downy mildews is possible and if not, will highlight important parameters for species-specific optimization that will direct the experiments in Aim 3. Because the experiments in this aim are straightforward, the labor invested in parallel studies will be relatively low, therefore the benefits will outweigh the costs.

Genes to be targeted: The optimization experiments will focus on two target genes from each pathogen. The first target gene is *Cellulose synthase A3* (*Hpa-CesA3* and *Pvp-CesA3*), for which SS-dsRNA silencing strongly reduces spore germination and virulence (Preliminary Data). These easily-assayed phenotypes will facilitate rigorous, quantitative comparisons of the various parameters to be tested and optimized in this Aim. The second target gene is *HpaT814784* (homolog of necrosis-inducing proteins) and its homolog in *PVP*. Silencing of this gene with a 30nt SS-RNA produces an interesting phenotype in which the percentage of spore germination is enhanced by approximately two-fold, compared to controls. Thus, by genetic criteria, this gene inhibits spore germination and represents a novel and interesting phenotype for further study. In infection assays, sporulation occurs consistently but at reduced levels compared to controls (unpublished). Considering that *CesA3* is essential for germination and that silenced isolates cannot mount a successful infection, assays on *T814784*-silenced strains will complement assays on *CesA3*-silenced strains for phenotypes that require infection of plants.

Experimental plan: Our first experiment will be to test whether NECT-dsRNAs can trigger silencing in the *CesA3* gene as efficiently as do SS-RNAs. We will produce NECT-dsRNAs using an *E. coli*-based system that co-PI Sherif has used to produce up to 5mg dsRNA/1L culture for several dsRNAs. Because the size of the dsRNA fragment can affect SIGS efficiency (e.g. (Koch et al., 2016)), we will design and PCR-amplify a series of five *CesA3* fragments that encompass the center of the target site of the validated SS-dsRNA and extend outward by 25, 75, 125, 250, and 500 bp on each side, resulting in fragments of 50, 150, 250, 500, and 1000 bp that contain the original SS-dsRNA target site of 25 nt at the center. We will also amplify a GFP fragment to serve as a control for non-specific effects of dsRNA application. These fragments will be inserted into plasmid pL4440, between two oppositely-oriented, IPTG-inducible T7 promoters, such that both strands of the cloned fragment are transcribed and dsRNA is produced. These dsRNA expression vectors will be transformed into E. coli strain “T7 Express HT115”, which is deficient for RNAseIII. The resultant recombinant strains will be cultured, induced with 2mM IPTG for 8 hours, pelleted, and lysed. Total RNA will be collected from the lystate and treated with nucleases to remove DNA and single-stranded RNA. The resultant product will comprise naked, ds-RNA.

We will apply these RNA species to spore suspensions and use the strong spore germination phenotype, resulting from *CesA* silencing (Fig. 3), as a rapid readout for the initial experiment. This phenotype can be assessed qualitatively and quantitatively as percent spore germination compared to the control sample. A concentration range of each NECT-dsRNAs will be compared to the effect of the 25nt SS-dsRNA applied at the previously-optimized concentration of 20 μM.

Our second experiment will focus on gene T814784, taking a similar approach to design, production, and application of a range of 50-1000 nt NECT-dsRNAs, with comparison to the 25nt SS-dsRNA. We will screen for enhanced spore germination. Additionally, we will collect spores after 8 hours of exposure to dsRNA and inoculate them onto seedlings. We will then assess conidiospore production and T814784 mRNA levels in leaves at seven days after inoculation. Key results will be confirmed with trypan blue staining and a DNA-qPCR test that the McDowell lab has developed to quantify *Hpa* vegetative biomass in infected leaves (manuscript in review at *Current Protocols*). A similar PCR assay will be developed to quantify *Pvp* biomass in infected pea leaves.

Predicted results, pitfalls and alternatives: Based on successes with using NECT-dsRNAs to trigger RNAi in fungi, we anticipate that NECT-dsRNAs will prove to be as good or better than SS-dsRNAs at triggering silencing in both genes in *Hpa* and *Pvp*. We also expect that long dsRNAs will be more efficient, up to a point that can only be determined empirically. We recognize that we might have to expand the range of some tested parameters (i.e., dsRNA fragment length, target site, and/or concentration) to optimize the method. Otherwise, we expect no major difficulties because the experiments are conceptually straightforward and rely on techniques that are well-established amongst the PIs. Should the NECT-

dsRNAs not induce RNAi efficiently, we will still be able to compare the efficacy of ME-dsRNAs to SS-dsRNA in Aim 1c.

Aim 1b: Quantify dsRNA internalization

Rationale: From first principles, RNAi begins with internalization of RNA into the targeted cells. This has not yet been directly demonstrated for downy mildew spores. Considering recent evidence that dsRNA uptake in *Phytophthora* spores is weak (Qiao et al., 2021), we feel that it is important to test this parameter and use the information in our optimizations. We will use results from Aim 1a to design experiments that test whether efficiency of silencing correlates with uptake in *Hpa*.

Experimental plan: Based on results from Aim 1a, we will select a minimal set NECT-dsRNAs that represent the full range of silencing efficiencies that we observe. We will then amplify these fragments with primers that incorporate the T7 promoter at the 5' end of both strands. We will transcribe these fragments in vitro using the MEGAscript RNAi Kit (Life Technologies, Carlsbad, CA). The resultant dsRNAs will be labelled with the fluorescein RNA Labeling Mix Kit (Sigma). Five µL of 150 ng/µL labelled dsRNA will be applied to 10 µL of 10^5 *Hpa* spores/mL on cellophane strips. Eight hours later, micrococcal nuclease enzyme will be applied for 30 min. at 37°C to degrade dsRNAs on the spore surface. This material will be transferred to a microscope slide and imaged on a Zeiss LSM 880, sited in the Virginia Tech core imaging facility. Fluorescence will be quantified, normalized to the number of spores, and compared to measures of silencing efficiency from Aim 1a.

Predicted results, pitfalls, and alternatives: Based on observations from (Qiao et al., 2021), we predict a significant correlation between uptake and silencing efficiency. We have not yet established RNA labelling or visualization in our labs, but the labeling protocols are straightforward and well-established, with robust kits and detailed methods papers available (e.g., (Wang et al., 2016; Hamby et al., 2020)). Visualization will be straightforward with resident expertise in the Virginia Tech confocal microscopy core facility. The worst-case outcome that we foresee is the absence of a correlation between uptake and silencing. However, this outcome, perhaps combined with insights from the RNAseq analysis in Aim 1d, might guide us to novel mechanisms of RNAi in downy mildews that could be investigated in a separate proposal (e.g., to the NSF-NIFA Plant-Biotic Interactions program).

Aim 1c: Compare silencing efficacy of "naked" dsRNA and dsRNA protected by *E. coli* minicells.

Rationale: It has been broadly documented that naked dsRNA is transient, and in some cases, inefficient for induction of RNAi. These experiments take advantage of co-PI Sherif's unique expertise with the *E. coli* minicell system. As shown in Fig. 9 and (Islam et al., 2021), Sherif has used this system with promising results against fungi. However, considering the pervasive biological differences between oomycetes and fungi (e.g., very different cell wall compositions, see Background), efficacy of ME-dsRNA in oomycetes is far from assured. We will test the efficiency ME-dsRNA-induced RNAi, using the data from Aim 1a and 1b as a solid baseline for comparison between naked and encapsulated dsRNA.

Experimental Plan: We will create plasmids with the same *CesA3*, *T814784*, and *GFP* fragments used in Aim 1c to represent a range of silencing efficiencies. In this context, the range encompassed by these fragments will provide a useful range to quantify differences, if any, when the same dsRNAs are encapsulated in mini-cells. Plasmids will be transformed into *E. coli* (*minCDE* and *rnc* knockout) and the resultant strains will be used to produce ME-dsRNAs as described in Preliminary Data and (Islam et al., 2021). We will then apply a range of concentrations of ME-dsRNA to *Hpa* and *Pvp* spores and will use the effects of naked EIVT-dsRNA and SS-RNAs as a baseline. We will assay spore germination as the primary readout for silencing of *CesA3*, with confirmation of the most interesting results using the same assays as described in Aim 1a. Assays for silencing of T814784 will also proceed as described in Aim 1a.

Expected results, pitfalls, and limitations: Based on the results from fungi (Islam et al., 2021), we expect that ME-dsRNA will be as least as efficient as naked dsRNAs, and perhaps significantly more efficient. If true, this bodes well for the applicability of this approach for functional genomic screens and for induction of SIGS. We do not expect technical pitfalls in this aim because of Sherif's experience with the system and access to advice from AgroSpheres (see letter of collaboration). The worst case is that ME-dsRNAs underperform. This will still be valuable information to streamline Aims 2 and 3.

Aim 1d: Test efficiency of multiplexed dsRNA to simultaneously silence multiple genes

Rationale: The capacity to multiplex would be of high value for reverse genetic assays and for

development of RNAi fungicides.

Approach: Using the optimized delivery methods and functional assays from Aim 1a, 1b and 1c, we will test the efficiency of simultaneously silencing the *CesA3* and *T814784* genes. The treatments and assays will follow procedures described above. If this trial produces promising results, then we will test additional genes for which silencing has been verified from the research under the Tör /McDowell BBSRC-funded project. We will test higher-order combinations until efficiency declines.

Predicted results, pitfalls, and alternatives: We predict that multiplexing will enable silencing of multiple genes and will succeed albeit with some upper limit that can only be determined empirically. This aim will be straightforward, utilizing the same assays as described above. If necessary, we will test different concentration ranges and constructs for the target genes.

Aim 2-Optimize in planta SIGS against downy mildews

Overview: For functional genomics of downy mildews, spray-induced gene silencing (SIGS) is a valuable complement to *in vitro* induction of RNAi in spores because SIGS can be induced in infected plants at specific stages of the disease cycle to investigate genes that are lethal when silenced in germinating spores (e.g., to examine the importance of *CesA3* during infection). Additionally, this Aim will provide valuable information for future applications of SIGS for disease control in greenhouse and field settings. SIGS has shown promise against *Phytophthora* (Kalyandurg et al., 2021) but has not been optimized against any oomycete and has not been tested against downy mildews. Previous success with HIGS against downy mildew (Govindarajulu et al., 2015) suggests that downy mildew pathogens are capable of internalizing sRNAs from the plant; Therefore, we are optimistic that SIGS will be effective. Another strength of this Aim is that it will be streamlined by the methods, constructs, and results from Aim 1. Accordingly, our experimental plan will parallel that of Aim 1, in which we establish a baseline of silencing efficiency triggered by applications of SS- vs. NECT-dsRNAs, followed by evaluation of ME-dsRNAs and tests of multiplexing. We will test foliar applications as well as seed treatments, with the latter being particularly advantageous to enable high-throughput functional genomics and to assess whether seed treatments with RNA are viable for disease control. We will focus on the same pathogens and the same target genes as in Aim 1, and will use the same assays to evaluate silencing efficacy.

Aim 2a: Test and optimize SIGS efficacy for SS-dsRNAs and NECT-dsRNAs.

Rationale: SS-dsRNAs are sufficient to induce gene silencing in downy mildew spores, but have not been tested for their capacity to trigger SIGS. As noted above, they are prohibitively expensive for large-scale functional genomic screens, particularly for SIGS in which much larger quantities are required compared to µL-scale applications to spore suspensions. NECT-dsRNAs can be produced inexpensively in an *E. coli*-based system as described in Aim 1a. We will compare efficiency of silencing *CesA3* and *T814784* using the following assays on infected plants: spore production (e.g. Figs. 1 & 3), in planta hyphal growth via trypan blue staining (Fig. 2), qPCR quantification of pathogen DNA, and RT-qPCR to assess expression of the targeted pathogen genes in planta during infection. Together, these assays will provide a robust assessment of the effectiveness of SIGS and direct comparison to the results from Aim 1.

Pathogen-host combinations: As in Aim 1, almost all of the experiments described below will be performed in parallel *Hpa - Arabidopsis* and *Pvp - pea* pathosytems to provide an immediate assessment of whether a single, broadly-applicable SIGS workflow is possible and if not, will highlight important parameters for species-specific optimization. As with Aim 1, we expect that the benefits of this parallelism will outweigh the costs.

Genes to be targeted: We will target the same genes as in Aim 1, based on the same rationales. The main difference is that we won't be able to use the in vitro spore germination assay. However, the other assays will robustly differentiate between reduction or enhancement of silencing.

Experimental plan: Our first experiment will be to test whether application of SS-dsRNAs and NECT-dsRNAs differ in the effectiveness in which they trigger SIGS in the *CesA3* gene. We will use the same plasmids and expression systems to produce the same sets of experimental and control dsRNAs described in Aim 1a. We will apply these RNA species to leaves of Arabidopsis or pea using high pressure spraying, which has been shown as an effective application method for SIGS in previous studies (Nerva et al., 2020). At 8 hours after application, we will apply spore suspension of *Hpa* or *Pvp* to dsRNA-treated leaves. We will collect samples of infected leaves for trypan blue staining to visualize pathogen

infection structures, qPCR of pathogen DNA, and qRT-PCR of target gene transcript abundance at 1, 3, and 5 days after infection. We will also quantify conidiospore production at 7 dai. A concentration range of each SS- and NECT-dsRNA will be tested, using previously published results from fungi and *P. infestans* to estimate an appropriate range (REFS). We will also test a range of intervals between dsRNA application and inoculation of leaves, to determine the time interval necessary to induce SIGS.

Predicted results, pitfalls and alternatives: Based on successes with using NECT-dsRNAs to trigger SIGS against fungi and *P. infestans*, we anticipate that NECT-dsRNAs will be effective against the downy mildew pathogens. As with Aim 1a, we recognize that we might have to expand the range of some tested parameters (i.e., dsRNA fragment length, target site, and/or concentration) to achieve optimality. Otherwise, we expect no major difficulties because the experiments are conceptually straightforward and rely on techniques that are well-established amongst the PIs. The worst-case scenario is that neither SS- nor NECT-dsRNAs induce RNAi efficiently. This seems unlikely because of previous successes with SIGS against fungi and *P. infestans*, and effectiveness of HIGS against lettuce downy mildew. However, even if that scenario comes to pass, we are still favorably positioned to test the efficacy of EC-dsRNAs.

Aim 2b: Compare SIGS efficacy of "naked" dsRNA and dsRNA protected by *E. coli* minicells.

Rationale: It has been broadly documented that naked dsRNA has transient, and in some cases, inefficient capacity for induction of SIGS. As in Aim 1, these experiments take advantage of co-PI Sherif's unique expertise with the ME-dsRNA system. These experiments will comprise an important extension of Sherif's pioneering description of how ME-dsRNA is much more efficient than naked RNA at triggering SIGS against fungi.

Experimental Plan: We will use the same *E. coli* strains to produce various ME-dsRNA fragments targeting *Ces3A*, *T814784*, and *GFP* as described in Aim 1c. We will then apply a range of concentrations of ME-dsRNA to pea or Arabidopsis leaves. We will perform the same assays for SIGS effectiveness as described in Aim 1a. If the initial trials suggest that ME-dsRNAs are efficient inducers of SIGS, then we will conduct a series of experiments to test the longevity of this effect. We will treat leaves with the best-performing ME-dsRNA and naked dsRNA fragments, and inoculate those plants at 1, 2, and 3 weeks after treatment. To streamline these comparisons, we will use pathogen sporulation as a primary assay, and follow up with additional assays to confirm the most interesting results.

Expected results, pitfalls, and limitations: Based on the results from fungi, we expect that ME-dsRNA will be as least as efficient as naked dsRNAs, and perhaps significantly more efficient. We also expect that longevity of the ME-dsRNA protection will significantly surpass that of naked dsRNAs. We do not expect technical pitfalls in this aim because of Sherif's experience with the system and access to advice from AgroSpheres (see letter of collaboration). The worst case is that ME-dsRNAs underperform. This will still be valuable information to inform strategies for RNAi-based experiments and disease control strategies against oomycetes.

Aim 2c: Test efficiency of multiplexed dsRNA to simultaneously inactivate multiple genes

Rationale: Multiplexing would be of high value for reverse genetic assays and for development of RNAi fungicides with stronger efficacy and durability because they target multiple, important pathways.

Approach: Using the optimized delivery methods and functional assays from previous aims, we will test the efficiency of simultaneously silencing the *Ces3A* and *T814784* genes. The treatments and assays will follow procedures described above. If this trial produces promising results, then we will test additional genes for which silencing has been verified from the research under the Tör /McDowell BBSRC-funded project. We will test higher-order combinations until efficiency declines.

Predicted results, pitfalls, and alternatives: We predict that multiplexing will enable silencing of multiple will succeed albeit with some upper limit that can only be determined empirically. This aim will be straightforward, utilizing the same assays as described above.

Aim 2d: Test whether seed coatings can induce SIGS

Rationale: Because ME-dsRNAs are protected against environmental stressors and can be desiccated, stored, and re-hydrated, we will test whether seed treatments with ME-dsRNAs are effective. This capacity would facilitate functional genomics studies by enabling construction of libraries consisting of seeds that have been treated with individual ME-dsRNAs, or with pools of multiplexed ME-dsRNAs. Such

libraries could be stored (e.g., in a stock center) and distributed to investigators for their phenotypic screens. Equally important, these experiments would provide the first proof-of-concept on whether dsRNA fungicides could be applied as a seed treatment, as is often done for chemical fungicides.

Experimental Plan: We will conduct the proof-of-concept experiments in *Arabidopsis* and pea seeds and will target the *Ces3A* and *T814784* genes, using the most effective fragments as established in Aims 2a and 2b. The GFP fragment will be included as a control. We will coat seeds with a range of ME-dsRNA concentrations by spraying or by imbibement. Seeds will then be dried and stored. Later, we will assay for induction of SIGS in freshly-treated seeds as well as in seeds stored at 1, 2, 4, 6, and 8 weeks after treatment. Each experiment will include leaf treatment of plants germinated from untreated seeds as a standard for comparison of SIGS induced by leaf spraying vs. seed treatment. If these initial trials are successful, then we will test seeds over longer storage periods until the end of the granting period.

Predicted results, pitfalls, and alternatives: Based on the initial successes with pea, we are optimistic that "fresh" treatments will be effective. The durability of such treatments remains to be seen. We recognize that we will test a very simplistic formulation; if necessary, we might test various adjuvants.

Aim 3: Test protocols for *in vitro* silencing and SIGS on downy mildews of lettuce, grape, and cucumber

Rationale: These experiments will test whether the optimized protocols from Aim 1b-d function on species that represent three of the most important downy mildew diseases and have distinctive biological attributes (see Introductory Material) that will significantly deepen and expand understanding of downy mildew evolution and virulence, with implications for effective (re-) design of disease prediction models and downstream interventions.

Experimental Plan: Based on the results from Aim 1 and 2, we will construct optimized protocols for *in vitro* silencing of spore and SIGS. We will target *CesA3* in all three species because homologs of this highly conserved gene are straightforward to identify in sequenced genomes. We will design and amplify fragments based on the most successful fragments from Aims 1 and 2, and we anticipate focusing on the ME-dsRNA system for dsRNA synthesis and delivery. For *in vitro* treatment of spores, we will generate cultures of isolates endemic in Virginia (grape, cucumber) or the UK (lettuce) by inoculating spores onto detached leaves of susceptible hosts, incubation for several days, and collection of new spores after the infected material has sporulated. We will then treat the spores with ME-dsRNAs, including the GFP control, and monitor spore germination as an initial readout. If this assay gives promising results, then we will repeat the experiments to include inoculation of detached leaves, followed by virulence assays and RT-qPCR of the target mRNA, as described in previous aims. For initial tests of SIGS, we will treat detached leaves of grapevines, lettuce, and cucumber with ME-dsRNAs, and then inoculate with spores and conduct virulence assays as described above. For lettuce and cucumber, we will also test seed treatments, guided by results from Aim 2d. Similar experiments will be conducted on attached grape and cucumber specimens grown within the controlled environment of the greenhouse. The grape plants will be obtained through the process of cutting propagation, while the cucumber cultures will initiate from sterilized, cold-stratified seeds. The SIGS methodology will be executed on plants with fully expanded foliage, utilizing a 0.2-gallon handheld garden pump sprayer. The materials, dosages, and timing of application for the dsRNA formulations to be used in these experiments will be established based on the outcomes of the in vitro assays performed on detached leaves, as well as the information generated from the other Aims of the study.

Predicted results, pitfalls, and alternatives: We anticipate a learning curve in setting up efficient propagation protocols and virulence assays for the cucumber and pea pathosystems, because we have not worked with these before. However, we have considerable experience working with diverse downy mildews and we can consult the literature as well as colleagues who have achieved success. Although the basic mechanisms underpinning RNAi are conserved in lettuce, grape, cucumber, and their respective downy mildews, it is conceivable that significant changes might be necessary to achieve success. Altogether, this is likely the riskiest aim in this proposal but the potential benefits, from fundamental and applied perspectives, are significant.

Broader Impacts

Impact on disease control: Downy mildews and other oomycete pathogens are very destructive to agriculture. Downy mildews account for ~25% of the global fungicide market (Clark and Spencer-Phillips,

2000; Gisi, 2002). Annual losses to *Phytophthora* diseases are estimated at 10's of billions of dollars (Tyler, 2007; Fry, 2008). The most sustainable method for disease control is genetic resistance; however, oomycetes are adept at overcoming classical gene-for-gene resistance because of their high evolutionary potential to mutate avirulence/effector genes (Fry, 2008). Many of the commercial fungicides developed against true fungi are ineffective against oomycetes because of their distinct biology. Additionally, oomycetes are adept at evolving resistance to chemical controls (Tyler, 2007; Fry, 2008). In this context, the functional genomic tools from this project could be applied to knowledge gaps on the molecular basis of fungicide resistance, as well as to identify leads for development of new fungicides. Moreover, SIGS itself is envisioned not only as a tool for functional genomics but as a means of delivering dsRNA as a bio-fungicide itself under field settings, as discussed in Background. In this context, the experiments in this proposal are designed to inform the application of dsRNA-based strategies for control of oomycete diseases. For example, a major motivation underpinning the design of Aim 3 is to develop SIGS protocols that could be field-tested for application to downy mildew of cucumber, grape, and lettuce, which are three of the most important downy mildew diseases. The SIGS protocols in Aims 2 and 3, while developed against downy mildews, are quite likely to have broader applicability to *Phytophthora* species and other oomycete pathogens in the *Albugo* and *Aphanomyces* genera. A distinctive advantage of SIGS, exemplified in Fig. 9, is that treatments can be applied post-harvest (e.g., to protect potato tubers against spoilage and reduce transmission of late blight disease due to shipping. Beyond oomycetes, we are excited (pp. 7-8) about the potential of ME-dsRNAs for SIGS against a variety of diseases, given the evidence supporting their efficacy and durability. As we develop our protocols, we will make every effort to attract industrial partners, to maximize the impact of our research on disease control strategies.

Impact on public perception of RNAi-based bioengineering: We have designed the Dissemination and Outreach Plan (see Single Copy Documents) with two, interconnected goals. The first is to disseminate the products of our research (i.e., protocols) to the research community, including industry so that the protocols will have maximum impact. The second is to engage in outreach to food producers and consumers with the goal of informing their perspective and collecting their feedback on the general utility of RNA-based fungicides. RNAi-based technologies are relatively new. Therefore, the attitudes of users and consumers on this new technology have not yet crystalized (Taning et al., 2020; Taning et al., 2021). In this context, balanced dialog about risks and benefits is critically important (Gupta et al., 2015) as are re-evaluations of stakeholder attitudes as new information emerges (Fischer et al., 2013). Thanks to parallels between dsRNA-based fungicides and the development of RNA vaccines against Covid-19, we foresee an unprecedented opportunity to frame our research by analogy to RNA vaccines. Such framing should be accessible and interesting to a variety of audiences, and we will fully leverage this analogy in our narratives. The extension expertise and contacts of Sherif, along with Tor's industrial and grower contacts, will enable engagement with end-users and other stakeholders at the very beginning of the project, to collect valuable feedback, address needs and reservations, and thereby promote buy-in. These dynamics are critically important for acceptance and adoption of new technologies (Davidson, 2008). Equally important, our outreach will leverage Sherif's access to forums that reach underserved groups.

Impact on training the next generation of scientists: This project will provide a distinctive training opportunity to three postdoctoral scholars and undergraduate researchers, within an international collaboration at the interface of basic and applied science that involves an industrial partner. We have budgeted for a paid position for an undergraduate at Virginia Tech and will recruit others through internal programs at Virginia Tech and the University of Worcester that fund undergraduate research and experiential learning. Our efforts to recruit project personnel will prioritize broadening participation. We will focus on advertising the postdoc positions in venues that reach underserved groups (e.g., the Minority Postdoc site) and will recruit undergraduates from programs at our respective institutions that forge such connections (e.g., the Multicultural Academic Opportunities Program at Virginia Tech, from which PI McDowell has recruited in the past). We have designed a Postdoctoral Mentoring Plan that accommodates a variety of career goals and maximizes the opportunities inherent in this project.

Relationship of the proposed research to currently funded projects

This proposal has benefitted from and could synergize with two grants held by the PIs. One grant, entitled "Deciphering pathogenicity and development in obligate downy mildew pathogen using small RNA approach" is funded by the BBSRC to Tör with a subcontract to McDowell. The goal of this project is to use SS-dsRNAs for a medium-scale functional genomics screen of *Hpa*, with emphasis on development

and pathogenicity. This project is off to a productive start and some of the data have informed the design of the NSF EDGE proposal (e.g., selection of the best target genes and baseline optimization of SS-dsRNA as an inducer of silencing). Importantly, the BBSRC focuses exclusively on SS-dsRNA rather than NEVT-dsRNAs or ME-dsRNAs, and does not attempt SIGS. Thus, there is no overlap between the BBSRC-funded project and the research in this proposal; However, if this proposal is funded, the resultant new methods could accelerate the progress during the later stages of the BBSRC-funded project. Sherif is completing (July 2023) a project with funding from the Virginia Biosciences Health Research Corporation entitled "Optimization and commercialization of environmentally-sustainable biofungicides." Sherif is collaborating with AgroSpheres to enhance the effectiveness of ME-dsRNA formulas in managing fungal diseases gray mold and powdery mildew. The project primarily focuses on testing various surfactants/additives for ME-dsRNA formulas and demonstrating their commercial viability under greenhouse and cold-storage conditions. This project does not investigate ME-dsRNA's use for oomycetes or any functional genomics aspects. Therefore, this project has informed the proposed experiments (e.g., Figs 7-9 but there is no overlap between this project and the current NSF proposal. Sherif also recently received a USDA-NIFA Biotechnology Risk Assessment Research Grant (BRAG) funding for “Investigating the Environmental Persistence and Target Specificity of Minicells-Encapsulated RNAi BioFungicides”. This project does not overlap with the proposed research but the experiments on persistence of ME-dsRNAs in the environment could inform our optimization experiments. Moreover, Sherif’s experience and perspectives on environmental impacts of SIGS will be an important component of the planned outreach and education efforts. Finally, Tör has submitted a collaborative planning grant to the BBSRC entitled: “siRNA for Disease control”. This small proposal is designed to strengthen collaboration between the Tör, McDowell, and Sherif groups by funding reciprocal visits and other planning activities. This is not a research proposal and therefore does not overlap with this proposal.

Justification for a foreign subaward

The requested budget for this proposal includes a subaward to the University of Worcester in the UK to fund co-PI Tör ’s activities. We recognize that foreign awards must be justified explicitly as per Chapter 1 E.6. of the PAPPG. In this context, co-PI Tör contributes “unique expertise, …(and) data resources, …not generally available to U.S. investigators (or which would require significant effort or time to duplicate)”; specifically, his expertise in SS-dsRNA-induced gene silencing in *Hpa* and particularly in pea and lettuce downy mildews. Neither McDowell or Sherif has experience with the latter two pathosystems, and none of the US researchers with expertise in pea and lettuce downy mildews possess Tör ’s expertise in SS-dsRNA gene silencing. Thus, his expertise is unique and would not available in the US without significant duplication of time and effort. Moreover, Tör and McDowell have a distinctively strong history of productive collaboration, enhancing the chance for success of this project. Finally, Tör ’s connections with European stakeholders will facilitate dissemination of the results on a global basis.

Results from prior support

Pilot (PI), McDowell, Helm and Okumoto. Mechanisms of nutrient transport from plants to biotrophic pathogens (NSF-IOS1353366; $761,563; 09/01/2014 – 08/31/2018). *Intellectual Merit*: This project was to discover sugar and amino acid transporters of Arabidopsis utilized by the pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) to extract carbon and nitrogen from its plant host. We developed a novel system to isolate the subset of mRNAs that are being translated into proteins specifically from haustoriated cells, leading to new information about how *Hpa* interacts with its host (in prep). We also tested disease susceptibility of knockout mutants of amino acid transporters in Arabidopsis. Analysis of one mutant provided evidence that Arabidopsis activates phloem loading to restrict amino acid availability to *Hpa* in leaves (in prep). We created a yeast strain to characterize amino acid transporter properties that has been distributed to 11 researchers worldwide. *Broader Impacts:* Our outreach involved mentoring ~600 students from two Governor’s schools in VA to design and perform original experiments on Arabidopsis mutants impaired in amino acid transport in the classroom. Involving high school students in authentic research broadened their understanding and appreciation of plants and science in general. This grant supported 13 publications (Anderson et al., 2015; Anderson and McDowell, 2015; Kamoun et al., 2015; Besnard et al., 2016; Kong et al., 2017; Michelmore et al., 2017; Anderson et al., 2018; Besnard et al., 2018; Dalio et al., 2018; Deb et al., 2018b; Deb et al., 2018a; Lai et al., 2018; Sonawala et al., 2018) and two more that are in preparation, as well as training of 2 graduate students and 6 undergraduates.

Co-PIs Sherif and Tor have not yet received NSF support.

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SUMMARY PROPOSAL BUDGET

YEAR 1

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months)	
	Proposed	Granted
AWARD NO.		

ORGANIZATION: Virginia Polytechnic Institute and State University

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR: John Mcdowell

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1.					
2.					
3.					
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.0			0	
7. ( ) TOTAL SENIOR PERSONNEL (1 - 6)	0.0			0	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (2) POST DOCTORAL SCHOLARS	12.0			50,000	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.0			0	
3. (0) GRADUATE STUDENTS				0	
4. (1) UNDERGRADUATE STUDENTS				9,600	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				59,600	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				18,064	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				77,664	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. U.S. POSSESSIONS)				1,000	
2. INTERNATIONAL				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				5,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				103,716	
6. OTHER				10,000	
TOTAL OTHER DIRECT COSTS				118,716	
H. TOTAL DIRECT COSTS (A THROUGH G)				197,380	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) F&A costs (Rate: 60.0, Base:118664)					
TOTAL INDIRECT COSTS (F&A)				71,198	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				268,578	
K. FEE				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				268,578	

M. COST SHARING PROPOSED LEVEL $ 0 AGREED LEVEL IF DIFFERENT $

PI/PD NAME: John Mcdowell

ORG. REP. NAME*: Jane Lee

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

*ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

SUMMARY PROPOSAL BUDGET

YEAR 2

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months)	
	Proposed	Granted
AWARD NO.		

ORGANIZATION: Virginia Polytechnic Institute and State University

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR: John Mcdowell

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1.					
2.					
3.					
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.0			0	
7. ( ) TOTAL SENIOR PERSONNEL (1 - 6)	0.0			0	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (2) POST DOCTORAL SCHOLARS	12.0			52,500	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.0			0	
3. (0) GRADUATE STUDENTS				0	
4. (1) UNDERGRADUATE STUDENTS				9,600	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				62,100	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				18,811	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				80,911	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. U.S. POSSESSIONS)				1,000	
2. INTERNATIONAL				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				228	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				228	
H. TOTAL DIRECT COSTS (A THROUGH G)				82,139	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) F&A costs (Rate: 60.0, Base:82139)					
TOTAL INDIRECT COSTS (F&A)				49,283	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				131,422	
K. FEE				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				131,422	

M. COST SHARING PROPOSED LEVEL $ 0 AGREED LEVEL IF DIFFERENT $

PI/PD NAME: John Mcdowell

ORG. REP. NAME*: Jane Lee

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

*ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

SUMMARY PROPOSAL BUDGET

Cumulative

ORGANIZATION	FOR NSF USE ONLY: PROPOSAL NO.	DURATION (months) Proposed	Granted
Virginia Polytechnic Institute and State University	2319757		
PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR: John Mcdowell	AWARD NO.		

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1.					
2.					
3.					
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)					
7. ( ) TOTAL SENIOR PERSONNEL (1 - 6)	0.0			0	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (4) POST DOCTORAL SCHOLARS	24.0			102,500	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.0			0	
3. (0) GRADUATE STUDENTS				0	
4. (2) UNDERGRADUATE STUDENTS				19,200	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				121,700	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				36,875	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				158,575	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. U.S. POSSESSIONS)				2,000	
2. INTERNATIONAL				0	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				5,228	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				103,716	
6. OTHER				10,000	
TOTAL OTHER DIRECT COSTS				118,944	
H. TOTAL DIRECT COSTS (A THROUGH G)				279,519	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)					
TOTAL INDIRECT COSTS (F&A)				120,481	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				400,000	
K. FEE				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				400,000	

M. COST SHARING PROPOSED LEVEL $ 0 AGREED LEVEL IF DIFFERENT $

PI/PD NAME	FOR NSF USE ONLY: INDIRECT COST RATE VERIFICATION		
John Mcdowell	Date Checked	Date Of Rate Sheet	Initials - ORG
ORG. REP. NAME* Jane Lee			

*ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

BUDGET JUSTIFICATION-Virginia Tech, McDowell and Sherif

A. Senior Personnel

No funds requested

B. Other Personnel

Funds are requested for two Postdoctoral Scholars, each for 12 months. One will work with the McDowell group, primarily responsible for the experiments with *Hpa* in Aims 1a, 1b, and 1d. The other Postdoctoral Scholar will work with the Sherif group, primarily responsible for construction of plasmids for Aims 1a and 1b and the mini-cell experiments in Aim 1c.

Funds are requested for one Undergraduate Student ($12/hr), 10 hrs/week during the Spring '24 and Fall '24 Semesters, and 40 hrs/week during the Summer '24 semester. We intend to recruit a sophmore or junior. The student will learn the techniques necessary for Aim 1 in Spring '24 and will then develop an independent research project, aligned with the goals of the proposal and with the student's career goals, during the Summer and Fall of 2024.

C. Fringe benefits

Fringe Benefits are calculated in accordance with Virginia Tech's federally negotiated fringe rate agreement which is available at https://osp.vt.edu/resources/rates/indirect-costs.html. The relevant rates are 35.52% for special research faculty (e.g., Postdoctoral Scholars) thru 6/30/24 & 35.10% on/after 7/1/24 and 6.34% % for wage employees (i.e., the undergraduate student's summer time) & 6.65% on/after 7/1/24.

E. Travel

Domestic

$1000/year is requested to cover attendance of McDowell and Sherif at one EDGE PI meeting (Note that VT is within driving distance of Alexandria).

G1. Materials and supplies

Funds are requested for consumables, chemicals, RT-PCR supplies, plant growth chamber fees, RNA oligos, molecular biology kits and enzymes. Total: $5,228

G2. Publication costs

We will target open access journals and apply to Virginia Tech funds to offset charges to incentivize publication in Open Access journals.

G6. Other

This line contains two items:

1- Subcontract to the University of Worcester, to provide essential expertise on experiments with pea and lettuce downy mildew. Total direct + indirect costs of $103,716.
2- Contractual services: A sum of $10,000 is requested for AgroSpheres, Inc. for production of minicell-encapsulated dsRNA formulas for the genes specified in Aim 1.

I. Indirect costs

The indirect rates applied have been negotiated and approved by Virginia Tech's Federal Cognizant Agency, ONR. The indirect rate through 7/21/24 is 60% Modified Total Direct Costs (MTDC) for on-campus research. A copy of VT's current federally negotiated indirect rate agreement can be found here: https://osp.vt.edu/resources/rates/indirect-costs.html.

The bases for indirect costs are $118,664 for year 1 and $82,139 for year 2.

Budget Impact Statement

Given the reduction of the proposed budget from $1,340,485 to $400,000, we will focus solely on **Aim 1: Optimize in vitro protocols for triggering RNAi in spores of diverse downy mildew species.** The goal of this aim is to develop protocols for robust, cost-effective of RNAi via application of dsRNA to suspensions of spores, and to optimize these protocols for applicability to a range of downy mildew species. The Aim is composed of the following sub-aims, each of which will be executed as described in the proposal:

Aim 1a: Compare silencing efficacy of small, synthetic, double-stranded RNAs (ss-dsRNAs) to naked, E. coli-transcribed- double-stranded RNAs (NECT-dsRNAs) and optimize length, dose, and target region of NECT-dsRNAs.

Aim 1b: Quantify dsRNA internalization of ss-dsRNAs and NECT-dsRNAs when applied to spores

Aim 1c: Compare silencing efficacy of “naked” dsRNA and dsRNA protected by *E. coli* minicells.

Aim 1d: Test efficiency of multiplexed dsRNAs to simultaneously silence multiple genes

We are confident that execution of this Aim will dramatically advance resources for functional genomics of downy mildews, thereby facilitating previously inaccessible genotype-phenotype understanding of downy mildew biology, evolution, and pathogenesis.

Below is a revision of the Project management plan:

Communication, evaluation, and planning

1. Zoom meetings of the entire group every two weeks: In these one-hour meetings each researcher will summarize progress and challenges since the previous meeting, and goals for the upcoming two weeks.

2. Quarterly Zoom meetings of the three PIs: In these two-hour meetings, the PIs will review progress towards milestones, troubleshoot bottlenecks, and reach consensus on alterations of the plan and timeline, as well as re-allocation of resources if necessary.

Responsibilities

McDowell Group, Virginia Tech:

- Collaborate with Sherif and Tör group to create all necessary plasmid constructs
- Lead role on experiments with Arabidopsis + *Hpa* in Aims 1a, 1b, and 1d

Sherif Group, Virginia Tech

- Lead role on plasmid construction
- Experiments with EM-dsRNAs in Aim 1c and 1d.
- Lead on role on extension programming as described in the Dissemination and Education Plan.

Tör Group, University of Worcester

- Collaborate with Sherif and McDowell group to create all necessary plasmid constructs

- Lead role experiments with Pea + *Pvp* in Aims 1a, 1b, and 1d

SUMMARY PROPOSAL BUDGET

YEAR 1

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months)	
	Proposed	Granted
AWARD NO.		

ORGANIZATION: UNIVERSITY OF WORCESTER

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR: John Mcdowell

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1.					
2.					
3.					
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.0			0	
7. ( ) TOTAL SENIOR PERSONNEL (1 - 6)	0.0			0	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (1) POST DOCTORAL SCHOLARS	12.0			73,287	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.0			0	
3. (0) GRADUATE STUDENTS				0	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				73,287	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				0	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				73,287	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. U.S. POSSESSIONS)				0	
2. INTERNATIONAL				2,000	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				19,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				19,000	
H. TOTAL DIRECT COSTS (A THROUGH G)				94,287	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) F & A (Rate: 10.0, Base:94287)					
TOTAL INDIRECT COSTS (F&A)				9,429	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				103,716	
K. FEE				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				103,716	

M. COST SHARING PROPOSED LEVEL $ 0 AGREED LEVEL IF DIFFERENT $

PI/PD NAME: John Mcdowell

ORG. REP. NAME*: Jane Lee

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

*ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

SUMMARY PROPOSAL BUDGET

Cumulative

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months)	
	Proposed	Granted
2319757		
AWARD NO.		

ORGANIZATION: UNIVERSITY OF WORCESTER

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR: John Mcdowell

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1.					
2.					
3.					
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)					
7. ( ) TOTAL SENIOR PERSONNEL (1 - 6)	0.0			0	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (1) POST DOCTORAL SCHOLARS	12.0			73,287	
2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.0			0	
3. (0) GRADUATE STUDENTS				0	
4. (0) UNDERGRADUATE STUDENTS				0	
5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				0	
6. (0) OTHER				0	
TOTAL SALARIES AND WAGES (A + B)				73,287	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				0	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				73,287	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				0	
E. TRAVEL 1. DOMESTIC (INCL. U.S. POSSESSIONS)				0	
2. INTERNATIONAL				2,000	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ 0					
2. TRAVEL 0					
3. SUBSISTENCE 0					
4. OTHER 0					
TOTAL NUMBER OF PARTICIPANTS (0) TOTAL PARTICIPANT COSTS				0	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				19,000	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				0	
3. CONSULTANT SERVICES				0	
4. COMPUTER SERVICES				0	
5. SUBAWARDS				0	
6. OTHER				0	
TOTAL OTHER DIRECT COSTS				19,000	
H. TOTAL DIRECT COSTS (A THROUGH G)				94,287	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)					
TOTAL INDIRECT COSTS (F&A)				9,429	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				103,716	
K. FEE				0	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				103,716	

M. COST SHARING PROPOSED LEVEL $ 0 AGREED LEVEL IF DIFFERENT $

PI/PD NAME: John Mcdowell

ORG. REP. NAME*: Jane Lee

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

*ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET

BUDGET JUSTIFICATION-University of Worcester, Tor

A. Senior Personnel

No funds requested

B. Other Personnel: We request 12 months of salary and fringe benefits for a post-doctoral researcher ($73,287). This project will require a wide range of knowledge and skills in the field of molecular genetics, pathology and bioinformatics. We have requested funding for an experienced PDRA in order to enhance significantly the prospects of recruiting a researcher with the requisite talents, allowing this project to move rapidly from the start. With this interdisciplinary project, PDRA will have valuable opportunities to make progress towards an independent scientist.

E. Travel

A total of $2000 is requested to offset the cost of the following:

Domestic

Attendance of both PIs and both Postdoctoral Scholars at one UK meeting to network and present their research (e.g., annual meetings of the British Society of Plant Pathology) $2000);

G1. Materials and supplies

A total of $19,000 is requested to offset the cost of following: 1) Estimated molecular consumable cost ($15,500) includes plasticware, enzymes, kits, oligonucleotides, molecular biology reagents, and standard sequencing. 2) The cost for access to growth rooms and cabinets, and related consumables (seeds, pots, soils, trays and etc.) amounting to $1500 is requested. 3) We request a computer ($1500) for the PDRA and licenses for software ($500), as sequence analysis, data compilation and analysis, slide construction, and paper writing are essential for this project.

G2. Publication costs

We will target open access journals and apply to University of Worcester funds to offset charges to incentivize publication in Open Access journals.

I. Indirect costs

A U.S. Federally negotiated indirect cost rate does not exist for the University of Worcester. Therefore, Virginia Tech requests the *de minimis* indirect cost rate recovery of 10% of modified total direct costs ($9,429) for the subaward to the University of Worcester.

Budget Impact Statement

Given the reduction of the proposed budget from $1,340,485 to $400,000, we will focus solely on **Aim 1: Optimize in vitro protocols for triggering RNAi in spores of diverse downy mildew species.** The goal of this aim is to develop protocols for robust, cost-effective of RNAi via application of dsRNA to suspensions of spores, and to optimize these protocols for applicability to a range of downy mildew species. The Aim is composed of the following sub-aims, each of which will be executed as described in the proposal:

Aim 1a: Compare silencing efficacy of small, synthetic, double-stranded RNAs (ss-dsRNAs) to naked, E. coli-transcribed- double-stranded RNAs (NECT-dsRNAs) and optimize length, dose, and target region of NECT-dsRNAs.

Aim 1b: Quantify dsRNA internalization of ss-dsRNAs and NECT-dsRNAs when applied to spores

Aim 1c: Compare silencing efficacy of “naked” dsRNA and dsRNA protected by *E. coli* minicells.

Aim 1d: Test efficiency of multiplexed dsRNAs to simultaneously silence multiple genes

We are confident that execution of this Aim will dramatically advance resources for functional genomics of downy mildews, thereby facilitating previously inaccessible genotype-phenotype understanding of downy mildew biology, evolution, and pathogenesis.

Below is a revision of the Project management plan:

Communication, evaluation, and planning

1. Zoom meetings of the entire group every two weeks: In these one-hour meetings each researcher will summarize progress and challenges since the previous meeting, and goals for the upcoming two weeks.

2. Quarterly Zoom meetings of the three PIs: In these two-hour meetings, the PIs will review progress towards milestones, troubleshoot bottlenecks, and reach consensus on alterations of the plan and timeline, as well as re-allocation of resources if necessary.

Responsibilities

McDowell Group, Virginia Tech:

- Collaborate with Sherif and Tör group to create all necessary plasmid constructs
- Lead role on experiments with Arabidopsis + *Hpa* in Aims 1a, 1b, and 1d

Sherif Group, Virginia Tech

- Lead role on plasmid construction
- Experiments with EM-dsRNAs in Aim 1c and 1d.
- Lead on role on extension programming as described in the Dissemination and Education Plan.

Tör Group, University of Worcester

- Collaborate with Sherif and McDowell group to create all necessary plasmid constructs

- Lead role experiments with Pea + *Pvp* in Aims 1a, 1b, and 1d

FACILITIES, EQUIPMENT, AND OTHER RESOURCES

John M. McDowell

Lab Space

1000 square feet of laboratory space in Latham Hall, a 15 year old building that houses most of the Molecular Plant Scientists at Virginia Tech. One certified Class II biohazard hood to prevent plant pathogen escape; one chemical fume hood; various refrigerators at 4°C, -20°C, and -80°C; Zeiss dissecting scope with epifluorescence, attached JVC digital camera, various centrifuges, thermal cyclers, incubators; BioRad gel imaging system and thermal printer; BioRad electroporation unit; Eppendorf speed vac; several DNA electrophoresis units (Owl) and power supplies.

Clinical:

Not applicable to this work.

Animal:

Not applicable to this work.

Computer:

All desktop units are connected to a backup external HD (1TB) and to centralized backup servers maintained by VT. Each student has a personal laptop. All computers are connected to the local and wireless network offered by Virginia Tech's Communications Network Services for file transfer, electronic mail and numerous support services via the Internet. These services include the Virginia Tech library catalog, TAIR, and Genevestigator.

Office:

McDowell has an office of 225 square feet located in Latham Hall.

Other:

Within Latham Hall, separate rooms are available for tissue culture, plant growth (Conviron and Percival), and conducting imaging experiments; 400 square feet of shared equipment room space as well as autoclaves, shared facilities for dishwashing, several gel imaging systems, a liquid scintillation counter (Beckman), ultracentrifuges (Beckman Optima MAX-XP and LX-P), Sorvall preparative centrifuge with rotors; growth space includes 1200 square feet in an adjacent greenhouse, a large array of Conviron growth chambers ($5 per day) and a room dedicated for experiments with plant pathogens including three plant growth chambers (Percival Scientific) for experiments with *Hpa*. Latham Hall receives an annual budget to cover service contracts, upgrades and maintenance of shared equipment, along with new equipment purchases.

Major Equipment

Real time PCR machines (two Applied Biosystems 7300, and one Applied Biosystems 7500) are available to all Latham Hall researchers and are regularly used by PI McDowell. A confocal microscope with spectral unmixing capacity (Zeiss LSM880 with AiryScan) is located in the neighboring building (Fralin Hall) and available for researchers on Campus.

Other Resources

An interdepartmental program in Translational Plant Sciences provides resources (e.g., seed grants, support for undergradate researchers) and more importantly, a vibrant intellectual climate. This is particularly true for students and postdocs, who participate in a dymanic professional organization that is provided with generous discretionary funds for retreats, trips to government and industry, and hosting external speakers for professional development.

Sherif Sherif

Dr. Sherif's laboratory spans 600 sq. ft. and can accommodate up to seven researchers. The space is fully equipped for plant pathogen culturing and manipulation, microbiology and molecular plant biology research, molecular cloning, and DNA analysis. The laboratory boasts a variety of molecular biology research equipment, including two Bio-Rad thermal cyclers, two qRT-PCR machines from Applied Biosystems and Bio-Rad, a droplet digital (ddPCR) machine, an HPLC with fraction collection, two fluorescent microscopes, two spectrophotometers, six temperature-controlled incubators. Additionally, the laboratory has a range of plant physiology research equipment, including a programmable Tenney freezer and associated data-acquisition hardware for cold hardiness assessment. Field research equipment includes a CIRAS-1 infra-red gas analyzer for field photosynthesis studies, pressure bombs for water potential measures, soil moisture probes, and basic lab apparatus for fruit chemistry analyses.

Dr. Sherif's laboratory is located in the Alson H. Smith Jr Ag Research and Extension Center, which offers a wealth of resources to support the proposed research. The center features dedicated greenhouse space, a walk-in growth room, and three identical growth chambers, providing ample resources for plant research. The center also includes a large office/lab building, offering administrative and scientists' offices, six laboratories, two walk-in coolers, and a variety of environmental chambers. Furthermore, the center features a high tunnel and a greenhouse for additional research options. With access to these facilities, Dr. Sherif has all the necessary resources to support the proposed research and ensure that the research is conducted to the highest standards of quality.

AgroSpheres

The facilities used for the project will be at the AgroSpheres lab in Charlottesville, Virginia. The 5,250 sq./ft. facility is located at 1180 Seminole Trail, Suite 100, Charlottesville, VA 22901, and contains lab and office space. The lab is capable of supporting pilot scale product manufacturing, high resolution fluorescence microscopy, absorbance/fluorescence analysis, characterization and composition analysis, DNA analysis, protein analysis, DNA polymerase chain reaction, enzyme assays, automated liquid handling, high-capacity downstream processing, encapsulation/release kinetic parameter testing and product storage. The lab contains the following major pieces of equipment, and associated kits and minor instruments that complement this list:

General Laboratory Equipment
- Thermo Scientific MaxQ 6000 Incubated/Refrigerated Stackable Shakers
- Sorvall XTR/TX1000 Centrifuge
- Bioteck-H1M Plate Reader
- Laxco LMC-4000 Series Brightfield and Fluorescence Compound Microscope System
- Safe Imager 2.0 Blue-light Transilluminator
- BioClave 16 Research Autoclave, 16 liter, 115V
- Laboratory Fume Hood with Blower, 70 in
- Bio-Rad iCycler iQ Real Time PCR Detection System
- Beckman Coulter Avanti J20 centrifuge
- Thermo Fisher Scientific Simpli Amp Thermocycler
- Beckman Coulter Multisizer 4e Coulter Counter
- Agilent 1100 HPLC System DAD with Quaternary Pump

Fermentation and Formulation Equipment
- KBiotech BIO-SIP Pilot Bioreactor (100L working volume bioreactor)
- Eppendorf Bioflo 120 bioprocess control station (2L working volume bioreactor)
- LabFreez Instruments SD-18A spray dryer (2 L/h)
- Fisherbrand RealTorqueDigital Overhead Stirrers (capacity up to 60L)
- GEA Group SD1 disc stack centrifuge

Mahmut Tör

Lab Space

The Molecular Plant and Microbial Bioscience Research Unit is located at St. John Campus at the UoW in Charles Darwin Building. Laboratories are purpose built for molecular and aerobiological studies. The Unit has a designated microbiology, plant science, cell biology laboratories and image analysis rooms. In addition, the unit contains a test chamber, which can be adjusted to different temperatures (-20 to 60 ˚C). Each laboratory is well equipped for designated works and following equipment are readily available: Two Certified Class II biohazard hoods for microbial work, 2 chemical fume hoods, various refrigerators (4˚C, -20˚C and -80˚C), Leica microscopes with monitors, various centrifuges, thermal cyclers, Roche and Agilent qPCR machines, gel imaging system, Agilent tape station and nanodrop, several gel electrophoresis units and their power supplies.

Clinical:

Not applicable to this work.

Animal:

Not applicable to this work.

Computer:

All desktop units within the laboratories and offices are served by the IT department and are connected to a backup external HD (1TB). University funded students are considered as staff and has been provided with a laptop. All computers are connected to the local LAN and wireless network offered by the IT department. A high-performance computing (HPC) server is also available for staff and students.

Office:

PIs and postdoctoral research fellows are offered offices. Tör has an office of 160 square feet located in Edward Elgar building.

Other:

Within Charles Darwin and Edward Elgar building, there are separate rooms for growth cabinets (Sanyo), 450 square feet of shared equipment room space as well as autoclaves, shared facilities for dishwashing, ultracentrifuges (Beckman Optima MAX-XP and LX-P), Sorvall preparative centrifuge with rotors; and a plant growth room dedicated for experiments with plant pathogens. School of Science and the Environment receives an annual budget to cover service contracts, upgrades and maintenance of shared equipment, along with new equipment purchases.

Major Equipment

Agilent MP-AES 4200, Agilent GC MS 7890A, Thermo scientific Centrifuge, Shimadzu GC-GC plus (GC2010), Shimadzu LC-MS 2020, Acta Molecular Dimension cristolography unit, various sizes shakers Thermofisher IR spectroscopy, Metrohm Thermo IC, Rotary evaporator, Concentrator (Speed vac) Agilent GC-MS are among the shared major equipment.

Other Resources

Expenses for day-to-day operations (phones, post, copying, and stationaries) are covered by the overhead in the school. IT and Facilities departments are in charge for repairs and maintenance of major structures and electricity. Repairs of major equipment are covered by the special contracts with the relevant companies.

PIs McDowell, Sherif, and Tor will collaborate to oversee the project's administration, advise project personnel, design and interpret experiments, co-author papers, and present at scientific conferences and extension and outreach events.

NSF BIOGRAPHICAL SKETCH

Provide the following information for the Senior personnel.
Follow this format for each person. **DO NOT EXCEED 3 PAGES.**

IDENTIFYING INFORMATION:

NAME: McDowell, John

ORCID: 0000-0002-9070-4874

POSITION TITLE: J.B. Stroobants Professor of Biotechology

ORGANIZATION AND LOCATION: Virginia Tech, Blacksburg, VA, US

Professional Preparation:

ORGANIZATION AND LOCATION	DEGREE (if applicable)	DATE RECEIVED	FIELD OF STUDY
University of Georgia, Athens, GA, US	Ph.D.	1995	Genetics
University of Tennessee, Knoxville, TN, US	B.A., Cell and Molecular Biology	1987	

Appointments and Positions

2018 - present J.B. Stroobants Professor of Biotechology, Virginia Tech, School of Plant and Environmental Sciences, Blacksburg, VA, US

2000 - present Professor (promoted from Assistant Professor in 2006 and Associate Professor in 2013), School of Plant and Environmental Sciences, Virginia Tech, Blacksburg, VA, United States

2021 - 2022 Program Director, National Science Foundation, Alexandria, VA, United States

2013 - 2020 Associate Scientific Director, Fralin Life Science Institute, Virginia Tech, Blacksburg, VA, United States

1995 - 1999 NIH and USDA Postdoctoral Fellow, University of North Carolina, Biology, Chapel Hill, NC, US

Products

Products Most Closely Related to the Proposed Project

1. Bilir Ö, Göl D, Hong Y, McDowell J, Tör M. Small RNA-based plant protection against diseases. Frontiers in Plant Science. 2022; 13:-. Available from: https://www.frontiersin.org/articles/10.3389/fpls.2022.951097/full DOI: 10.3389/fpls.2022.951097
2. Dinkeloo K, Pelly Z, McDowell J, Pilot G. A split green fluorescent protein system to enhance spatial and temporal sensitivity of translating ribosome affinity purification. The Plant Journal. 2022 May 10; 111(1):304-315. Available from: https://onlinelibrary.wiley.com/doi/10.1111/tpj.15779 DOI: 10.1111/tpj.15779
3. Telli O, Jimenez-Quiros C, McDowell J, Tör M. Effect of light and dark on the growth and development of downy mildew pathogen *Hyaloperonospora arabidopsidis*. Plant Pathology.

2020 May 27; 69(7):1291-1300. Available from: https://onlinelibrary.wiley.com/doi/10.1111/ppa.13207 DOI: 10.1111/ppa.13207

4. Deb D, Anderson RG, How-Yew-Kin T, Tyler BM, McDowell JM. Conserved RxLR Effectors From Oomycetes Hyaloperonospora arabidopsidis and Phytophthora sojae Suppress PAMP- and Effector-Triggered Immunity in Diverse Plants. Mol Plant Microbe Interact. 2018 Mar;31(3):374-385. PubMed PMID: 29106332.
5. Baxter L, Tripathy S, Ishaque N, Boot N, Cabral A, Kemen E, Thines M, Ah-Fong A, Anderson R, Badejoko W, Bittner-Eddy P, Boore J, Chibucos M, Coates M, Dehal P, Delehaunty K, Dong S, Downton P, Dumas B, Fabro G, Fronick C, Fuerstenberg S, Fulton L, Gaulin E, Govers F, Hughes L, Humphray S, Jiang R, Judelson H, Kamoun S, Kyung K, Meijer H, Minx P, Morris P, Nelson J, Phuntumart V, Qutob D, Rehmany A, Rougon-Cardoso A, Ryden P, Torto-Alalibo T, Studholme D, Wang Y, Win J, Wood J, Clifton S, Rogers J, Van den Ackerveken G, Jones J, McDowell J, Beynon J, Tyler B. Signatures of Adaptation to Obligate Biotrophy in the *Hyaloperonospora arabidopsidis* Genome. Science. 2010 December 10; 330(6010):1549-1551. Available from: https://www.science.org/doi/10.1126/science.1195203 DOI: 10.1126/science.1195203

Other Significant Products, Whether or Not Related to the Proposed Project

1. Wang W, Liu J, Mishra B, Mukhtar M, McDowell J. Sparking a sulfur war between plants and pathogens. Trends in Plant Science. 2022 December; 27(12):1253-1265. Available from: https://linkinghub.elsevier.com/retrieve/pii/S136013852200187X DOI: 10.1016/j.tplants.2022.07.007
2. Wilson R, McDowell J. Recent advances in understanding of fungal and oomycete effectors. Current Opinion in Plant Biology. 2022 August; 68:102228-. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1369526622000577 DOI: 10.1016/j.pbi.2022.102228
3. Cui C, Herlihy J, Bombarely A, McDowell J, Haak D. Draft Assembly of *Phytophthora capsici* from Long-Read Sequencing Uncovers Complexity. Molecular Plant-Microbe Interactions®. 2019 December; 32(12):1559-1563. Available from: https://apsjournals.apsnet.org/doi/10.1094/MPMI-04-19-0103-TA DOI: 10.1094/MPMI-04-19-0103-TA
4. Deb D, Mackey D, Opiyo SO, McDowell JM. Application of alignment-free bioinformatics methods to identify an oomycete protein with structural and functional similarity to the bacterial AvrE effector protein. PLoS One. 2018;13(4):e0195559. PubMed Central PMCID: PMC5895030.
5. Michelmore R, Coaker G, Bart R, Beattie G, Bent A, Bruce T, Cameron D, Dangl J, Dinesh-Kumar S, Edwards R, Eves-van den Akker S, Gassmann W, Greenberg J, Hanley-Bowdoin L, Harrison R, Harvey J, He P, Huffaker A, Hulbert S, Innes R, Jones J, Kaloshian I, Kamoun S, Katagiri F, Leach J, Ma W, McDowell J, Medford J, Meyers B, Nelson R, Oliver R, Qi Y, Saunders D, Shaw M, Smart C, Subudhi P, Torrance L, Tyler B, Valent B, Walsh J. Foundational and Translational Research Opportunities to Improve Plant Health. Molecular Plant-Microbe Interactions®. 2017 July; 30(7):515-516. Available from: https://apsjournals.apsnet.org/doi/10.1094/MPMI-01-17-0010-CR DOI: 10.1094/MPMI-01-17-

0010-CR

Synergistic Activities

1. Editorships: Deputy Editor, Science Advances (2019-present); Editorial Board, Annual Review of Phytopathology, (2020-21; Co-Editor-in-Chief 2022-26); Editor-in-Chief, Molecular Plant-Microbe Interactions (2016-18); Senior Editor, Molecular Plant-Microbe Interactions (2010-15); Editorial Board, PLOS Genetics (2012-15); Editorial Board, Molecular Biotechnology (2008-15); Advisory Board, The Plant Journal (2004-08); Editorial Board, Molecular Plant Pathology (2000-05); Associate Editor, Molecular Plant-Microbe Interactions (2000-05).
2. Invited Presentations: International Society for Molecular Plant-Microbe Interactions Congresses, 2009, 2014, 2016; International Congress for Plant-Biotic Interactions, 2015; Brazilian Phytopathologial Society Conference, 2015; International Soilborne Oomycete Diseases Conference, 2015; Phytophthora Molecular Genetics Meeting, 2004, 2006, 2009, 2012, 2014, 2019; Practical Summer Workshop in Functional Genomics, Ohio State University, 2014; Keystone Symposia, Plant Immunity, 2008, 2013; International Congress for Arabidopsis Research, 2012, American Society of Plant Biologists, 2011, Plant and Animal Genome, 2010; International Congress of Developmental and Comparative Immunology, 2006; American Phytopathological Society Annual Meeting, 2001, 2006, 2020; Keystone Symposium: Functions and Control of Plant Cell Death, 2003.
3. Outreach: Partnership in Research and Education Using Plants (PREP). This outreach program enables high school students to conduct experiments in their classrooms using mutants of Arabidopsis. I advise on experimental design and execution, produced an interactive video about my research, and travel to high schools to interact with participants and discuss my research. Also numerous presentations about agricultural research to various groups within and outside the university, and testimony to US Senate and House Committees on Agriculture.
4. Education: Academy of Faculty Service, Virginia Tech, 2017; Certificate for Teaching Excellence, Virginia Tech, 2010; Extensively revised graduate-level courses in Molecular Biology and Molecular Plant-Microbe Interactions; Created new graduate course in Translational Plant Science, 2013. Chair, Molecular Plant Sciences Interdepartmental Ph.D. Program, Virginia Tech, 2008-09. PI of successful, internally funded proposal for graduate program in Translational Plant Science.

Certification:

When the individual signs the certification on behalf of themselves, they are certifying that the information is current, accurate, and complete. This includes, but is not limited to, information related to domestic and foreign appointments and positions. Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§ 3729-3733 and 3802.

Certified by McDowell, John in SciENcv on 2023-02-10 15:35:50

NSF BIOGRAPHICAL SKETCH

Provide the following information for the Senior personnel.
Follow this format for each person. **DO NOT EXCEED 3 PAGES.**

IDENTIFYING INFORMATION:

NAME: Tor, Mahmut

ORCID: 0000-0002-4416-5048

POSITION TITLE: Professor

ORGANIZATION AND LOCATION: University of Worcester, Worcester, Worcestershire, GB

Professional Preparation:

ORGANIZATION AND LOCATION	DEGREE (if applicable)	DATE RECEIVED	FIELD OF STUDY
University of London, Ashford, Kent, GB	PhD	1991	Plant Biotechnology
University of London, Ashford, Kent, GB	MSc	1987	Applied Plant Sciences
Çukurova Üniversitesi Ziraat Fakültesi, Adana, Adana, TR	BSc	1984	Plant Protection

Appointments and Positions

2010 - 2014 Senior Lecturer, University of Worcester, Institute of Science and the Environment, Worcester, Worcestershire, GB

2003 - 2010 Group Leader, University of Warwick, Warwick-HRI, Coventry, Coventry, GB

2000 - 2003 Post-doctoral Research Fellow, University of Warwick, Warwic-HRI, Coventry, Coventry, GB

1997 - 2000 Post-doctoral Research Fellow, University of Warwick, HRI, Coventry, Coventry, GB

1994 - 1997 Assistant Prof., Akdeniz Üniversitesi, Ziraat Fakultesi, Plant Protection, Antalya, TR

1991 - 1994 Post-doctoral Research Fellow, Imperial College London - Wye Campus, Biological Sciences, Ashford, Kent, GB

Products

Products Most Closely Related to the Proposed Project

1. Bailey K, Cevik V, Holton N, Byrne-Richardson J, Sohn KH, Coates M, Woods-Tör A, Aksoy HM, Hughes L, Baxter L, Jones JD, Beynon J, Holub EB, Tör M. Molecular cloning of ATR5(Emoy2) from Hyaloperonospora arabidopsidis, an avirulence determinant that triggers RPP5-mediated defense in Arabidopsis. Mol Plant Microbe Interact. 2011 Jul;24(7):827-38. PubMed PMID: 21361788.
2. Zhang X, Kang L, Zhang Q, Meng Q, Pan Y, Yu Z, Shi N, Jackson S, Zhang X, Wang H, Tor M, Hong Y. An RNAi suppressor activates in planta virus–mediated gene editing. Functional &

Integrative Genomics. 2019 December 18; 20(4):471-477. Available from: http://link.springer.com/10.1007/s10142-019-00730-y DOI: 10.1007/s10142-019-00730-y

3. Bilir Ö, Telli O, Norman C, Budak H, Hong Y, Tör M. Small RNA inhibits infection by downy mildew pathogen *Hyaloperonospora arabidopsidis*. Molecular Plant Pathology. 2019 September 26; 20(11):1523-1534. Available from: https://onlinelibrary.wiley.com/doi/10.1111/mpp.12863 DOI: 10.1111/mpp.12863
4. Telli O, Jimenez-Quiros C, McDowell J, Tör M. Effect of light and dark on the growth and development of downy mildew pathogen *Hyaloperonospora arabidopsidis*. Plant Pathology. 2020 May 27; 69(7):1291-1300. Available from: https://onlinelibrary.wiley.com/doi/10.1111/ppa.13207 DOI: 10.1111/ppa.13207
5. Bilir Ö, Göl D, Hong Y, McDowell J, Tör M. Small RNA-based plant protection against diseases. Frontiers in Plant Science. 2022; 13:-. Available from: https://www.frontiersin.org/articles/10.3389/fpls.2022.951097/full DOI: 10.3389/fpls.2022.951097

Other Significant Products, Whether or Not Related to the Proposed Project

1. Lotze MT, Zeh HJ, Rubartelli A, Sparvero LJ, Amoscato AA, Washburn NR, Devera ME, Liang X, Tör M, Billiar T. The grateful dead: damage-associated molecular pattern molecules and reduction/oxidation regulate immunity. Immunol Rev. 2007 Dec;220:60-81. PubMed PMID: 17979840.
2. Wang W, Barnaby J, Tada Y, Li H, Tör M, Caldelari D, Lee D, Fu X, Dong X. Timing of plant immune responses by a central circadian regulator. Nature. 2011; 470(7332):110-114. Available from: http://www.nature.com/articles/nature09766 DOI: 10.1038/nature09766
3. Kamoun S, Furzer O, Jones J, Judelson H, Ali G, Dalio R, Roy S, Schena L, Zambounis A, Panabières F, Cahill D, Ruocco M, Figueiredo A, Chen X, Hulvey J, Stam R, Lamour K, Gijzen M, Tyler B, Grünwald N, Mukhtar M, Tomé D, Tör M, Van Den Ackerveken G, McDowell J, Daayf F, Fry W, Lindqvist-Kreuze H, Meijer H, Petre B, Ristaino J, Yoshida K, Birch P, Govers F. The Top 10 oomycete pathogens in molecular plant pathology. Molecular Plant Pathology. 2015 May; 16(4):413-434. Available from: https://onlinelibrary.wiley.com/doi/10.1111/mpp.12190 DOI: 10.1111/mpp.12190
4. Johansson O, Fantozzi E, Fahlberg P, Nilsson A, Buhot N, Tör M, Andersson M. Role of the penetration-resistance genes *PEN1, PEN2* and *PEN3* in the hypersensitive response and race-specific resistance in *Arabidopsis thaliana*. The Plant Journal. 2014 August; 79(3):466-476. Available from: https://onlinelibrary.wiley.com/doi/10.1111/tpj.12571 DOI: 10.1111/tpj.12571
5. Mohamed A, Jin Z, Osman T, Shi N, Tör M, Jackson S, Hong Y. Hotspot siRNA Confers Plant Resistance against Viral Infection. Biology. 2022 May 06; 11(5):714-. Available from: https://www.mdpi.com/2079-7737/11/5/714 DOI: 10.3390/biology11050714

Synergistic Activities

1. Committees, Societies and Editorships: Core Member of BBSRC Grant Committee, 2020-Present; BSPP (British Society for Plant Pathology) Treasurer, 2017-2022; Associate Editor, Frontiers in Plant–Microbe Interactions, 2014-Present; Editorial Board of Journal of Environment and Agricultural Studies, 2014-Present.

2. Invited Presentations: 1st International Congress on Biotech Studies, 2022; 21st Annual Meeting of the Oomycete Molecular Genetics Network, 2022; 3rd International, Biological & Life Science Conference, Edirne, Turkey, 2022; Plant Biology World-wide Summit, 2021;
3. Education: 2010-2021 Module Leader, BIOS2024 Infectious Agents and Allergens for second year students; 2010-2015: Module Leader, HORT 3010 Plant Breeding and Gene Technology; 2011-Present: Supervising 3rd year undergraduate students for their independent studies; 2008-2010: Weekly tutorials to 1st year students; 2007-2009 Post graduate certificate in Academic and Professional Practice (PCAPP).
4. Education: 2010-2021 Module Leader, BIOS2024 Infectious Agents and Allergens for second year students; 2010-2015: Module Leader, HORT 3010 Plant Breeding and Gene Technology; 2011-Present: Supervising 3rd year undergraduate students for their independent studies; 2008-2010: Weekly tutorials to 1st year students; 2007-2009 Post graduate certificate in Academic and Professional Practice (PCAPP).

Certification:

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Certified by Tor, Mahmut in SciENcv on 2023-02-14 08:09:17

NSF BIOGRAPHICAL SKETCH

Provide the following information for the Senior personnel.
Follow this format for each person. **DO NOT EXCEED 3 PAGES.**

IDENTIFYING INFORMATION:

NAME: Sherif, Sherif

ORCID: 0000-0002-2801-0679

POSITION TITLE: Assistant Professor

ORGANIZATION AND LOCATION: Virginia Tech, Winchester, VA, United States

Professional Preparation:

ORGANIZATION AND LOCATION	DEGREE (if applicable)	DATE RECEIVED	FIELD OF STUDY
University of Guelph, Guelph, ON, Canada	PHD	2011	Molecular Plant-Microbe Interactions
Alexandria University, Damanhour, Beheira, Egypt	MS	2004	Tree Fruit Breeding and Genetics
Alexandria University, Damanhour, Beheira, Egypt	BS	2000	Pomology

Appointments and Positions

2017 - present Assistant Professor, Virginia Tech, School of Plant and Environmental Sciences, Winchester, VA, United States

2014 - 2016 Postdoctoral Research Assosciate, University of Guelph, Department of Plant Agriculture, Guelph, Ontario, Canada

2012 - 2014 Postdoctoral Research Fellow, University of Guelph, The Gosling Research Institute for Plant Preservation, Guelph, Ontario, Canada

2012 - 2012 MITACS Accelerate Fellow, University of Guelph, Department of Plant Agriculture, Guelph, Ontario, Canada

Products

Products Most Closely Related to the Proposed Project

1. Nigg M, de Oliveira TC, Sarmiento-Villamil JL, de la Bastide PY, Hintz WE, Sherif SM, Shukla M, Bernier L, Saxena PK. Comparative Analysis of Transcriptomes of *Ophiostoma novo-ulmi* ssp. *americana* Colonizing Resistant or Sensitive Genotypes of American Elm. J Fungi (Basel). 2022 Jun 16;8(6) PubMed Central PMCID: PMC9224576.
2. Islam MT, Coutin JF, Shukla M, Dhaliwal AK, Nigg M, Bernier L, Sherif SM, Saxena PK. Deciphering the Genome-Wide Transcriptomic Changes during Interactions of Resistant and Susceptible Genotypes of American Elm with *Ophiostoma novo-ulmi*. J Fungi (Basel). 2022 Jan 26;8(2) PubMed Central PMCID: PMC8874831.

3. Islam MT, Davis Z, Chen L, Englaender J, Zomorodi S, Frank J, Bartlett K, Somers E, Carballo SM, Kester M, Shakeel A, Pourtaheri P, Sherif SM. Minicell-based fungal RNAi delivery for sustainable crop protection. Microb Biotechnol. 2021 Jul;14(4):1847-1856. PubMed Central PMCID: PMC8313293.
4. Sherif SM, Shukla MR, Murch SJ, Bernier L, Saxena PK. Simultaneous induction of jasmonic acid and disease-responsive genes signifies tolerance of American elm to Dutch elm disease. Sci Rep. 2016 Feb 23;6:21934. PubMed Central PMCID: PMC4763294.
5. Sherif S, Jones AM, Shukla MR, Saxena PK. Establishment of invasive and non-invasive reporter systems to investigate American elm-Ophiostoma novo-ulmi interactions. Fungal Genet Biol. 2014 Oct;71:32-41. PubMed PMID: 25139300.

Other Significant Products, Whether or Not Related to the Proposed Project

1. Sherif S, Erland L, Shukla M, Saxena P. Bark and wood tissues of American elm exhibit distinct responses to Dutch elm disease. Scientific Reports. 2017 August 02; 7(1):-. Available from: https://www.nature.com/articles/s41598-017-07779-4 DOI: 10.1038/s41598-017-07779-4
2. Sherif S, El-Sharkawy I, Mathur J, Ravindran P, Kumar P, Paliyath G, Jayasankar S. A stable JAZ protein from peach mediates the transition from outcrossing to self-pollination. BMC Biol. 2015 Feb 13;13:11. PubMed Central PMCID: PMC4364584.
3. Sherif S, El-Sharkawy I, Paliyath G, Jayasankar S. PpERF3b, a transcriptional repressor from peach, contributes to disease susceptibility and side branching in EAR-dependent and -independent fashions. Plant Cell Rep. 2013 Jul;32(7):1111-24. PubMed PMID: 23515898.
4. Sherif S, El-Sharkawy I, Paliyath G, Jayasankar S. Differential expression of peach ERF transcriptional activators in response to signaling molecules and inoculation with Xanthomonas campestris pv. pruni. J Plant Physiol. 2012 May 1;169(7):731-9. PubMed PMID: 22410465.
5. Sherif S, Paliyath G, Jayasankar S. Molecular characterization of peach PR genes and their induction kinetics in response to bacterial infection and signaling molecules. Plant Cell Rep. 2012 Apr;31(4):697-711. PubMed PMID: 22101723.

Synergistic Activities

1. Education: served as a guest lecturer for undergraduate and graduate courses at a number of institutions, including Virginia Tech (2018-Present), American University of Beirut (2021), and the University of Guelph (2011-14); worked as a Teaching Assistant for five pomology courses from 2001-06; had the privilege of serving as academic advisor and co-advisor for 12 graduate students from various ethnic and cultural backgrounds; organized and co-organized panels and workshops at various institutions, including Tamil Nadu Agricultural University in India in 2013, American Society of Horticultural Sciences (ASHS) from 2020-22, and Virginia Tech in both 2018 and 2019.
2. Outreach: manage the "Tree Fruit Horticulture Updates" blog website for over 320 tree fruit producers, and use modern technology to send subscribers instant email notifications when new blog posts are released; manage the "Virginia Tech Tree Fruit Extension and Outreach" Facebook page with over 1,100 dedicated followers; co-organize an annual open-house day that brings together 80-100 attendees from diverse backgrounds; and co-organize an annual field-day to showcase our field trial findings to agrichemical company representatives and a wider audience.

3. Professional organizations: served as an executive board member for the Cumberland Shenandoah Fruit Workers Conference (2022-present), secretary (2020), chair-elect (2021), then chair (2022) of the pomology interest group- ASHS; Shepard award committee member- American Pomological Society (2018-21); and full member in Sigma Xi, the Scientific Research Honor Society (2022-present).
4. Consultant: for the Examiner Education Office, Washington DC, United States, Federal Financial Institutions Examination Council (FFIEC) (2018). The purpose of this activity was to help financial institution examiners at the national level identify areas of risk in various types of agricultural loans and to discuss ways for bankers to minimize those risks.
5. Editorial Boards: Guest editor; Frontiers in Plant Science (2020-21), Editorial board member; Horticulturae (2022-Present) and Ad-hoc reviewer for 12 scientific journals, including New Phycologist, Horticulture Research and Microbial Biotechnology.

Certification:

When the individual signs the certification on behalf of themselves, they are certifying that the information is current, accurate, and complete. This includes, but is not limited to, information related to domestic and foreign appointments and positions. Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§ 3729-3733 and 3802.

Certified by Sherif, Sherif in SciENcv on 2023-02-14 12:06:11

Other Personnel Biographical Information

Data Not Available

CURRENT AND PENDING (OTHER) SUPPORT INFORMATION

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person.

*NAME: McDowell, John

PERSISTENT IDENTIFIER (PID) OF THE SENIOR/KEY PERSON: https://orcid.org/0000-0002-9070-4874

*POSITION TITLE: J.B. Stroobants Professor of Biotechnology, School of Plant and Environmental Sciences

*ORGANIZATION AND LOCATION: Virginia Tech, Blacksburg, Virginia, United States

Projects/Proposals

***Project/Proposal Title:** J.B. Stroobants Professorship
***Status of Support:** current
Proposal/Award Number:
***Source of Support:** Virginia Tech Endowed Professorship
***Primary Place of Performance:** Virginia Tech
***Project/Proposal Support Start Date: (MM/YYYY):** 01/2023
***Project/Proposal Support End Date: (MM/YYYY):** 12/2027
***Total Award Amount:** $20,000

*** Person Months (Calendar/Academic/Summer) per budget period Committed to the Project:**

Year	Person Months
2023	1

***Overall Objectives:** This unrestricted funding is used to support several on-going projects in the McDowell lab.

***Statement of Potential Overlap:** These funds will not be used to support the proposed research if this proposal is funded.

***Project/Proposal Title:** Examining the use of biochar to improve lavender disease resistance in Virginia
***Status of Support:** current
Proposal/Award Number:
***Source of Support:** Virginia Department of Agriculture & Consumer Services
***Primary Place of Performance:** Virginia Tech
***Project/Proposal Support Start Date: (MM/YYYY):** 10/2021

***Project/Proposal Support End Date: (MM/YYYY):** 09/2023

***Total Award Amount:** $68,383

*** Person Months (Calendar/Academic/Summer) per budget period Committed to the Project:**

Year	Person Months
2023	1

***Overall Objectives:** This project is to examine the efficacy of biochar soil treatments in promoting disease-resistance in a model specialty crop system using greenhouse and field studies. This project will develop diagnostic tools and growing recommendations for lavender farmers afflicted with Phytophthora infections that will be shared with lavender growers via U. S. Lavender Grower newsletters, social media, and conferences.

***Statement of Potential Overlap:** N/A

***Project/Proposal Title:** Deciphering pathogenicity and development in obligate downy mildew pathogen using small RNA approach

***Status of Support:** current

Proposal/Award Number:

***Source of Support:** UK-BBSRC

***Primary Place of Performance:** Virginia Tech

***Project/Proposal Support Start Date: (MM/YYYY):** 03/2021

***Project/Proposal Support End Date: (MM/YYYY):** 02/2024

***Total Award Amount:** $40,097

*** Person Months (Calendar/Academic/Summer) per budget period Committed to the Project:**

Year	Person Months
2023	1

***Overall Objectives:** The aim of this project is to use a small RNA (sRNA) approach to increase our understanding of plant - biotrophic oomycete microbe interactions. We are using Arabidopsis-Hyaloperonospora model as the model system and representative of the obligate oomycetes. We are using a sRNA-based genetic screen to identify and study genes specifically involved in processes that are poorly understood in obligate oomycetes. Objective 1: Investigate the properties of sRNA-mediated silencing, optimize, and test in other oomycetes. Objective 2: Generate gene-specific sRNAs for highly regulated genes in spores, during germination, mycelial development and sporulation. Objective 3: Identify genes showing a phenotype upon silencing. Objective 4: Investigate some of the well-known effector genes under native conditions.

***Statement of Potential Overlap:** This experiments in this project are focused exclusively on SS-dsRNAs as triggers of RNAi. Data from this project was used for selection of the Ces3A and T814784 target genes and provides a baseline for optimization of protocols that employ NIVT- and EM-dsRNAs. There is no overlap between the BBSRC-funded project and the experiments described in the NSF proposal.

***Project/Proposal Title:** EDGE FGT: RNAi-based tools to unlock functional genomics of obligate oomycete plant pathogens

***Status of Support:** pending

Proposal/Award Number: (this proposal)

***Source of Support:** National Science Foundation

***Primary Place of Performance:** Virginia Tech

***Project/Proposal Support Start Date: (MM/YYYY):** 11/2023

***Project/Proposal Support End Date: (MM/YYYY):** 10/2025

***Total Award Amount:** $400,000

*** Person Months (Calendar/Academic/Summer) per budget period Committed to the Project:**

Year	Person Months
2023	0.2
2024	1
2025	1

***Overall Objectives:** As described in the project summary

***Statement of Potential Overlap:** N/A

***Project/Proposal Title:** Next-Generation Biofungicides for Application in Controlled Environment Agriculture

***Status of Support:** pending

Proposal/Award Number: PP3N3VHV

***Source of Support:** Virginia Dept. of Agriculture & Consumer Services

***Primary Place of Performance:** Virginia Tech

***Project/Proposal Support Start Date: (MM/YYYY):** 10/2023

***Project/Proposal Support End Date: (MM/YYYY):** 09/2025

***Total Award Amount:** $74,939

*** Person Months (Calendar/Academic/Summer) per budget period Committed to the Project:**

Year	Person Months
2023	0.2
2024	0.5
2025	0.4

***Overall Objectives:** A team of researchers at Virginia Tech will develop and evaluate the use of biofungicides for control of common diseases of food crops produced in controlled environment agriculture which is limited int eh number of pesticides that can be utilized. Specific Objectives are to: Objective 1: Develop minicell-enclosed dsRNAs. Target genes in the pathogen will be identified with computational tools.

E. coli strains that express dsRNAs against these genes will be engineered and mini-cells will be isolated for Objectives 3 and 4. Objective 2: The RNase3 gene will be deleted/replaced with a double-strand RNA expression construct in the PsJN genome. The engineered PsJN strain is expected to be able to express and deliver dsRNAs targeting genes as described in Objective 1. Objective 3: Evaluate the efficacy of the minicell-encapsulated RNAi and endophytes in the control of grey mould in strawberry and downy mildew in spinach under a range of environmental conditions in growth chamber settings. Objective 4: Test the efficacy of developed minicell-encapsulated RNAi and endophytes in the control of gray mold in strawberry and downy mildew in spinach produced in a greenhouse under typical greenhouse conditions.

***Statement of Potential Overlap:** The two proposals do not overlap, but the VDACS project (if funded) could be enhanced by the tool development funded by the NSF EDGE proposal.

Certification:

When the individual signs the certification on behalf of themselves, they are certifying that the information is current, accurate, and complete. This includes, but is not limited to, information related to current, pending, and other support (both foreign and domestic) as defined in 42 U.S.C. §§ 6605. Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§ 3729- 3733 and 3802.

Certified by McDowell, John in SciENcv on 2023-07-05 08:45:05

CURRENT AND PENDING (OTHER) SUPPORT INFORMATION

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person.

*NAME: Tor, Mahmut

PERSISTENT IDENTIFIER (PID) OF THE SENIOR/KEY PERSON: https://orcid.org/0000-0002-4416-5048

*POSITION TITLE: Professor

*ORGANIZATION AND LOCATION: University of Worcester, Worcester, United Kingdom

Projects/Proposals

***Project/Proposal Title:**	Microbial biological control agents for downy mildew diseases (MBCA4DM)
***Status of Support:**	current
Proposal/Award Number:	BB/X018253/1
***Source of Support:**	BBSRC
***Primary Place of Performance:**	University of Worcester
***Project/Proposal Support Start Date: (MM/YYYY):**	06/2023
***Project/Proposal Support End Date: (MM/YYYY):**	06/2026
***Total Award Amount:**	$36,272

*** Person Months (Calendar/Academic/Summer) per budget period Committed to the Project:**

Year	Person Months
2023	0.5
2024	0.5
2025	0.5
2026	0.5

***Overall Objectives:** The aim of this partnering is to develop a long-term UK-TR team with a mutual interest on controlling downy mildew diseases and a unique combination of expertise on plant pathology, omics, plant breeding and microbiology. Partner's translational objectives and research cultures are well aligned. This partnership would allow to bring experts together for knowledge exchange. We will focus on the use of MBCA against the obligate oomycete downy mildew pathogens. Our specific objectives are as follows. Objective 1: Consolidate existing connections between the collaborators to provide complementary competencies to our own capabilities. Objective 2: Generate new scientific insight to translate laboratory-based research on MBCA into application. Objective 3: Provide a platform for knowledge exchange between academics and industry.

***Statement of Potential Overlap:** N/A

***Project/Proposal Title:** siRNA for Disease control

***Status of Support:** current

Proposal/Award Number: BB/X018245/1

***Source of Support:** BBSRC

***Primary Place of Performance:** University of Worcester

***Project/Proposal Support Start Date: (MM/YYYY):** 05/2023

***Project/Proposal Support End Date: (MM/YYYY):** 05/2027

***Total Award Amount:** $60,762

*** Person Months (Calendar/Academic/Summer) per budget period Committed to the Project:**

Year	Person Months
2023	0.1
2024	0.1
2025	0.1
2026	0.1
2027	0.1

***Overall Objectives:** The aim of this partnering is to develop a UK-US team with a unique combination of expertise in plant-oomycete and plantfungal interactions, and novel, newly developed sRNA-based disease control methods.. We will focus on the oomycete crop pathogens including PVP (pea DM) and the two Phytophthora species, P. parasitica (a root pathogen with broad host range) and P. capsici (blight and fruit rot of peppers and other important commercial crops). The partners' fundamental and translational objectives, and research cultures are well-aligned. This partnership would allow the optimisation of resource uses and mitigate risks. Objective 1: Consolidate existing connections between the collaborators to provide complementary competencies to our own capabilities. Objective 2: Generate new scientific insight to translate laboratory-based research on sRNA into application. Objective 3: Provide a platform for knowledge exchange between academics and industry.

***Statement of Potential Overlap:** This pending proposal is for partnering with US collaborators and includes a workshop. It is not research project proposal and thus there is no overlapping.

***Project/Proposal Title:** NGS-Assisted plant genome editing using CRISPR technology

***Status of Support:** current

Proposal/Award Number: 21/22-0125

***Source of Support:** Manier Seed Ltd.

***Primary Place of Performance:** University of Worcester

***Project/Proposal Support Start Date: (MM/YYYY):** 09/2022

***Project/Proposal Support End Date: (MM/YYYY):** 08/2025

***Total Award Amount:** $13,350

*** Person Months (Calendar/Academic/Summer) per budget period Committed to the Project:**

Year	Person Months
2022	0.1
2023	0.1
2024	0.1
2025	0.1

***Overall Objectives:** Generate transformation constructs with guide RNA

***Statement of Potential Overlap:** N/A

***Project/Proposal Title:** Deciphering pathogenicity and development in obligate downy mildew pathogen using small RNA approach

***Status of Support:** current

Proposal/Award Number: BB/V014609/1

***Source of Support:** BBSRC

***Primary Place of Performance:** University of Worcester

***Project/Proposal Support Start Date: (MM/YYYY):** 01/2022

***Project/Proposal Support End Date: (MM/YYYY):** 12/2024

***Total Award Amount:** $763,146

*** Person Months (Calendar/Academic/Summer) per budget period Committed to the Project:**

Year	Person Months
2022	1
2023	1
2024	1

***Overall Objectives:** Deciphering the gene functions in the obligate oomycetes has been difficult due to the lack of an efficient genetic manipulation method. Our preliminary results clearly show that we established a method that can be used to reveal functions of many genes within the downy mildew pathogen Hyaloperonospora arabidopsidis (Hpa). The AIM of this proposal is to use a small RNA (sRNA) approach to increase our understanding of plant - biotrophic oomycete microbe interactions. We will use Arabidopsis-Hyaloperonospora model as the model system and representative of the obligate oomycetes. We will use a high-throughput, sRNA-based genetic screen to identify and study genes specifically involved in processes that are poorly understood in obligate oomycetes. Objective 1: Investigate the properties of sRNA-mediated silencing, optimize, and test in other oomycetes. Objective 2: Generate gene-specific sRNAs for highly regulated genes in spores, during germination, mycelial development and sporulation. Objective 3: Identify genes showing a phenotype upon silencing. Objective 4: Investigate some of the well-known effector genes under native conditions.

***Statement of Potential Overlap:** There is very little overlap with the project as this one uses high throughput reverse genetics to identify and study genes specifically in development and pathogenicity. We

will use the knowledge generated in this project to accelerate the research described in the proposal.

***Project/Proposal Title:** Pulse-Downy Mildew Pathosystem: deploying disease resistance, pathogenomics and microbial biocontrol

***Status of Support:** current

Proposal/Award Number: BB/T016043/1

***Source of Support:** BBSRC

***Primary Place of Performance:** University of Worcester

***Project/Proposal Support Start Date: (MM/YYYY):** 11/2021

***Project/Proposal Support End Date: (MM/YYYY):** 10/2023

***Total Award Amount:** $493,119

*** Person Months (Calendar/Academic/Summer) per budget period Committed to the Project:**

Year	Person Months
2021	1
2022	1
2023	1

***Overall Objectives:** This project focuses on the identification of new R-genes for breeding purposes, the development of tools for accurate detection and diagnostics of Pvp/Pvf isolates and the evaluation of microbial biological control agents to suppress downy mildew pathogens. The use of appropriate molecular tools will enable breeders, epidemiologists, modellers and growers to: a) identify the prevailing virulent isolates; b) investigate the epidemics of disease; c) monitor pathogen movement and d) deploy the appropriate cultivar(s) resistant to the prevailing isolate(s) rapidly and thus control the disease in an environmentally friendly and sustainable manner. The protection of valuable resistance sources through novel biocontrol agents is likely to further prolong their effectiveness, reducing the chances of damaging pathotypes arising and becoming established. Specifically, the project will: 1) Characterise the genetic basis of resistance in pea and broad bean to Pvp and Pvf, respectively. 2) Construct annotated genomes of Pvp/Pvf to enable pathogenomics to generate molecular markers for monitoring DM races. 3) Develop isolate-specific diagnostic tools to increase speed/accuracy of detection of Pvp/Pvf and decrease reliance on lengthy differential testing. 4) Identify effectors that differentiate Pvp and Pvf races. 5) Explore microbial biological control agents to suppress Pvp/Pvf.

***Statement of Potential Overlap:** N/A

***Project/Proposal Title:** RNAi-based tools to unlock functional genomics of obligate oomycete plant pathogens

***Status of Support:** pending

Proposal/Award Number: 2319757

***Source of Support:** NSF

***Primary Place of Performance:** University of Worcester

***Project/Proposal Support Start Date: (MM/YYYY):** 11/2023

***Project/Proposal Support End Date: (MM/YYYY):** 10/2025

***Total Award Amount:** $103,716

*** Person Months (Calendar/Academic/Summer) per budget period Committed to the Project:**

Year	Person Months
2023	0.1
2024	0.3

***Overall Objectives:** As described in the project summary

***Statement of Potential Overlap:** N/A

Certification:

When the individual signs the certification on behalf of themselves, they are certifying that the information is current, accurate, and complete. This includes, but is not limited to, information related to current, pending, and other support (both foreign and domestic) as defined in 42 U.S.C. §§ 6605. Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§ 3729- 3733 and 3802.

Certified by Tor, Mahmut in SciENcv on 2023-07-05 11:35:34

Effective 01/30/2023 NSF CURRENT AND PENDING (OTHER) SUPPORT OMB-3145-0058

*Name: Sherif M Sherif

ORCID ID (Optional): 0000-0002-2801-0679

*Position Title : Assistant Professor

*Organization: Virginia Tech

*Location: Winchester, VA

Certification:

When the individual signs the certification on behalf of themselves, they are certifying that the information is current, accurate, and complete. This includes, but is not limited to, information related to current, pending, and other support (both foreign and domestic) as defined in 42 U.S.C. §§6605. Misrepresentations and/or omissions may be subject to prosecution and liability pursuant to, but not limited to, 18 U.S.C. §§ 287, 1001, 1031 and 31 U.S.C. §§3729-3733 and 3802.

Signature
(Please type out full name): Sherif M. Sherif Date: 02/15/2023

***Required fields**

Note: NSF has provided 15 project/proposal and 10 in-kind contribution entries for users to populate. Please leave any unused entries blank.

Projects/Proposals Section:

According to 42 U.S.C. §§ 6605, Current and Pending (Other) Support (A) means all resources made available, or expected to be made available, to an individual in support of the individual's research and development efforts, regardless of (i) whether the source of the resource is foreign or domestic; (ii) whether the resource is made available through the entity applying for a research and development award or directly to the individual; or (iii) whether the resource has monetary value; and (B) includes in-kind contributions requiring a commitment of time and directly supporting the individual's research and development efforts, such as the provision of office or laboratory space, equipment, supplies, employees, or students. If the time commitment or dollar value is not readily ascertainable, reasonable estimates should be provided.

Projects/Proposals

1. *Project/Proposal Title : Investigating the Environmental Persistence and Target Specificity of Minicells-Encapsulated RNAi BioFungicides

*Status of Support : (•) Current ( ) Pending

Proposal/Award Number (if available):

*Source of Support: USDA-NIFA-BRAG

*Primary Place of Performance : Virginia Tech

*Project/Proposal Start Date (MM/YYYY) : 09/2022

*Project/Proposal End Date (MM/YYYY) : 08/2025

*Total Award Amount : $ 494,581.00

*Person-Month(s) (or Partial Person-Months) Per Year Committed to the Project

If the time commitment is not readily ascertainable, reasonable estimates should be provided.

*Year (YYYY)	*Person Months (##.##)	Year (YYYY)	Person Months (##.##)
1. 2023	1.00	4.	
2. 2024	1.00	5.	
3. 2025	1.00		

*Overall Objectives : The research project aims to address regulatory concerns related to the use of minicell-encapsulated dsRNA (ME-dsRNA) in biopesticides, as outlined by FIFRA. The project will provide science-based guidelines for regulating encapsulated-dsRNAs, with objectives that include studying its environmental fate, effects on non-target organisms, and ensuring it has no adverse effects on beneficial microorganisms and insects.

*Statement of Potential Overlap : The project's primary focus is to study the stability of minicell-encapsulated dsRNA in soil, water sources, and plant surfaces, with no consideration for its use in disease control. Thus, there is no potential overlap between this project and the functional genomics or disease control aspects of the current NSF proposal

Projects/Proposals

2. *Project/Proposal Title : Optimization and commercialization of environmentally-sustainable biofungicides

*Status of Support : (●) Current ( ) Pending

Proposal/Award Number (if available):

*Source of Support: Virginia Biosciences Health Research Corporation

*Primary Place of Performance : Virginia Tech

*Project/Proposal Start Date (MM/YYYY) : 11/2021

*Project/Proposal End Date (MM/YYYY) : 06/2023

*Total Award Amount : $ 600,000.00

*Person-Month(s) (or Partial Person-Months) Per Year Committed to the Project

If the time commitment is not readily ascertainable, reasonable estimates should be provided.

*Year (YYYY)	*Person Months (##.##)	Year (YYYY)	Person Months (##.##)
1. 2023	0.50	4.	
2.		5.	
3.			

*Overall Objectives : AgroSpheres has successfully developed a new biopesticide that controls gray mold on strawberries in greenhouse conditions for at least 12 days. This project's primary goal is to optimize the target genes' size, structure, and application timing for consistent >80% species-specific control of gray mold and powdery mildew diseases and test ME-dsRNA formulas on commercial farms to demonstrate commercial viability in harsh open field

*Statement of Potential Overlap : The project focuses solely on optimizing commercial minicell-encapsulated dsRNA (ME-dsRNA) formulas for gray mold and powdery mildew pathogens, with no investigation into ME-dsRNA's use for oomycetes or any functional genomics aspects. Therefore, there is no expected overlap between the project and the current NSF proposal. Moreover, the project is set to conclude by June of this year

Projects/Proposals

3. *Project/Proposal Title : Evaluation of Cellulose Nanocrystals (CNCs) for Frost Protection of Apple and Stone Fruits

*Status of Support : (•) Current ( ) Pending

Proposal/Award Number (if available):

*Source of Support: Virginia Department of Agriculture and Consumer Services

*Primary Place of Performance : Virginia Tech

*Project/Proposal Start Date (MM/YYYY) : 10/2021

*Project/Proposal End Date (MM/YYYY) : 09/2023

*Total Award Amount : $ 60,000.00

*Person-Month(s) (or Partial Person-Months) Per Year Committed to the Project

If the time commitment is not readily ascertainable, reasonable estimates should be provided.

*Year (YYYY)	*Person Months (##.##)	Year (YYYY)	Person Months (##.##)
1. 2023	0.50	4.	
2.		5.	
3.			

*Overall Objectives : This project explores the effectiveness of cellulose nanocrystals (CNC) in mitigating spring frost damage to apple and grape crops in response to the growing threat of climate change. CNC is a promising nanomaterial that possesses valuable properties to counteract frost damage. The project will examine the optimal rate and timing of CNC application to improve cold hardiness by 2-6°C, providing substantial protection against spring frosts

*Statement of Potential Overlap : N/A

Projects/Proposals

4. *Project/Proposal Title : Expediting rootstock and scion breeding for cold and drought tolerance by predicting hormone signaling interactions

*Status of Support : (•) Current ( ) Pending

Proposal/Award Number (if available):

*Source of Support: USDA-NIFA-AFRI

*Primary Place of Performance : Virginia Tech

*Project/Proposal Start Date (MM/YYYY) : 12/2021

*Project/Proposal End Date (MM/YYYY) : 11/2023

*Total Award Amount : $ 300,000.00

*Person-Month(s) (or Partial Person-Months) Per Year Committed to the Project

If the time commitment is not readily ascertainable, reasonable estimates should be provided.

*Year (YYYY)	*Person Months (##.##)	Year (YYYY)	Person Months (##.##)
1. 2023	0.50	4.	
2.		5.	
3.			

*Overall Objectives : The project has two primary objectives: (1) to identify hormone-mediated interactions between rootstock and scion by analyzing transcriptomics and hormonal profiling of a range of rootstock-scion pairs, and (2) to verify high throughput molecular assays for characterizing hormone perception in deciduous fruits. The combination of systems and synthetic biology will enable the identification and validation of breeding targets and grafting

*Statement of Potential Overlap : N/A

Projects/Proposals

5. *Project/Proposal Title : Precision Crop Load Management for Apples

*Status of Support : (•) Current ( ) Pending

Proposal/Award Number (if available):

*Source of Support: USDA-NIFA-SCRI

*Primary Place of Performance : Cornell University

*Project/Proposal Start Date (MM/YYYY) : 09/2020

*Project/Proposal End Date (MM/YYYY) : 08/2024

*Total Award Amount : $ 4,800,000.00

*Person-Month(s) (or Partial Person-Months) Per Year Committed to the Project

If the time commitment is not readily ascertainable, reasonable estimates should be provided.

*Year (YYYY)	*Person Months (##.##)	Year (YYYY)	Person Months (##.##)
1. 2023	0.50	4.	
2. 2024	0.50	5.	
3.			

*Overall Objectives : The project aims to develop computer models and strategies, implement machine vision and robotic tools, design autonomous vehicles, and evaluate the economic and sociological impacts of adopting precision crop load management for sustainable apple production. The project also seeks to increase the adoption of this management technique through rapid Extension efforts.

*Statement of Potential Overlap : N/A

Projects/Proposals

6. *Project/Proposal Title : Integrating multidisciplinary and translational approaches to manage postharvest rots on apples and pears in major U.S. pome fruit growing regions

*Status of Support : ○ Current ◉ Pending

Proposal/Award Number (if available):

*Source of Support: USDA-NIFA-SCRI

*Primary Place of Performance : Oregon State University

*Project/Proposal Start Date (MM/YYYY) : 10/2023

*Project/Proposal End Date (MM/YYYY) : 09/2027

*Total Award Amount : $ 4,200,000.00

*Person-Month(s) (or Partial Person-Months) Per Year Committed to the Project

If the time commitment is not readily ascertainable, reasonable estimates should be provided.

*Year (YYYY)	*Person Months (##.##)	Year (YYYY)	Person Months (##.##)
1. 2023	0.10	4. 2026	0.50
2. 2024	0.50	5. 2027	0.50
3. 2025	0.50		

*Overall Objectives : The overall objective of this research is to develop innovative approaches, tools, and strategies for effectively managing the economically impactful postharvest fruit rot diseases, such as blue mold and gray mold, in apples and pears. This includes investigating the influence of the preharvest orchard environment, developing advanced decay management tools, assessing their effectiveness during storage and post-storage periods,

*Statement of Potential Overlap : N/A

Projects/Proposals

7. *Project/Proposal Title : Preserving Apples In The Face Of Frost: Evaluating The Efficacy Of Cryoprotectants

*Status of Support : ○ Current ◉ Pending

Proposal/Award Number (if available):

*Source of Support: Virgina Department of Agriculture and Consumer Services

*Primary Place of Performance : Virginia Tech

*Project/Proposal Start Date (MM/YYYY) : 10/2023

*Project/Proposal End Date (MM/YYYY) : 10/2025

*Total Award Amount : $ 69,700.00

*Person-Month(s) (or Partial Person-Months) Per Year Committed to the Project

If the time commitment is not readily ascertainable, reasonable estimates should be provided.

*Year (YYYY)	*Person Months (##.##)	Year (YYYY)	Person Months (##.##)
1. 2023	0.10	4.	
2. 2024	0.20	5.	
3. 2024	0.20		

*Overall Objectives : The project aims to investigate the effectiveness of cryoprotectants (frost protectants) in reducing frost damage in apple trees. These compounds have the potential to lower the freezing point and prevent ice-crystal formation, minimizing cell dehydration-induced damage caused by cold temperatures.

*Statement of Potential Overlap : N/A

Projects/Proposals

8. *Project/Proposal Title : Next Generation Biofungicides For Application In Controlled Environment Agriculture

*Status of Support : ○ Current ◉ Pending

Proposal/Award Number (if available):

*Source of Support: Virgina Department of Agriculture and Consumer Services

*Primary Place of Performance : Virginia Tech

*Project/Proposal Start Date (MM/YYYY) : 10/2023

*Project/Proposal End Date (MM/YYYY) : 10/2025

*Total Award Amount : $ 75,000.00

*Person-Month(s) (or Partial Person-Months) Per Year Committed to the Project

If the time commitment is not readily ascertainable, reasonable estimates should be provided.

*Year (YYYY)	*Person Months (##.##)	Year (YYYY)	Person Months (##.##)
1. 2023	0.10	4.	
2. 2024	0.20	5.	
3. 2025	0.20		

*Overall Objectives : The objective of this project is to explore the effectiveness of dsRNA formulas targeting two specific genes in the Botrytis cinerea fungus. The aim is to reduce both the pathogenicity of the fungus and the development of grey mold disease in strawberry plants grown under controlled environment conditions.

*Statement of Potential Overlap : If granted funding, this research project will primarily center on evaluating the effectiveness of dsRNA formulas, which have been previously employed by PI Sherif and the research team, as biofungicides. It is important to note that the pathogen and the target genes studied in this research do not overlap with those proposed for the NSF project.

Projects/Proposals

9. *Project/Proposal Title : EDGE FGT: RNAi-based tools to unlock functional genomics of obligate oomycete plant pathogens

*Status of Support : ○ Current ◉ Pending

Proposal/Award Number (if available): This proposal

*Source of Support: National Science Foundation

*Primary Place of Performance : Virginia Tech

*Project/Proposal Start Date (MM/YYYY) : 11/2023

*Project/Proposal End Date (MM/YYYY) : 10/2025

*Total Award Amount : $ 400,000.00

*Person-Month(s) (or Partial Person-Months) Per Year Committed to the Project

If the time commitment is not readily ascertainable, reasonable estimates should be provided.

*Year (YYYY)	*Person Months (##.##)	Year (YYYY)	Person Months (##.##)
1. 2023	0.20	4.	
2. 2024	0.50	5.	
3. 2025	0.50		

*Overall Objectives : As described in the project summary

*Statement of Potential Overlap : N/A

Table 1

1	Your Name:	Your Organizational Affiliation(s), last 12 mo	Last Active Date
	McDowell, John M	Virginia Tech	

Table 2

2	Name:	Type of Relationship	Optional (email, Department)	Last Active Date

Table 3

3	Advisor/Advisee Name:	Organizational Affiliation	Optional (email, Department)
T	Anderson, Ryan	BASF	
T	Burbidge, Toni	Univ. of California, Berkeley	
T	Davis, Colin	FDA	
T	Deb, Devdutta	Mercy College, NY	
T	Dinkeloo, Kasia	Univ. of Texas, Austin	
T	Fedkenheur, Kevin	NIH	
T	Fedkenheur, Micheael	NIH	
T	Herlihy, John	Virginia Tech	
T	Hoff, Troy	Virginia Commonwealth Univ.	
G	Meagher, Richard	Univ. of Georgia	
T	Simon, Stacey	US Dept. of Education	
T	Sonnawala, Unnati	Cambridge Univ.	
T	Sun, Jian	Genscript, China	
T	Wang, Wei	Virginia Tech	
T	Castillo-Machuca, Iliana	Virginia Tech	

Table 4

4	Name:	Organizational Affiliation	Optional (email, Department)	Last Active Date
A	Altarawy, Dimitri	Virginia Tech, USA		01/01/19
A	Ansariola, M	Celgene		01/01/19
A	Besnard Julian	Texas A & M Univ.		01/01/22
A	Bilir, Özlem	Univ. of Worcester		01/01/23
A	Bombarely, Aureliano	Univ. of Milan		01/01/20

A	Collakova Eva	Virginia Tech, USA		01/02/21
C	Cristian Danna	Univ. of Virginia, USA		
A	Cui, Chenming	Virginia Tech, USA		01/01/20
A	Cuzick, A.	Rothamstead Research Insitute, UK		
A	Dalio, Ronaldo	Centro de Citricultura Sylvio Moreira		01/31/18
A	Daron Johqin	Ohio State Univ.		01/01/20
A	Dimitrova, Elenea	Clemson Univ.		01/01/19
A	Finlayson Scott	Texas A & M Univ.		01/03/21
A	Glazebrook, Jabe	Univ. of Minnesota		01/01/19
A	Göl, Deniz	Univ. of Worcester		01/01/23
A	Ha, Sunhook	Virginia Military Institute		01/01/19
A	Haak, David	Virginia Tech, USA		01/01/20
A	He, Sheng Yang	Duke Univ.		01/01/20
A	Hillmer, Rachel	Univ. of California, Davis		01/01/19
A	Hong, Yiguo	Univ. of Worcester		01/01/23
A	Hoops, Stephan	Virginia Tech, USA		01/01/19
A	JimenezQuiros, Catherine	Univ. of Worcester		01/01/19
A	Katagiri, Fumaki	Univ. of Minnesota		01/01/19
A	Katiyar, Neerja	Univ. of California, Riverside		01/01/19
A	Kim, Theresa	Virginia Tech, USA		01/01/19
A	Lai Yan	Univ. of Connecticut		01/01/20
A	Laubenbacher, Reinhard	Univ. of Connecticut		01/01/19
A	Le Roch, Karine	Univ. of California, Riverside		01/01/19
A	Liu, Jinbao	Univ. of Alabama, Birmingham		01/01/22
A	Long Terri	North Carolina State Univ.		01/01/20
A	Lu Xueqing	Univ. of California, Riverside		01/01/20
A	Ludwig, N.R.	Deceased		01/01/20
A	Ma, Wenbo	Sainsbury Laboratories, UK		01/31/18
A	Machado, Marcos	Centro de Citricultura Sylvio Moreira		01/31/18
A	Mackey, Dave	Ohio State Univ.		01/01/19
A	Maharjan Bal	Texas A & M Univ.		01/01/21
A	Megraw, Molly	Oregon State University, USA		01/01/19
A	Mishra, Bharat	Univ. of Alabama, Birmingham		01/01/22
A	Mukhtar, Shahid	University of Alabama at Birmingham, USA		
A	Mukhtar, Shahid	Univ. of Alabama, Birmingham		01/01/22
A	Okumoto Sakiko	Texas A & M Univ.		01/04/21
A	Oliveira, Tiago	Centro de Citricultura Sylvio Moreira		01/31/18

A	Opiyo, Stephen	Ohio State Univ.		01/01/19
A	Pan Songqin	Univ. of California, Riverside		01/01/20
A	Pelly Zoe	Virginia Tech, USA		01/01/22
A	Pilot, Guillaume	Virginia Tech, USA		
C	Sagai Maroof	Virginia Tech, USA		
A	Setubal, Joao	Univ. of Sao Paulo		01/01/19
A	Shahin, H	Virginia Tech, USA		01/01/19
A	Slotkin, Keith	Ohio State Univ.		01/01/20
A	Telli, Osman	Univ. of Worcester		01/01/20
A	Tsuchiya, Tokuji	Nijon Univ., Japan		01/01/19
A	Tyler, Brett	Retired		01/01/19
C	Van Den Ackerveken, Guido	Utrecht University, NL		
A	Wang Jianqiang	Univ. of California, Riverside		01/01/20
A	Wang, Yuanchao	Nanjing Agricultural Univ.		01/31/18
A	Webb, Anne	NIAB		01/01/23
A	Wilson, Richard	Univ. of Nebraska		01/01/22
A	Withers, John	Duke Univ.		
A	Wood, Tom	NIAB		01/01/23

Table 5

5	Name:	Organizational Affiliation	Journal/Collection	Last Active Date
B	Thorpe, Holden	Science Family of Magazines	Science Magazine	
E	Klosterman, Steven	Univ. of California, Davis	Phytofrontiers	

Table 1

1	Your Name:	Your Organizational Affiliation(s), last 12 mo	Last Active Date
	Tör, Mahmut	University of Worcester	

Table 2

2	Name:	Type of Relationship	Optional (email, Department)	Last Active Date
R				

Table 3

3	Advisor/Advisee Name:	Organizational Affiliation	Optional (email, Department)
G	Charles Ainsworth	University of London	
T	Sinclair Mantell	University of London	

Table 4

4	Name:	Organizational Affiliation	Optional (email, Department)	Last Active Date
A	Hong, Yiguo	Hangzhou Normal University, China; University of Worcester, UK,		02/14/23
A	Studholme,David	Exeter University, UK		02/14/23
A	Wood, Tom	NIAB, UK		02/14/23
C	Domoney, Claire	John Innes Research Centre, UK		02/14/23
A	McDowell, John	VT, USA		02/14/23
C	Jones, Jonathan	The Sainsbury Lab, UK		02/14/23
A	Jackson, Steve	Warwick University, UK		02/14/22
A	Laxalt Ana	National University Marta del Plata, Argentina		05/05/22
A	Gol, Deniz	University of Worcester, UK		02/14/23
A	Webb, Anne	NIAB, Cambridge, UK		02/14/23
A	Di Palma, Andres Arruebarrena	Instituto de Investigaciones Biologicas, CONICET - Universidad Nacional de Mar del Plata, Mar del Plata, Argentina		03/23/22
A	Perk, Enzo A.	Instituto de Investigaciones Biologicas, CONICET - Universidad Nacional de Mar del Plata, Mar del Plata, Argentina		03/23/22
A	Carboni, Martin E.	Museo Argentino de Ciencias Naturales "Bernardino Rivadavia"CONICET, Buenos Aires, Argentina		03/23/22
A	Carlos, García-Mata	Instituto de Investigaciones Biologicas, CONICET - Universidad Nacional de Mar del Plata, Mar del Plata, Argentina		03/23/22
A	Budak, Hikmet	Montana BioAgriculture, Inc., Missoula, Montana, USA		03/23/22

A	Bilir, Ozlem	Trakya Agricultural Research Institute, Turkey		09/18/22
A	Jimenez-Quiros, Catherine	University of Worcester, UK		07/31/22
A	Okechukwu, Emeka C.	University of Worcester, UK		07/31/22
A	Baysal, Omur	Mugla Sitki Kocman University, Turkey		07/31/22
A	Atef, Mohamed	University of Warwick, UK Fayoum University, Egypt		06/05/22
A	Jin, Zhenhui	Hangzhou Normal University, China; University of Worcester, UK		06/05/22
A	Toba, Osman	University of Warwick, UK Fayoum University, Egypt		06/05/22
A	Nongnong, Shi	University of Warwick, UK		06/05/22
A	Yu, Zhiming	Hangzhou Normal University, China		03/10/22
A	Wang, Yue	Hangzhou Normal University, China		03/10/22
A	Mei, Fengling	Hangzhou Normal University, China		03/10/22
A	Yan, Haiting	Hangzhou Normal University, China		03/10/22
A	Jin, Zhenhui	Hangzhou Normal University, China University of Worcester, UK		03/10/22
A	Zhang, Pengcheng	Hangzhou Normal University, China; University of Worcester, UK		10/03/22
A	Zhang, Xian	Hangzhou Normal University, China		10/03/22
A	Shi, Nongnong	Hangzhou Normal University, China		10/03/22
A	Kahveci, Erdem	M.Y. Genetik Tarim Tek. Lab. Tic. Ltd. Sti., Turkey		08/18/22
A	Devran, Zubeyir	Faculty of Agriculture, University of Akdeniz, Turkey		08/18/22
A	Ozkaynak, Ercan	Yüksel Tohum Tarim San. ve Tic. A. S., Turkey		08/18/22

Table 5

5	Name:	Organizational Affiliation	Journal/Collection	Last Active Date
B	Mauch-Mani, Brigitte	Universite de Neuchatel, Switzerland	Frontiers	02/12/22
E	Dean, Ralph	North Caroline State University	MPP	02/15/22

Table 1

1	Your Name:	Your Organizational Affiliation(s), last 12 mo	Last Active Date
	Sheirf, Sherif M	Virginia Tech	

Table 2

2	Name:	Type of Relationship	Optional (email, Department)	Last Active Date

Table 3

3	Advisor/Advisee Name:	Organizational Affiliation	Optional (email, Department)
G	Subramanian, Jayasankar	University of Guelph	jsubrama@uoguelph.ca
T	Sapkota, Sangeeta	Michigan State University	sangee7@vt.edu
T	Zhou, Dongfang	Virginia Tech	dfezhou@vt.edu
T	Hezema, Yasmine	University of Guelph	yhezema@uoguelph.ca

Table 4

4	Name:	Organizational Affiliation	Optional (email, Department)	Last Active Date
A	Cline, John	University of Guelph		12/12/16
A	Elsharkawy, Islam	Florida A&M University		01/01/23
A	El Kayal, Walid	American University of Beirut		02/02/21
A	Goel, Alok	University of Guelph		08/05/21
A	Ravindran, Pratibha	National University of Singapore		10/15/21
A	Singh, Amritpal	Agriculture and Agri-Food Canada		12/12/22
A	Artlip, Tim	USDA/ARS		02/15/23
A	Ayyanath, Murali M	University of Guelph		08/05/21
A	Banks, Travis	Vineland Research and Innovation Centre		05/17/19
A	Bartlett, Kira	AgroSpheres		02/24/21
A	Bastide, Paul Y de la	University of Victoria		06/16/22
A	Bernier, Louis	Université Laval		06/16/22
A	Carballo, Sergio M	AgroSpheres		02/24/21
A	Chen, Lisa	AgroSpheres		02/24/21
A	Cheng, Lailiang	Cornell University		11/15/22
A	Choi, Dana	Penn State University		11/15/22

C	Clements, Jon	Univ. of Massachusetts		11/15/22
A	Coutin, Jose Freixas	University of Guelph		01/26/22
A	Darwish, Ahmed G.	Florida A&M University		04/16/22
A	Das, Protiva	AgroSpheres		02/15/23
A	Davis, Zachery	AgroSpheres		02/24/21
A	Dhaliwal, Amandeep Kaur	University of Guelph		01/26/22
A	Duan, Hui	North Carolina State University		12/22/22
C	Einhorn, Todd	Michigan State University		11/15/22
A	Englaender, Jacob	AgroSpheres		02/24/21
A	Frank, Joseph	AgroSpheres		02/24/21
A	Gajjar, Pranavkumar	Florida A&M University		04/16/22
A	Gao, Liwei	North Carolina State University		12/22/22
A	Gomez, Miguel	Cornell University		11/15/22
A	Haak, David C.	Virginia Tech		06/18/22
A	Haikal, Amr M.	Damanhour University		04/16/22
C	He, Long	Penn State University		11/15/22
C	Heinemann, Paul	Penn State University		11/15/22
A	Hintz, Will E	University of Victoria		06/16/22
A	Huang, Debao	North Carolina State University		12/22/22
A	Islam, Md Tabibul	AgroSpheres		02/24/21
A	Islam, Tabibul	AgroSpheres		02/15/23
A	Ismail, Ahmed	Damanhour University		04/16/22
A	Jackson, Daniel L	University of Georgia		07/23/19
C	Jiang, Yu	Cornell University		11/15/22
C	Kahlke, Craig	Cornell University		11/15/22
A	Kester, Mark	AgroSpheres		02/24/21
A	Kon, Tom	North Carolina State University		11/15/22
A	Laliberte, Suzanne	Virginia Tech		06/18/22
A	Latimer, Joyce Griffin	Virginia Tech		07/23/19
C	Layer, Chris	MOOG Inc., Space Group		11/15/22
A	Liu, Jianyang	USDA/ARS		02/15/23
A	Liu, Wusheng	North Carolina State University		12/22/22
A	Lu, Hongyan	North Carolina State University		12/22/22
A	Martin, Jeremy	North Carolina State University		12/22/22
A	McAdams, Jeremy	North Carolina State University		12/22/22
A	Miranda, Mario	Cornell University		11/15/22
A	Musacchi, Stefano	Washington State University		11/15/22

A	Nigg, Martha	Université Laval		06/16/22
A	Oliveira, Thais C de	Université Laval		06/16/22
A	Owen, W Garrett	Michigan State University		07/23/19
A	Peck, Greg	Cornell University		11/15/22
A	Pourtaheri, Payam	AgroSpheres		02/24/21
A	Prakash, Kumar	National University of Singapore		10/15/21
A	Robinson, Terence	Cornell University		11/15/22
A	Sarmiento-Villamil, Jorge L	Université Laval		06/16/22
A	Saxena, Praveen K	University of Guelph		06/16/22
C	Schmidt, Tory	Wash. Tree Fruit Res. Com.		11/15/22
C	Schwallier, Philip	Michigan State University		11/15/22
A	Scoggins, Holly L	Virginia Tech		07/23/19
A	Serra, Sara	Washington State University		11/15/22
A	Shakeel, Ameer	AgroSpheres		02/24/21
A	Sheikh, Mehboob B.	Florida A&M University		04/16/22
A	Shukla, Mukund	University of Guelph		06/16/22
A	Soliman, Karam F.A.	Florida A&M University		04/16/22
A	Somer, Daryl	Vineland Research and Innovation Centre		05/17/19
A	Somers, Elisabeth	AgroSpheres		02/24/21
A	Tsolova, Violeta	Florida A&M University		04/16/22
A	Wright, Caly	Virginia Tech		03/15/23
A	Wu, Yonghui	North Carolina State University		12/22/22
A	Zhao, Fangzhou	North Carolina State University		12/22/22
A	Zomorodi, Sepehr	AgroSpheres		02/24/21

Table 5

5	Name:	Organizational Affiliation	Journal/Collection	Last Active Date
B	Jones, Brian	University of Sydny	Frontiers in Plant Science	01/12/21

Data Management Plan

Types of Data

Data and samples that will be collected include: 1) Protocols and methodologies, e.g., detailed descriptions of protocols for induction of RNAi in vitro or via SIGS with SS-, NIVT-, and ME-dsRNA, digital image analysis e.g., germinating spores and infected plants (.docx Word files and .tif image files); 2) Micrographs: tiled Z-stacks of spores uptaking fluorescently labelled dsRNA (.lsm AIMApplication Document files); 3) Computational tools: Python and R code and workflows for obtaining and processing of sequencing data (.rmd RMD files and .py PY files); 4) RNA-seq data e.g., .fastq raw reads files and .bam mapped reads files; 5) synthetic RNAs, PCR amplicons and DNA constructs for production of dsRNAs; 6) E. coli strains for production of mini-cells with specific dsRNAs; 7) General laboratory data: including physiological, and molecular assessment data of traits and molecular signatures from infected plants (.xlsx spreadsheet files, .docx Word files, .one OneNote files, .tif image files).

The amount of data that will be generated from this project will vary depending on the nature of the experiment. For RNA-seq data, the size of raw data will vary from 5Gb to 100Gb depending on the sequencing depth. Data will shared between groups as necessary via secure Sharepoint sites at the respective institutions. The PIs of this project will be in charge of collecting data, dissemination of data, and biological resources until the time of publication after which these resources will be deposited in publicly available repositories.

Standards

For RNAseq data, expression raw reads (.fastq), mapped reads (.bam) and associated metadata files (to be deposited to the NCBI Gene Expression Omnibus/Short Read Archive database). For other data, there are not established standards for data formats and meta-data; as much as possible, data will be generated and stored in digital format (see above).

Related Tools, Software, and/or Code

Python, and R code and workflows for obtaining and processing, and analysis of RNAseq data will be available on the open access Protocols.io repository (https://www.protocols.io/) and through the participating labs' open access GitHub repository (https://github.com/maoli0923) along with proper documentation. Code will be freely accessible within the project team before publication and code will be open source and freely available for research use after publication.

Access, Sharing, and Dissemination

The participating institutions are committed to the open and timely dissemination of research findings. Our results will be made as accessible as in accordance with the principles stated in the Office of Science Statement on Digital Data Management (https://science.osti.gov/funding-opportunities/digital-data-management). Data may be presented before publication at international conferences. All data collected in the project will be submitted for publication in international journals during or after the duration of the granting period. Sequencing data generated will be deposited in the Gene Expression Omnibus at NCBI for public use. Data will be embargoed before acceptance of the publications and the embargo will be released as soon as publication is accepted. Upon publication of the results, all tabulated text-format data will be deposited to public data repositories such as FigShare (https://figshare.com/) with permanent Digital Object Identifier (DOI), for preservation and bulk sharing. Protocols and pipelines will be deposited in Protocols.io and GitHub (https://www.protocols.io/ and https://github.com/) and peer-reviewed methods and protocol papers (*e.g.*, the Journal of Visualized Experiments or Plant Methods).

Specifically for methods and tools developed for induction of RNAi, extra care will be taken to ensure sustainability, continued access, maintenance, and/or operation of services past the lifetime of the award. Results of the work, including relevant negative results that may not make into peer-reviewed publications, will be deposited in the bioRxiv preprint server (https://www.biorxiv.org/). Plasmids with effective dsRNA constructs will be made available through the Addgene plasmid repository (https://www.addgene.org/) and also maintained at VT for distribution upon request.

Data Preservation

Long-term data preservation will be achieved through publications and public data repositories such as NCBI SRA, which is maintained by the National Center for Biotechnology Information (NCBI) with support from the NIH. Data will be submitted to public repositories prior to the submission of manuscripts describing the data generation and data analysis processes. We expect the data will be preserved indefinitely or depend on the availability of the public repositories. We will strive to publish and release the data to the public as soon as possible, and typically, the process of data analysis and manuscript preparation, manuscript submission, and review would take from 1 year to 3 years post data generation. Data will be curated to ensure the accuracy of meta-data and will be formatted according to standards set by the data repository.

Data Protection: Security and Integrity

At Virginia Tech, Advanced Research Computing (ARC) provides all Virginia Tech research groups with redundant data backup using tape backup for unlimited storage. The tape backup is located in two physical locations to avoid single-point failures. All data will be stored in the ARC clusters. Data from all computers from Virginia Tech are backed up by Crash Plan, an automated backup service provided by the College of Agriculture and Life Sciences at Virginia Tech. Similarly, data at UW…Data access will be managed by the PIs, and all team members will have read access to raw data, processed data, and source code. Team members directly involved in data analysis will have read and write access to the processed data and source code. Raw data will only have read access to all members once they have been downloaded from the sequencing facility to ensure raw data will not be accidentally modified.

Oversight of Data Management

The PIs, together with team members involved in the collection of such data or samples, will consider requests for use, which will include any re-use, re-distribution, and production of derivatives. Usage decisions will be communicated in writing to the requester and copied to all members of the team and will reflect requirements that address intellectual property rights, other rights, protection of privacy, confidentiality, and all other requirements for use of such data or project products. Decisions of data management will try to avoid negative impacts on innovation and U.S. competitiveness; and be consistent with all applicable laws, and regulations.

Rationale

This data management plan is developed according to a commonly adopted standard for publication of research data. The host institutions and public repositories provide the resources for data storage, maintenance, and dissemination to the public. These resources are freely available to the PI, co-PIs, and collaborators and no budget is requested for long-term storage of the data. Budgets are requested for publication and for traveling to conferences, which are elements of the data-sharing plan of this project.

Postdoctoral Mentoring Plan

This project will support three postdoctoral researchers (PR) each for three years full-time. Two PRs will work at Virginia Tech, one in PI McDowell's lab and the other in co-PI Sherif's lab. The third PI will work in co-PI Tor's lab at the University of Worcester (UW). This Mentoring Plan will provide the skills, knowledge, and experiences to prepare these PRs to excel in their chosen career path, be it academic or other.

Orientation will include in-depth conversations between each PI and the supervising PR. The PR and PI will complete an Initial Meeting Template/Worksheet for Postdoc and Mentor as well as annual review meetings. In addition to the orientation meeting between the PR and PI, the VT Postdoctoral Association offers recurring New Postdoc Orientation programs. At the University of Worcester, the PR is enrolled automatically for a second Research Mentor by Human Resources (HR). It is University policy that all mentors attend a university mentoring workshop prior to the commencement of a mentoring relationship.

Career Development Plans will be completed for all three PRs within the first three months of their appointments, with explicit milestones that address the elements below and others that are pertinent to the PRs career goals.

Career Counseling/Advising will be provided in part by the PIs, who will leverage their collective research, teaching and extension experiences. The PRs will also be encouraged to leverage counseling resources and networks at their respective institutions, e.g. the VT Postdoctoral Association and a comprehensive workshop schedule that includes both career and professional development events.

Cross-training opportunities will be provided by the collaborative, international nature of this project. Each PR will be encouraged to undertake short-term stays in each of the other two labs. The timing and duration of these visits will be determined in part by the need for transfer of skills between labs. We anticipate that these experiences will provide a valuable opportunity for PRs to obtain broad skill sets and exposure to distinctive cultures. Moreover, we will encourage the PRs to bond as a "cohort" to provide mutually beneficial advice and support.

Experience with Grant Proposals will be gained by direct involvement in proposals prepared by the PIs. The PRs will have an opportunity to learn best practices in proposal preparation, including identification of key research questions, definition of objectives, description of approach and rationale, and construction of a work plan, timeline, and budget.

Publications and Presentations will be prepared under the collaborative direction of all three PIs, as appropriate. The PRs will receive guidance and training in the preparation of manuscripts for scientific journals and presentations at conferences. Additionally, the PR will have access to courses on effective writing and presentation skills at their respective institutions.

Teaching, Mentoring, and Extension Skills will be developed in the context of weekly meetings within the collaborative research groups during which graduate students and PRs describe their work to colleagues within the group and assist each other with solutions to challenging research problems, often resulting in cross- fertilization of ideas. If desired, each PR will have the opportunity to gain substantial teaching experience by designing and leading lectures in classes taught by the PIs. PRs will have access to extensive resources for effective teaching and mentoring in place at each institution. Co-PI Sherif can provide exposure to and advice on extension faculty responsibilities, if desired.

Instruction in Professional Practices will include fundamentals of the scientific method, laboratory safety, and other standards of professional practice. PRs will be required to complete Responsible Conduct of Research training courses at their respective institutions.

Technology Transfer experience will be provided by the collaboration with Agrospheres. If desired, VT PDs can access additional training in confidentiality requirements and preparation of invention disclosure applications at their institutions, e.g., VT's Link, License, and Launch Program.

Success of the Mentoring Plan will be assessed by tracking the PRs' progress toward their research and career goals in the form of quarterly meetings with a defined assessment agenda based on each PRs career development plan, including discussion of the progress made on milestones relating to all of the specific elements outlined above.

Dissemination and Education Plan

The dissemination of our research findings and the education of growers and stakeholders regarding the efficacy and limitations of SIGS as a disease management strategy will be achieved through the deployment of a multitude of outreach approaches, the most prominent of which will be the already-established extension program presided over by PI Sherif at Virginia Tech. The Extension team, comprised of Sherif and Virginia cooperative extension agents, actively engages with a diverse array of stakeholders, including fruit and grape producers of varying sizes, fieldmen, consultants, and industry leaders. Sherif oversees an ambitious yearly extension program, marked by a weeklong series of fruit schools and complemented by farm visits, in-class presentations, and twilight meetings. In addition to reaching commercial fruit and vegetable producers, he will employ the "Virginia Tech Tree Fruit Extension and Outreach" Facebook page and the "Tree Fruit Horticulture Updates" blog website he administers to disseminate the practical and theoretical knowledge acquired throughout thjs project, with the aim of providing valuable insights into RNAi-enabled biofungicides and their applications, as well as their potential benefits for pollinators and beneficial organisms. These outreach efforts will begin in Year 1.

Sherif's leadership in the domain of RNAi biofungicides is validated by his status as the principal investigator in a research endeavor funded by BRAG (see Current and Pending Support and p. 15 of the Project Description), which delves into the environmental persistence and target specificity of minicell-encapsulated RNAi biofungicides. Harmonization of the BRAG-funded research with the findings from the proposed research will produce a profusion of synergistic data, providing conventional and organic growers with informed decisions regarding the utilization of SIGS as an effective, eco-friendly, and species-specific disease management strategy. Moreover, Sherif will host an annual public open-house (year 3), which is expected to draw participants from a wide range of backgrounds, and he will collaborate with PI McDowell to organize a field-day (year 3) to share the results of Aim 3’s greenhouse trials with representatives from agrochemical industry and other interested stakeholders. Furthermore, our team, leveraging the expertise of our extension agents of Hispanic heritage, will proactively engage with the Hispanic farmer population in Virginia through the judicious dissemination of informational resources and extension presentations, tailored to meet their specific cultural and linguistic needs. At the termination of the extension program in the third year, a comprehensive impact survey shall be executed to evaluate the impact of our initiatives in elevating the growers' comprehension of the new class of biofungicides, including their applications and potential.

Our second outreach/education approach is to design and conduct a meeting workshop to provide a platform for knowledge exchange between academics and industry on the topic of “RNA Technologies for Crop protection”. We are aware that other scientists in the US, UK and Europe work on DM control using biological control agents and sRNAs. We want to disseminate the knowledge we obtained from our collaborative efforts and gain further information from colleagues in a workshop with aim to;

- Highlight the benefits and use of sRNAs for crop protection.
- Compare new technologies that could help to deliver faster pathogen and pest control strategies
- To develop new connections between the plant protection research communities.
- Explore different ways to translate laboratory-based research into application, especially in DM control.
- Increase the awareness of problems that the plant health industry faces.
- Discuss the latest developments in the field and disseminate these cutting-edge scientific outcomes to a wider community.
- Provide a platform for knowledge exchange between academics and industry.
- Foster new research collaborations that are focused on delivering benefits for plant health improvement.
- Enable participants to develop links to assist their professional development.

Third, the PI Tör and his group will participate in The Pulse Crop Genetic Improvement Network (PCGIN) in the UK, interfacing with breeders, growers, and academics in the field and disseminating the project's outcomes. Finally, the PIs and their laboratory associates and students will ensure a continual exchange of information and results through their participation in regional, national, and international conferences and by publishing refereed journal articles and fact sheets.

We are committed to share outcomes of this project in a timely manner with the research community. Research findings will be disseminated through research publications, including deposition of manuscripts in bioRxiv prior to submission, through (social) media releases associated with research publications, and presentations at conferences. In addition, we will deposit raw data in public databases (e.g. Genbank, EMBL). These activities will involve the PI, CO-I as well as post-doctoral researchers on the project.

We will keep track of Article metrics (e.g., reads, downloads, citations, Altmetric Attention Scores) associated with publications. In addition, hits to websites with media releases will be monitored and recorded (e.g. Google analytics). For conference presentations we will record the audience (size, type of audience) of events. These data will evaluated during our quarterly and yearly meetings, to assess the impact of the Outreach and Dissemination plan and alter it if necessary.

Project Management Plan

Communication, evaluation, and planning

1. Zoom meetings of the entire group every two weeks: In these one-hour meetings each researcher will summarize progress and challenges since the previous meeting, and goals for the upcoming two weeks.
2. Quarterly Zoom meetings of the three PIs: In these two-hour meetings, the PIs will review progress towards milestones, troubleshoot bottlenecks, and reach consensus on alterations of the plan and timeline, as well as re-allocation of resources if necessary.
3. In person annual meetings of the entire team: These will take place over two days, hosted by Worcester in Year 1 and Virginia Tech in Years 2 and 3. Each researcher will give a formal presentation on progress and challenges in the previous years. Additionally, the PIs will give a "big picture" discussion of progress towards milestones, followed by a moderated, whole-group discussion in which consensus is reached on strategic realignment, if necessary. The meetings will include tours of local attractions and other social activities to build interpersonal comfort amongst participants.

Responsibilities

McDowell Group, Virginia Tech:

- Aims 1 and 2: All experiments with Arabidopsis + *Hpa*, including construction of plasmids. constructs and production of NECT-dsRNAs, phenotyping of *Hpa* spores and *Hpa*-infected plants
- Aim 1b: experiments on the uptake of labelled RNA in Aim 1c.
- Aim 3: Assist the Sherif group in setting up pathosystems for grape and cucumber downy mildew.

Sherif Group, Virginia Tech

- Aims 1 and 2: Produce EM-dsRNAs
- Aim 3: Experiments to optimize protocols for grape and cucumber downy mildew.

Tör Group, University of Worcester

- Aims 1 and 2: All experiments with Pea + *Pvp*, including construction of plasmids. constructs and production of NECT-dsRNAs, phenotyping of Hpa spores and Hpa-infected plants
- Aim 1d: experiments on the uptake of labelled RNA in Aim 1c.
- Aim 3: Experiments to optimize protocols for lettuce downy mildew.

Timeline

		Fall 2023	Winter 2024	Spring 2024	Summer 2024	Fall 2024	Winter 2025	Spring 2025	Summer 2025	Fall 2025	Winter 2026	Spring 2026	Summer 2026
Aim 1-Silencing in spores													
Aim 1a-Hpa	Testing NECT-dsRNA	JM	JM	JM	JM								
Aim 1a-Pvp		MT	MT	MT	MT								
Aim 1b-Hpa	Uptake of dsRNA in spores			JM	JM	JM	JM						
Aim 1b-Pvp				MT	MT	MT	MT						
Aim 1c-Hpa	Testing ME-dsRNA				JM + SS	JM + SS	JM + SS	JM + SS					
Aim 1c-Pvp					MT + SS	MT + SS	MT + SS	MT + SS					
Aim 1d-Hpa	Multiplexing dsRNAs						JM + SS	JM + SS	JM + SS				
Aim 1d-Pvp							MT + SS	MT + SS	MT + SS				
Aim 2-SIGS of intact plants													
Aim 2a-Hpa	Testing NECT-dsRNA						JM + SS	JM + SS	JM + SS	JM + SS			
Aim 2a-Pvp							MT + SS	MT + SS	MT + SS	MT + SS			
Aim 2b-Hpa	Testing ME-dsRNA								JM + SS	JM + SS	JM + SS	JM + SS	
Aim 2b-Pvp									MT + SS	MT + SS	MT + SS	MT + SS	
Aim 2c-Hpa	Multiplexing dsRNAs										JM + SS	JM + SS	JM + SS
Aim 2c-Pvp											MT + SS	MT + SS	MT + SS
Aim 3	Protocols for grape, cucumber, lettuce	MT + SS	MT + SS	MT + SS	MT + SS	MT + SS	MT + SS	MT + SS	MT + SS	MT + SS	MT + SS	MT + SS	MT + SS

McDowell	JM
Sherif	SS
Tor	MT

AgroSpheres INC.
1180 Seminole Trail, STE 100
Charlottesville, Virginia 22901

Dear NSF-EDGE Committee,

If the proposal submitted by Dr. John McDowell entitled "*Development of RNAi-based tools for functional genomics of obligate downy mildew plant pathogens"* is selected for funding by NSF, it is my intent to collaborate and/or commit resources as detailed in the Project Description or the Facilities, Equipment and Other Resources section of the proposal.

Sincerely,

Payam Pourtaheri
Chief Executive Officer
AgroSpheres INC.

Date: 02/07/2023

LIST OF SUGGESTED REVIWERS

Laura Grenville-Briggs Swedish University of Agricultural Scientists laura.grenville.briggs@slu.se

Expert on Ces3A, RNAi in oomycetes

Scot Hulbert Washington State University scot_hulbert@wsu.edu

Expert on RNAi for functional genomics of obligate rust fungi

Roger Innes Indiana State University rinnes@indiana.edu

Expert on cross-kingdom RNA trafficking mechanisms and implications for HIGS/SIGS

Richard Michelmore Univ. of California, Davis rwmichelmore@ucdavis.edu

Expert on downy mildews and HIGS

LIST OF REVIWERS TO EXCLUDE

Hailing Jin Univ. of California, Riverside

Competitor