

PROJECT SUMMARY

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

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Project Directors

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Project Title:

INVESTIGATION AND EXPLOITATION OF LIGHT AS A NON-CHEMICAL MEANS TO MANAGE POWDERY MILDEWS

PROPOSAL TYPE

USDA NE-IPM

Project type: Research

SUMMARY

This is a research project. Our long-term goal is to exploit light as a natural and non-chemical means of controlling powdery mildews. Powdery mildews are an internationally important pathogen group attacking a broad variety of crops. The driving force of epidemics is abundant sporulation. Disease management is problematic due to widespread resistance to most fungicides. We have discovered a non-chemical means to disrupt sporulation, and have considerable preliminary data to show that light in the visible spectrum as well as brief exposures to ultraviolet B (UV-B) can be used to suppress disease. We will investigate the use of low-cost, low energy LEDs that produce specific wavebands to disrupt sporulation, both alone and in conjunction with UV-B. A diverse group of powdery mildews of rose, grapevine, strawberry and cucumber will be explored as model systems with potential for spinoffs and adaptation to other crops. The project is highly focused, short-term (12 months), and is well aligned with NE-IPM goals, and priorities. The research problem encompasses many of the top priorities of several stakeholder groups within the northeast region, nationally, and internationally. The project involved international collaboration, and represents a novel approach to disease management. The project directors and cooperators are internationally recognized leaders in research and outreach in this area. Effective outreach is inherent in the design of the project, and is seamlessly integrated into the transition between objective I (treatment investigation, quantification, and refinement) and objective II (demonstration).

PROJECT NARRATIVE

PROBLEM, BACKGROUND, AND JUSTIFICATION

The problem. Our project innovatively addresses a fundamental challenge in IPM: discovery, development, and exploitation of non-chemical means to manage pests and diseases. The microbial group under investigation (the *Erysiphales*) is comprised of powdery mildews: an internationally important and destructive group of plant pathogens. Copious production of asexual spores is the driving force of powdery mildew epidemics, and also represents the Achilles Heel of the pathogen group (2, 5). Suppress sporulation and you suppress the disease.

Our proposal has the potential to provide an entirely new approach to the management of plant diseases: the regulation of spore production by specific wavelengths and intensities of light. In several parallel studies involving a diverse group of powdery mildew, we have demonstrated several ways in which we can manipulate light intensity and quality to significantly suppress disease (4, 9-13). Our proposal builds upon these preliminary studies, with an aim towards a demonstration of practical application of the technology, to demonstrate feasibility, and to enable further development by stakeholders.

A breakdown of chemical control has already impacted powdery mildews in many pathosystems where fungicides are a significant component of disease management, and will likely worsen with each passing year (5). There is a paucity of new fungicides in the research pipeline. Resistance to the DMI fungicides and strobilurins will inevitably increase. Two recently registered materials (quinoxifen and boscalid) are at high risk for developing resistance (5), and the sustainability of their use should be considered limited. Remaining broad-spectrum fungicides (sulfur, copper compounds, and various oils) possess relatively modest intrinsic activities and are inherently phytotoxic to certain cultivars or in closely spaced sprays (5). Therefore, there is a heightened need for targeted biological research that will enhance control of this pathogen group with a greatly reduced arsenal of fungicides.

Over the last 25 years, improved control of major fungal diseases has come from two principle sources: (i) new fungicide chemistry and (ii)-increased knowledge of pathogen biology and ecology. Contributions from fungicides have flowed and ebbed as new materials are first registered and then lost due to fungicide resistance. In contrast, new knowledge of pathogen biology and ecology has provided improvements that are both substantial and stable. Consider the impacts of steady increases in our understanding of infection requirements, inoculum sources, and natural sources of resistance such as ontogenic resistance. Aspects of the foregoing research are now embedded in IPM programs for apple, grapevine, and strawberry worldwide. Much of this work was supported by the NE-IPM program. Our research group has a good track record of producing results that benefit stakeholders. Nonetheless, much of the foregoing work on pathogen biology was commercially relevant only in that it allowed us to precisely focus fungicide treatments to provide maximum benefits. If fungicides themselves are lacking, then we must look elsewhere for sustainable methods to suppress the disease. This is the context for our present research project. It is based upon a substantial body of our published preliminary research, and the project will be completed in a relatively short time (12 months).

Background and Justification. The following are offered as documentation of need and stakeholder support for the proposed research:

- Northeastern IPM Center: Vegetable IPM Working Group – Under General Needs and Priorities, listed “*Conduct research on how to promote plant health and suppress insect, disease and weed problems through cultural and biological production practices*”, and under Priority Pests, listed “*Powdery mildew of Cucurbits*”. See: <http://www.northeastipm.org/working-groups/vegetable/priorities-and-reports/>.
- The New York IPM Program ranked “*Development and testing of alternative materials and non-pesticide options for pest and crop management*” as the 4th highest priority out of 33 for tree fruit. “*Development and testing of alternative materials and non-pesticide options*” was ranked the 3rd highest priority out of 27 for grapevine, and “*Powdery Mildew biology and management*” was ranked the 6th highest priority out of 27 for grapevine. “*IPM in high tunnels*” (where powdery mildews are often the major limiting factor for sustainable culture) was ranked the 3rd top priority out of 30 for berry crops. See: http://www.nysipm.cornell.edu/grantspgm/rfp_ag/fruitpri.asp-grapes.
- Powdery Mildews were listed as one of the top 4 diseases threatening greenhouse production in the *Pest Management Strategic Plan for Commercial Greenhouses in the Northeastern United States*. See: http://www.northeastipm.org/rese_pmspbystate.cfm
- Preliminary research that served as the foundation of the present project has received broad sponsorship from commodity and applied research entities, including the New York Wine and Grape Foundation, The North American Strawberry Growers Association, the USDA Viticulture Consortium, and the Norwegian Research Council. See: *Current and pending support pages of project directors*.
- In cooperation with the North American Strawberry Growers Association (NASGA - http://www.nasga.org/about_us.htm), we conducted a nationwide survey of strawberry growers in early 2010 to collect data on efficacy of management programs for powdery mildew, economic loss, and trends towards high tunnel production. NASGA currently has approximately 300 members in 40 states. Over 100 survey responses were returned to us from 14 states. This represents a response rate of over 30%, and indicates a highly-motivated respondent population. Nationwide, 85% of respondents reported "unsatisfactory" control of powdery mildew, and 74% reported “substantial economic loss” due to powdery mildew during one or more years during the previous 5-year period. Furthermore, 27% of respondents reported such losses in 3 or more of the previous 5 years. Thus, losses were both widespread and among over one quarter of all respondents losses were occurring year after year. Respondent acreage ranged from 0.5 to 4,446 acres, with one-half of managing between 5 and 50 acres. Nearly 60% indicated they are producing strawberries in high tunnel systems, or are considering moving production to high tunnels in the next 5 years. Thus, at the very time that performance of fungicides is eroding, it appears that disease pressure is likely to increase over time due to the shift towards high tunnel production.

- Our project is directly relevant to 2 national needs identified in the National IPM Roadmap (www.ipmcenters.org/ipmroadmap.pdf) as follows:
 1. Elucidating how we can suppress sporulation using light will "*identify vulnerable stages in the pest life cycle*".
 2. Exploitation of a natural environmental factor such as light illustrates "*advanced management tactics... that prevent or avoid pest attack*".
- Our project is closely aligned with the purposes and priorities of the NE-IPM Program as provided in the 2012 RFA, Part I-B, specifically priorities 1 (reduced environmental risk), 3 (stakeholder support and priority), 4 (focuses on pests and crops found in ALL states of NE region), 5 (fills a niche for which no such tactic exists), 7 (will advance IPM in a few years), 8 (is interdisciplinary), 9 (reduces dependence on chemical pesticides), and 10 (has significant economic implications). See: <http://www.northeastipm.org/>

The role of light as a modifier of asexual development is a common theme in filamentous fungi. In *Aspergillus nidulans*, asexual sporulation is repressed in the dark, reduced in blue or red light, and stimulated by white light (7). Photoinduction of conidiation by blue light has also been demonstrated in *Paecilomyces fumosoroseus*, *Alternaria tomato*, and *Trichoderma atroviride* (6-8). Our research group has recently demonstrated that conidiation in the rose powdery mildew pathogen *Podosphaera pannosa* was stimulated by blue light (420 to 520 nm) but repressed by red light (620 to 720 nm), and that daylight extension with supplementary lighting was an effective means of suppressing rose powdery mildew via the suppressive effects of long daylength on sporulation (10, 12). In *Erysiphe necator*, we have recently found that light operates as a signal to initiate sporulation once the colonies reach a critical age or density (4). Most recently, we have found that ultraviolet-B (UV-B, 280 to 315 nm) lamps can be operated during the night period for as little as 5 minutes to suppress powdery mildew of cucurbit (11). UV-B lamps are now used in a variety of applications in human medicine, but have not yet been exploited for management of plant diseases. Furthermore, the suppressive effects of UV-B can be enhanced by red light or reversed by blue light. We have used low cost, low energy light-emitting diodes (LEDs) to supply specific wavelengths for this purpose (reference). All of the foregoing opens several possible avenues by which light can be manipulated to suppress powdery mildews. Although our proposed research focuses only on powdery mildews, there may be several spinoff applications for other fungal plant pathogens, as the regulatory effects of light on sporulation appear to apply broadly among fungi. Recent advances in production of low-cost LEDs, together with new biological knowledge on how light controls fungal development may open a new and non-pesticidal pathway for controlling plant diseases.

We have selected four diverse pathosystems for our project: powdery mildews of grapevine, strawberry, rose, and cucurbit. We chose this group for the following reasons: (i) we have considerable experience in manipulating the hosts and pathogens in experimental settings, (ii) they represent diverse commodities, (iii) our preliminary studies have identified target responses to light that might be exploited to suppress disease, and (iv) the systems within which the plants are cultured, while diverse, may lend themselves to supplemental lighting; particularly in organic or low-pesticide systems (i.e., greenhouse, high tunnel, or trellised field plots). We anticipate

that a successful project will serve as a model for follow up studies and expansion to include powdery mildews in other amenable crops.

OBJECTIVES AND ANTICIPATED IMPACTS

The objectives of the proposed project and their anticipated impacts are:

- I. To determine an optimum quality of light and time of exposure that will significantly and substantially disrupt conidiation and suppress disease in the following powdery mildew pathosystems: rose (*Sphaerotheca pannosa*), cucurbit (*Podosphaera xanthanii*), grapevine (*Erysiphe necator*), and strawberry (*Podosphaera aphanis*) without deleterious effects on plant growth and development.

Anticipated impacts: Fulfillment of Objective I is directly connected to the NE-IPM goals of (i) “*encouraging science-based pest management*” and (ii) “*promoting economic benefits*”. Metrics applied to quantify the impacts relative to the foregoing goals (i) and (ii) respectively will be:

- Attendance at presentations on project to stakeholder groups (i)
- Analysis and reporting of internet traffic on webpage devoted to project (i)
- Number of fungicide applications eliminated as a consequence of light treatments (ii)
- Comparative disease and loss levels in light-treated vs fungicide treated plots (ii)
- Comparison of cost of conventional fungicide treatment vs light treatments (ii)
- Number of publications (refereed and popular) attributable to the project (i)

- II. To demonstrate commercially relevant levels of powdery mildew suppression in the above pathosystems in small-plot trials through manipulation of lighting.

Anticipated impact: Fulfillment of Objective II is directly connected to the NE-IPM goal of “*furthering the implementation of IPM*”. Metrics applied to quantify the impacts relative to the foregoing goal will be:

- Attendance at field day tours of demonstration plots
- Analysis and reporting of internet traffic on webpage devoted to project data from demonstration plots

APPROACH AND PROCEDURES

Novelty of the approach: Our project embodies a novel and innovative approach to the management of a diverse pathogen group: we will exploit the naturally evolved photoregulatory systems of powdery mildews to disrupt the normal process of spore production. A second novel aspect is that we will use short nighttime exposures to UV-B light to suppress powdery mildews. Powdery mildews are unique among fungal pathogens as the vegetative body of the pathogen is wholly external to the host, making them uniquely susceptible to UV-B at doses that have little to no measurable affect on host plants. A third novel aspect is that we will use specific wavelengths of light to synergistically increase the sensitivity of powdery mildew fungi to UV-B light. Our preliminary work involving *Podosphaera xanthanii* on cucumber has indicated that

LEDs producing blue light in the waveband 420 to 490 nm greatly increase the suppressive effects of a 5 minute exposure to UV-B applied during the normal night interval (11).

Objective I: To determine an optimum quality of light and time of exposure that will significantly and substantially disrupt conidiation in the following powdery mildew pathosystems: rose (*Sphaerotheca pannosa*), cucurbit (*Podosphaera xanthanii*), grapevine (*Erysiphe necator*), and strawberry (*Podosphaera aphanis*) without deleterious effects on plant growth and development.

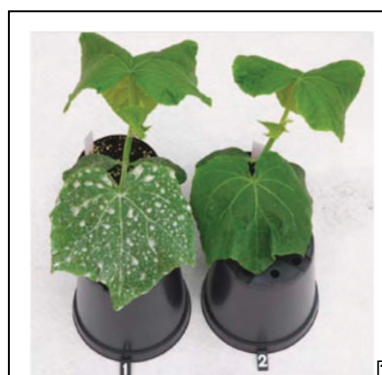


Fig. 1. Cucumber inoculated with powdery mildew conidia and untreated for 12 days (left) or exposed to 1W/m² UV-B for 5 min during the night interval.

Determination of active wavebands. We will use combinations of narrow waveband LEDs identified in our preliminary studies to evaluate the impact of light quality in 100 nm steps across the visible spectrum as described in our preliminary studies (11,12). Once the broader wavebands are identified based upon various pathogen responses, we will selectively eliminate one or more of the contributing colored LEDs that make up a composite waveband, narrowing the resultant waveband in each successive evaluation. For example, in the first-pass studies exposed to 100 nm bandwidths, we anticipate that only one treatment will produce suppression. We can then focus on narrowing the waveband in successive experiments to identify the specific wavelengths involved in the response. Preliminary studies (11, 12) have indicated phytochrome systems in *Sphaerotheca pannosa* and *Podosphaera xanthanii* that respond to 420 to 490 nm (blue) and 610 to 660 nm (red). These studies have also indicated that the principal impact of light treatments is exerted directly upon the pathogen, and not through enhanced host resistance, i.e., light treatments are not protective in nature, but rather inhibit the pathogen. Thus, out of necessity given the scope of the proposed project, we will focus upon pathogen responses wherein the host is exposed to light treatments *after* inoculation. While we could conceivably also evaluate light intensity, at a practical level this will be predetermined by the intensity of available LED and UV-B sources. Thus light quantum delivered will, in practice, be more likely regulated by duration of exposure, rather than regulation of intensity.



Fig. 2. LED light sources that produce a variety of wavebands across the visible spectrum are inexpensive and widely available. They can also be configured in screw in fixtures as above, large light banks, or in strings, as in holiday lighting.

Our preliminary experiments have also illustrated that brief exposure to UV-B lamps (e.g., as little as 5 min for cucumber powdery mildew) have a substantial suppressive effect. We will expand these studies to include powdery mildews or rose, strawberry and grapevine. As in our preliminary studies, the UV-B treatments will first be evaluated in isolation from supplemental lighting from specific LEDs, and later in combination with specific wavebands in the visible spectrum produced by LEDs.

Host plants of each species will be grown in 10 cm plastic pots of a peat/soil mix in greenhouses to bear several true leaves. Ontogenically susceptible leaves of each host plant will be inoculated with an aqueous conidial suspension containing 10^5 conidial per ml of the appropriate powdery mildew pathogen species prior to light treatments as previously described (9-13).

Responses to be assessed and recorded in each of the above four pathosystems include infection efficiency (percentage of germinated spores that develop secondary hyphae within 48 hrs), colony expansion rates (colony expansion from a $5 \mu\text{l}$ droplet of conidial suspension 5 days after inoculation (reference)), latent period (days from inoculation to first production of secondary inoculum), and disease severity (percentage of leaf surface mildewed). Plants under light treatments, as well as controls that are untreated or treated with conventional fungicides will be assessed for possible growth or horticultural effects of light treatments.

Timeline/Milestones: Proposed work will begin in October 2012 and will be completed by December of 2012. Order of task progression is indicated above. By January of 2013, we should know how specific wavelengths of light within the visible spectrum affect sporulation of the four candidate pathogens, and will have identified LEDs that supply the active wavebands.

Determination of optimal exposure time. Optimal time of exposure has two aspects: the duration of exposure to a specific waveband, and the hour of the day during which the exposure occurs. The latter aspect may be of particular significance with respect to red or blue light treatments combined with brief exposures to UV-B. Our preliminary experiments with cucumber powdery mildew indicate that the suppressive effect of UV-B is counteracted by blue light (a component of natural sunlight). Thus, UV-B treatments are most effective when applied during the night (Fig. 3); and have been rendered even more effective when applied in combination with red light (11).

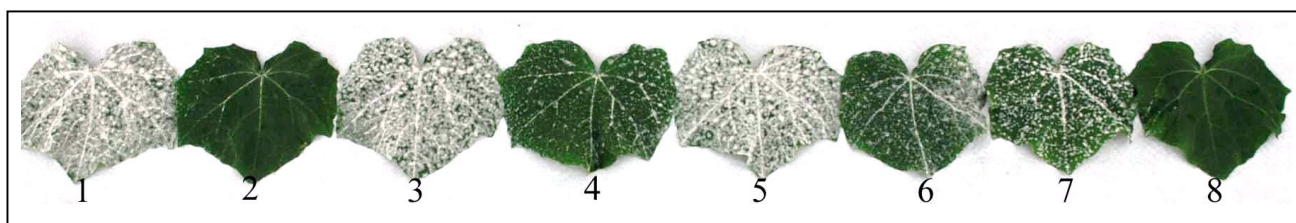


Fig. 3. Effect of background or end of day light quality on the efficiency of UV-B on powdery mildew severity of cucumber cv. Confida 12 days after inoculation. Inoculated plants were immediately exposed to treatments of 16 h W L (1), 16 h W L + UV-B (2), 16 h W L + UV-A (3), 16 h W L + UV-B + UV-A (4), 16 h W L + blue (5), 16 h W L + UV-B + blue (6), 16 h W L + red (7) and 16 h W L + UV-B + red (8)

Wavebands in the visible spectrum identified as suppressive to powdery mildew in the initial section under this objective will be further evaluated for optimal duration and timing of exposure. Duration of exposure to specific suppressive wavebands will be evaluated by sequentially halving the duration of exposure until the treatment effect is lost, or we reach a minimal exposure time (e.g., 5 min, or less). Each exposure duration of less than 12 hrs will be evaluated during daylight hours, and during the normal night period. Pathogen responses recorded as above.

Timeline/Milestones: We anticipate that active wavebands will be identified early in the first section under Objective I, thus work on timing of exposure will begin concurrently in November 2012, and will be completed by January of 2013, with task progression as above. By February 2013, we will know how to use UV-B alone or in combination with specific wavebands against each pathogen group for optimal efficacy, and will have preliminary data on possible growth and horticultural effects of light treatments.

Objective II. To demonstrate commercially relevant levels of powdery mildew suppression in the above pathosystems in small-plot greenhouse trials through manipulation of lighting.

Optimal light treatments (either LED alone, UV-B alone, or LED + UV-B) identified under Objective I will be evaluated in a long-term production cycle for each of the four crops under study: rose, grapevine, cucumber, and strawberry. Mildew susceptible and commercially relevant cultivars for each crop will be selected and raised through a production cycle in greenhouses at the New York State Agricultural Experiment Station (NYSAES) in Geneva. In the case of grapevine, large vines in 5-gal pots will be used. Roses will be of a pot rose cultivar in 1-gal containers. Strawberries will be of a day-neutral variety amenable to high-tunnel production, and will also be cultivated in 1-gal containers. A cucumber variety amenable to greenhouse production will also be reared in 1 gal containers, and trellised within the greenhouse. Control/comparison treatments will consist of untreated plants, and plants treated with a conventional fungicide program to suppress powdery mildew.

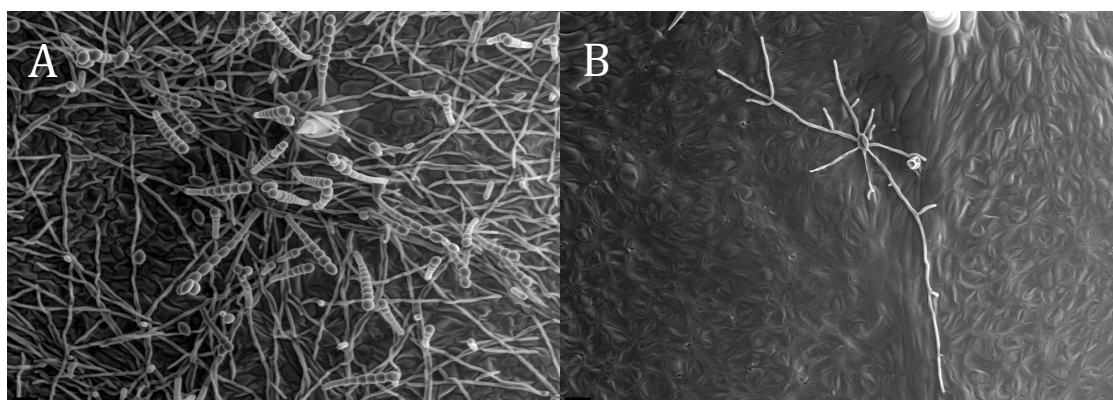


Fig. 4. Scanning micrograph showing effect of UV-B treatment on development of *Podosphaera xanthii*, causal agent of powdery mildew in cucumber six days after inoculation. Plants were inoculated and were either maintained in 16 h white light/ 8 hrs darkness (A) or were maintained under the same daytime lighting, but were exposed to UV-B for 5 min at 1 W m⁻² at 36 h after inoculation (B). Treatment efficacy will be demonstrated to stakeholders using a variety of display methods and media, ranging from demonstration plants to videomicroscopy.

Demonstration plots in greenhouses and tunnel production systems will be exhibited to stakeholders in regularly scheduled field days for the respective commodities held at NYSAES (Fig. 4). In addition, oral presentations will be made on the project to stakeholder group annual meetings (e.g., Lake Erie Regional Grape Program, and North American Strawberry Growers Association). Articles on the project will be published in stakeholder directed newsletters and journals. Technical and scientific aspects of the project will be presented at the annual meeting of the American Phytopathological Society (APS), and published as a component of journal articles in the refereed publications of APS.

A website devoted to the project will be developed and linked to the eXtension communities of practice for the relevant commodities. The website will host descriptive information on the research, news on field days and visits to NYSAES, and links to manufacturers of the UV-B and LED light sources, along with technical data and advisory information on how growers can conduct their own evaluations of the technology; which can then be reported back to project leaders.

Timeline/Milestones: Website construction will be completed in October 2012, the first month of the project. Propagation/planting of plants for the greenhouse trials will begin in February 2013 (grapevine and rose) and March (cucumber and strawberry). Data collection on comparative disease levels among control, light, and fungicide treatments will begin as soon as symptoms are noted on controls, and will continue throughout the experiment at 1 to 2 wk intervals. Field days to view the trials will begin in June and continue through July 2013. Publication of results in extension newsletter and refereed journals will begin in June 2013, with project concluding in September 2013.

EVALUATION PLAN

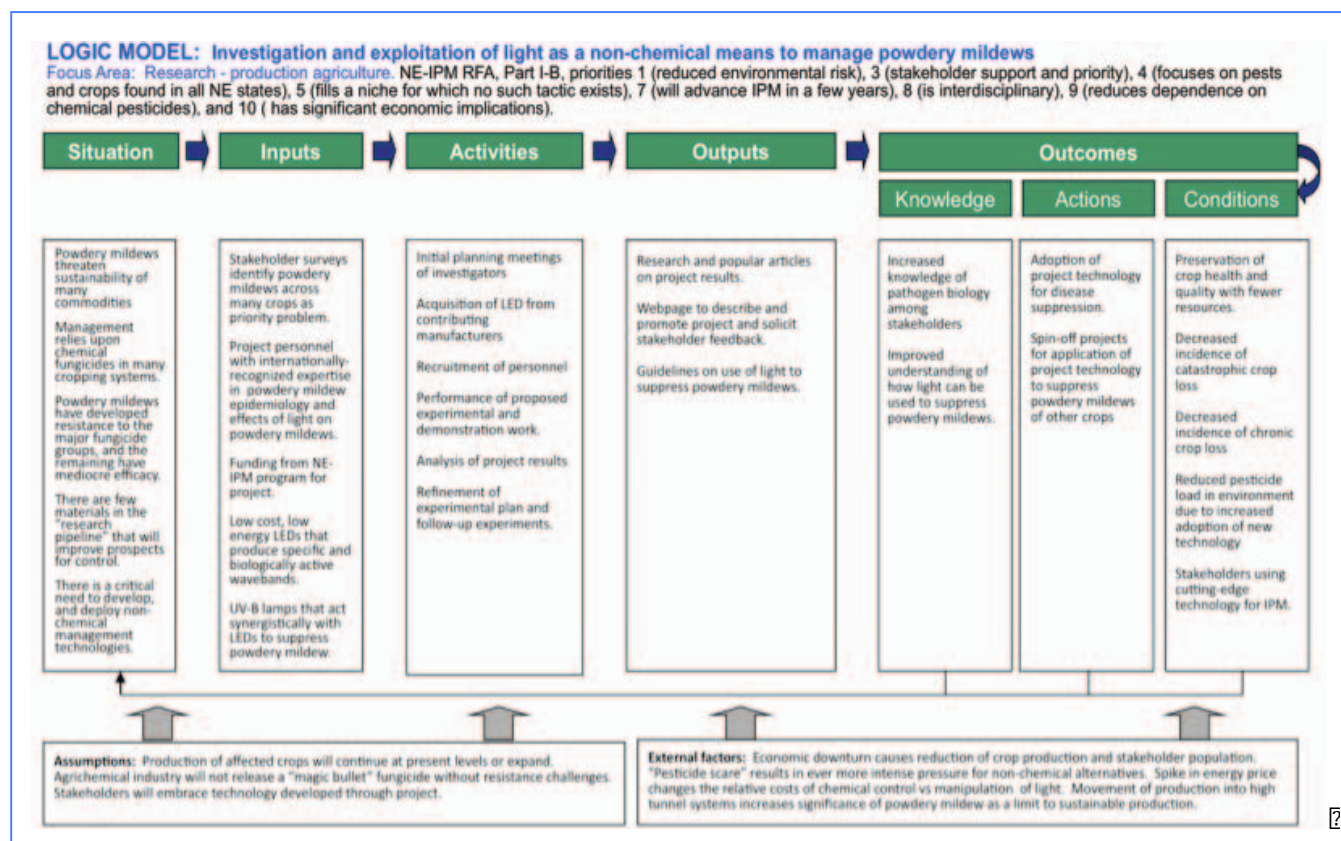
This is a relatively short-term project, built upon a considerable body of preliminary data. We have proposed a 12-month time line for the project from initiation to completion. The knowledge, action, and condition outcomes listed in our logic model are reasonable extensions of the project if it is successful. Our preliminary studies indicate, in our opinion, a high probability of project success. However, the logic model outcomes are still largely prophetic in nature.

The projected impacts for each objective are a different matter. They are comparatively straightforward and will be quantified by the metrics listed in Table 1. The short time line of the project requires that evaluation begin at the project outset, and continue over the life of the project.

Given the present limitations to management of powdery mildews as described in the project narrative, and as indicated in the logic model, we anticipate that the UV-B and LED technology will be further evaluated by research and extension personnel in other regions based upon publication of the proposed experiments, and promotion of the demonstrations to be conducted under objective II. Success of the present project is likely to therefore spur further research, and may lead to commercialization of the technology. However, it is important to note that commercialization of the technology, given the low cost of the apparatus that may be required, is not necessarily a limiting factor. The lamps we are using in the performance of the proposed experiments are inexpensive and commonly available from a variety of commercial sources. Thus, the foundational studies that we will conduct could lead directly to commercial use, assuming our experiments are successful, and the demonstrations are effectively promoted to the stakeholder community.

Table 1. Objectives, relevant NE-IPM goals, anticipated impacts, and metric proposed to verify anticipated impact for proposed project.

Objective	Relevant NE-IPM Goal	Anticipated impact	Metric to verify impact
I: Light quality and time	<i>Encourage science-based pest management</i>	Publication of results	Number of publications
		Presentation of results at stakeholder meetings	Number attending meetings
		Creation of website to extend results and inform stakeholders	Quantify website traffic related to project
	<i>Promote economic benefits</i>	Reduced number of fungicide applications	Reduction in number of fungicide treatments allowed by light treatments and cost thereof
		Reduced loss due to disease	Dollar value attributable to severity of disease in non-treated, light-treated, and fungicide treated
II. Demonstrate suppression in small plots	<i>Furthering implementation of IPM</i>	Stakeholder exposure to demonstration plots from field days and tours	Attendance at field days and tours
		Stakeholder exposure to project results from website	Quantify website traffic related to project.



KEY PERSONNEL

Dr. David M. Gadoury (PD) is a senior research associate in Cornell University's Department of Plant Pathology and Plant-Microbe Biology at the New York State Agricultural Experiment Station. He has 26 years experience in research and outreach program on powdery mildews of various crops. In collaboration with Drs. Suthaparan, Stensvand (*See: COLLABORATIVE ARRANGEMENTS for further detail and Biosketches*), and Seem, he has published the groundwork articles on the use of light for management of powdery mildews. Dr. Gadoury is the Project Director. He has primary responsibilities for overall project management, for design of experiments, and to assist the technicians as postdoctoral scientist (Dr. Suthaparan) assigned to the project. He will lead the design of the website, and all outreach efforts to stakeholders.

Dr. Robert C. Seem (Co-PD) is a professor in Cornell University's Department of Plant Pathology and Plant-Microbe Biology at the New York State Agricultural Experiment Station. Dr. Seem is a plant epidemiologist and authority on the relationship between environmental factors and disease development, and in the precise quantification of environmental factors, and measurement of disease incidence and severity. He also has extensive experience in research on powdery mildew fungi, including the preliminary studies on use of LED and UV-B lamps to suppress powdery mildews. Dr. Seem will assist in the design of experiments, analysis of results, and in the supervision of technical support staff.

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