

BIOAg **FINAL** report due June 30, 2016

TITLE:

A BIOLOGICALLY INTENSIVE APPROACH TO INDUCE THE NATURAL IMMUNITY OF POTATOES AGAINST THE PATHOGENS INCITING LATE AND VERTICILLIUM WILT

PRINCIPAL INVESTIGATOR(S) AND COOPERATOR(S):

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KEY WORDS

WILD YEASTS, INDUCED IMMUNITY, VERTICILLIUM DAHLIAE, PHYTOPHTHORA INFESTANS

ABSTRACT

The success of this research is indicated by the advances realized on the primary objectives and the resulting publications. The overall objective of this proposal was to develop procedures for improving the natural immunity of potato adequate to ward off the destructive pathogens causing Late Blight and *Verticillium* wilt. In lab tests we successfully elicited the potato immune response against Late Blight infections with cell suspensions of “Wild Yeasts” Also protection from a chemical known as EDTA was demonstrated in the laboratory and greenhouse. The commercial development of the EDTA chemical compound is a massive financial effort and currently has not been realized. However the results from potatoes have been published in the American Journal of Potato Research (Wild Yeast paper) and Molecules (EDTA paper) and those on auxiliary systems elsewhere (see below). This successful results prompted an effort to initiate Yeast treatments against potato late blight under field conditions. The first year test was not conclusive because of the presence of environmental conditions adversely opposite of those conducive for infection. The generation of the Wild Yeast treatment appeared to be **economical enough** to serve as protection for Organic Growers as the yeasts can be grown exclusively on potato extracts that are by-products of the potato market material. The EDTA treatment as a synthesized organic molecule does not conform to the National Organic Program guide lines, and was used to further understand the molecular mechanisms for inducing immunity. Three separate trials for inducing improved immunity against the potato wilt, *Verticillium dahlia* were conducted by treating potato seed pieces with varying concentrations of the “Wild Yeast”. The yeast was beneficial to potato growth and there were incremental reductions in wilt symptoms. The corresponding studies on the induction of immunity in a legume system contributed to our understanding of how the immunity in all plants may be generated.

PROJECT DESCRIPTION:

As indicated in the abstract, the main objective of our research is to develop foliar treatments for inducing the natural immunity of potatoes with technologies that especially benefit the organic grower. These growers, except for some copper fungicides, have little practical defense against the potato late blight. When conditions are right, Late Blight can totally destroy potato crops. Our potato facilities enabled the testing of immunity generation in response to both pathogens. Similar immunity increases against *Verticillium* Wilt were detected, however the treatments were administered to potato seed-pieces rather than the foliage and will likely reduce the cost of administering an immunity inducing component.

OUT PUTS:

WORK COMPLETED:

We were fortunate to obtain Wild Yeast strains discovered locally. We now know that microorganisms such as these can release elicitors capable of inducing immune responses in plant tissues if they are applied prior to the arrival of infecting pathogen spores. Our screening of 10 yeast strains selected "Strain 22" with the genus-and-species name, *Metschnikowia pulcherrima*. We developed the assays that identified this optimal strain 22. This data was published in the May 2015 issue of the Am. J. Potato Res.(Vol.92:379) The use of these assays has also allowed us to know the yeast cell concentrations most likely to be successful under actual field conditions, as well as the duration of the protection. I developed a yeast preparation that totaled approximately 24 liters with a concentration of 10^8 cells per milliter. This preparation was developed for the field test of August 2015.

PUBLICATIONS:

With this grant, adequate data was collected to enable the following manuscripts to be published in the American Journal of Potato Research, and to an organic chemical journal called Molecules.

Hadwiger, L. A., McDonel, H., Glawe, D. 2015. Wild Yeast strains as prospective candidates to induce resistance against potato late blight. Am. J. Potato Res. DOI 10.1007/s12230-015-9443-y

Hadwiger, L. A., Tanaka, K. 2015. EDTA a novel inducer of pisatin, a phytoalexin indicator of the non-host resistance in peas. Molecules 20, 24-34: doi:10.3390/molecules20010024

Publications related to the response of plants to pathogens releasing elicitors that are not considered specific for the plant species challenged (non-host resistance) are as follows:

Hadwiger, L. A. 2015. Nonhost resistance: Self-inflicted DNA damage by fungal DNase accumulation is a major factor in terminating fungal growth in the pea –*Fusarium solani* f sp. *phaseoli* interaction. Physiol. and Molecular Plant Pathology 92:79-87.

Hadwiger, L. A. 2015. Anatomy of a nonhost disease resistance response of pea to *Fusarium solani*: PR gene elicitation via DNase, chitosan and chromatin alterations. Frontiers in Plant Science 6:373-400.

Hadwiger, L. A. 2015. Chitosan: The preliminary research and the host/parasite system that led to the discovery of its antifungal and gene inducing properties. J. Mol. Genet. Med. 9:158. doi: 10.4172/1747-0862.1000158.

Hadwiger, L. A. 2015. Low level DNA damage occurs as PAMPs, chitin and flg22, activate PR genes, and increases pisatin and disease resistance in pea endocarp tissue. New Negat. Plant Sci.1-2:6-15.

OUTREACH:

Outreach had been mainly to pursue complementary funding and was directed, primarily to the consortium of Northwest Potato commissions –(Portland Oregon,Fall 2015). We developed a poster and presented data at the BIOAg symposium in November and at the WSU Showcase.

Subsequently the director of the North west Consortium of potato commissions invited me to present this research at the annual meeting of the Washington Idaho and Oregon potato growers (held in the Tri-Cities last fall) and explain how basic research such as this can be developed into practical applications.

The ultimate outreach to the scientific community was via the extensive publications listed above.

IMPACTS:

SHORT TERM:

Our data were accepted well by the Northwest Consortium of potato commissions and this organization will also provide some support for research directed towards the benefits of organic growers. The data also provides us a start for aggressively pursuing the mechanisms of immunity induction. The data has been and is currently a basis for obtaining the commission funding.

INTERMEDIATE-TERM:

The National Organic Program reluctantly allows some copper compounds to be legally used by organic growers probably since they are not chemically synthesized beyond mining. We previously developed a successful copper treatment using the natural component, chitosan as a sticker (Hadwiger and McBride, Plant Health Progress doi:10.1094/PHP-2006-01-RS). Without any late blight protection, potatoes in some rainy areas simply can not be produced. The prospect for having protection in the absence of a heavy metal (copper) may result in the NOP removing their copper allowance. This would of course cause organic growers to seek the bio-control methods such as those I am developing.

LONG TERM:

Those pursuing biological controls are aware that developing a control-- that can end up as a commercial agriculture practice-- is a tall order. In the case of protection from potato Late Blight, it will be worth the effort if there is to be disease-free organic potatoes available to the consumer. Researchers also know that what works nicely in the lab is much more of a challenge to see similar results under field conditions. It will depend on how the field conditions are worked out as to what the long-term benefit can be. There are some things that have been worked out, such as concentration of yeast cells required and the methods of their culture and preservation. The field testing contracted to a very competent private researcher, did not

develop since the environmental conditions were not conducive for a late blight epidemic the summer of 2015. The potato research locations available to Washington State University can not allow the dispersal of Late Blight inocula, because of the chance of initiating epidemics effecting local growers. I am optimistic that this yeast control can be developed to the point where potatoes can be grown organically and yield reasonable if not bountiful harvests. This yeast microbe can be reproduced to levels that can economically viable in a very short period of time and has no environmentally detrimental properties.

ADDITIONAL FUNDING SECURED:

I received minimal support from the Washington State Potato Commission and will continue to apply for funds from this source in the future. To date the commission has funded 3 years at 7,000/per annum.

GRADUATE STUDENTS FUNDED.

The budget of this BIOAg grant was intended to be used for one year of a graduate student stipend, however the student being recruited decided on a career change and took an available position. The timing was not good to pursue another applicant. The original funding was reduced to 10,000 and re-categorized through the CAHNRS office since funds were not sufficient to support a graduate student. The funding **has** supported an undergraduate work study individual.

RECOMMENDATION FOR FUTURE RESEARCH:

The future research will continue to employ field testing (when conditions allow) of the Late Blight control and additional greenhouse testing for *Verticillium* wilt control. If Wild yeast cells or the EDTA chelating agent can induce immunity against Late Blight infections in field grown potatoes, there is hope that a practical application is possible. My personal interest is to more aggressively pursue the biochemical and genetic bases for how the Wild Yeast is able to induce immunity in potatoes.

It is likely that the yeast releases components that induce the immunity and that these components can be isolated and identified. I will start by purifying the DNase 1 that all yeasts have the potential to produce and release. It has been shown in another system to be a major elicitor of plant defense responses. I will try to obtain a constant supply of the DNase 1 enzyme in an expression vector so that both the optimal levels and DNA damage caused in the plant tissue can be assessed. We have observed in other host–parasite interactions that the single strand nicking by DNase 1 is capable of activating defense genes called pathogenesis genes (PR genes). We have access to the DNA primers for the PR genes of potato and have developed an induction tissue that can detect the rapid plant responses – both those induced by the DNase 1 and those induced by the intact yeast cells. The funding obtained by this BIOAg grant has brought to the forefront components that we can now study in detail, knowing that we are involved with processes that are actually involved in plant immunity induction.

It is likely that our research results will benefit other labs looking at potato immune responses. The benefits of this research will be usable throughout the potato growing regions.