San Luis Valley Research Center Committee

Project Outline

Project Title: Exogenous and endogenous late-blight resistance while providing antioxidant qualities to potatoes

Principal Investigator: Dr. Jorge M. Vivanco (Department of Horticulture and Landscape Architecture)

Keywords: antimicrobial compounds, ecologically-derived fungicides, antioxidants, added value in potatoes, post-harvest

I would like to respectfully request support from the San Luis Valley Research Center Committee (SLVRCC) to continue research on the antifungal proteins from *Phytophthora infestans* (Late Blight)-resistant potato progenitors. We believe our research may accelerate the development of resistant potato varieties. I'd also like to point out that no late-blight resistant gene endogenous to potato has been cloned to date. My laboratory specializes in studying metabolism and biochemistry of biologically active secondary metabolites and proteins produced in plant roots and tubers, and the uses of plants for nutritional, pharmaceutical and agrochemical applications. The projects described below will fit into my research duties as an assistant professor of horticulture, and will provide useful applications for San Luis Valley agriculture.

Funding provided by the SLVRCC during 2002 has allowed us to identify a natural product (Rosmarinic acid) with strong antioxidant activity as an ecologically-benign fungicide against *P. infestans*. Last year, my laboratory reported the isolation of Rosmarinic acid (RA) from sweet basil (*Ocimum basilicum* L.) root exudates. RA showed antimicrobial activity against a range of soil-borne microorganisms including several fungal and bacterial plant pathogens. We have obtained evidence that RA is a strong fungicide that inhibits the growth of *P. infestans*. The proposed research explores the possibility of prophylactically treating potato plants with an ecologically benign fungicide; RA could additionally provide value-added properties such as increased antioxidant potential in the tubers.

Work performed in this project has been conducted by a Master's degree candidate (Hope Gruszewski) co-advised by Drs. Vivanco and David Holm, and a post-doctoral fellow (Dr. Neelam Sharma).

Results from 2002

Copies of the progress report for our 2002 work will be handed to the Committee during our annual meeting (March 2003).

A. Endogenous Late-Blight resistance in potatoes

In the effort to develop resistant varieties, a somatic hybrid (The Wisconsin J series) between potato (*Solanum tuberosum*) and a wild relative (*S. bulbocastanum*) was created, and has been found to convey durable resistance against *P. infestans*. Through chromatographic separation and an *in vitro* bioassay to assess inhibition of spore germination, we screened tubers of a Late Blight-resistant progenitor (Wisconsin J 138) for bioactivity against *P. infestans*. Utilizing the J138 genetic material developed by Dr. Holm and currently available at the CSU' San Luis Valley research center, we have purified a 40kD protein from potato tubers with *in vitro* antimicrobial activity against this pathogen. The N terminal sequence of this protein was found to have exact amino acid homology to patatin, the major storage protein of potato tubers. The inhibitory protein has the same molecular weight and cross-reacts with patatin antibodies. Comparison of amino acid composition with patatin from a susceptible cultivar suggests that the putative patatin protein

has a unique profile and may be a new isoform. In a research communication to be submitted shortly to the journal *Planta*, we report for the first time antimicrobial activity of patatin against *P. infestans*.

B. Effect of Rosmarinic as a fungicide against *P. infestans*
Rosmarinic acid (RA), an abundant antioxidant present in members of the Lamiaceae family, was previously reported by our group as a natural product that exhibits antimicrobial activity against a broad array of plant pathogens. Our findings suggest that RA is a potent antifungal agent against *P. infestans* biotypes US1, US6, US8, and US11. Fungistat assays showed that RA (80 to 250 mM) inhibited the mycelial growth rate of *P. infestans* by 60 to 80%. Scanning imaging and fluorescence microscopy of *P. infestans* hyphae and sporangia treated with RA (250 mM) revealed that the fungus was morphologically compromised by changes in hyphal surfaces and sporangial development. All treatments from 0.1 to 10 mM RA inhibited spore germination and subsequent hyphal growth of these biotypes in *in vitro* assays. We have recently conducted experiments in collaboration with Dr. Jeff Miller (University of Idaho, Aberdeen Experiment Station) to test the potential of RA as a fungicide to control *P. infestans* infection on potatoes. Preliminary data from greenhouse spray experiments suggest that a crude extract from common sweet basil (*Ocimum basilicum)* containing ~ 2mM of RA has fungicidal activity against *P. infestans* US8, US1, US6 and US11.

Experimental Plan:

A. Endogenous Late Blight resistance in potatoes

Year 3 (2003): Gene cloning and characterization of the antifungal patatin isoform. Primers will be designed based on the isoform's internal peptide sequences, which will allow us to clone the antifungal patatin by RT-PCR. We will use the PCR product to screen a potato (J1 series) cDNA library in order to obtain a full-length clone. Using the cDNAs as probes we will isolate genomic clones for this gene.

Year 4 (2004). Characterization of the antifungal patatin's specific promoters and *Agrobaterium*-mediated transformation. The availability of a genomic clone for the antifungal protein will enable us to obtain insight into the regulation of its specific promoter. For example, over-expression of these antifungal proteins will be assayed in experimental systems such as cell cultures using different stress responses. Once we have obtained this preliminary information, we will transform commercial potato varieties with this Late Blight-specific gene. Two different promoters will be used: CaMV 35S, which is a strong and constitutive promoter, and the antifungal patatin-specific promoter. After transformation the plants will be screened and different potato lines will be selected. We will transform potato varieties used commercially in the SLV, as well as varieties determined to be resistant.

Year 5 (2005). Pathogenic challenge and screening. The different potato transformants will be challenged with *P. infestans* in the greenhouse to confirm their resistance. Subsequently, these plants will be taken to the San Luis Valley for disease resistance trials. These plants will also be taken to the Toluca Valley in Mexico, the area with the highest diversity and concentration of *P. infestans* inoculum. We will use the antibodies raised against the antifungal protein to screen for Late Blight-resistant potato progenitors developed by CSU's Potato Breeding program.

B. Effect of Rosmarinic acid as a fungicide against *P. infestans*

Year 1 (2003). Application of Rosmarinic acid as a curative and preventive fungicide under field and post-harvest conditions. Extracts containing different concentrations of Rosmarinic acid (RA) will be sprayed under field and post-harvest conditions to control the spread of potato diseases in the San Luis Valley. We are particularly interested in analyzing the effect of RA as a fungicide against *Alternaria solani* and *Spongospora subterranea*, and other pathogens that usually infect potatoes in the San Luis Valley. Hauser,

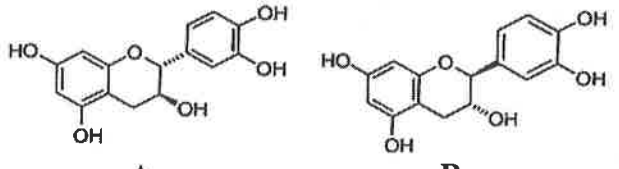
a natural product company located in Longmont (CO), has agreed to provide RA-containing extracts from rosemary at a reduced price. The antimicrobial effect of these extracts will be compared to the effect of commercial fungicides usually sprayed in the San Luis Valley. Drs. David Holm, Richard Zink and Robert Davidson (CSU) will collaborate in the development of these studies. Additionally, we will continue our collaboration with Dr. Jeff Miller (University of Idaho) to evaluate the effectiveness of RA against *P. infestans* infectivity in potatoes.

As previously outlined, RA is a strong antioxidant; thus we will evaluate if treatment with RA (under field and post-harvest conditions) confers to the tubers an increased antioxidant content. If indeed RA can be translocated to the tubers while controlling disease, it will confer a value-added property to potatoes that could be used as a health benefit and as a strong marketing tool.

Year 2-4 (2004-2006). Characterize the biosynthetic pathway of Rosmarinic acid. We will isolate the enzymes and genes involved in the biosynthesis of RA with the tentative goal of engineering those genes into potato cultivars. If successful, we will confer broad-spectrum disease resistance to potatoes, and a value-added, antioxidant and medicinal property to the crop.

C. Evaluation of (-)-catechin for weed control in potatoes

The Asian native *Centaurea maculosa* L. (spotted knapweed) is one of the most economically destructive exotic invaders of western North America. It rapidly displaces native vegetation and improved pasture species with the help of phytotoxic root exudates (Callaway et al., 2000). Although allelopathy (secretion of phytotoxic chemicals) has been suggested as the main displacing mechanism, until recently there has been little if any success in characterizing the responsible allelochemical. Our laboratory has determined that an exudate of *C. maculosa* roots is phytotoxic. The active fraction of the exudate was found to be composed of racemic catechin, a 50:50 mixture of (+)-catechin (**A**) and (-)-catechin (**B**) (See Figure 1). Although (+)-catechin is widespread throughout the plant kingdom, both (-)-catechin and the racemic form have only rarely been isolated. Further, we found that the phytotoxicity and germination-inhibiting action of the exudate was entirely due to **B**, while **A** was found to have antimicrobial properties. (-)-Catechin (**B**) was toxic to several weeds, soybean, sugar beet and the model plant species *Arabidopsis thaliana*, but did not affect seedlings of *C. maculosa*. Our manuscript titled "Enantiomeric dependent phytotoxic and antimicrobial activity of (±)-catechin; a rhizosecreted racemic mixture from *Centaurea maculosa* (spotted knapweed)" was published in the April 2002 issue of *Plant Physiology*. This manuscript was selected among all the papers in the April issue for a special Editorial Note, and has received prolonged attention in the popular media.



A **B**

Figure 1. Chemical structures of (+)-(2*R*,3*S*)-catechin (**A**) and (-)-(2*S*,3*R*)-catechin (**B**).

We have also recently undertaken preliminary greenhouse studies which show that (-)-catechin is phytotoxic when sprayed on the foliage of susceptible plants. In these studies, (-)-catechin significantly reduced the height of some weed species at 0.02 lb ai/ac and significantly reduced the height of four broadleaf weeds at 0.16 lb ai/ac. The two most sensitive weeds were lambsquarters and hairy nightshade, while barnyard grass showed only a moderate reduction in plant height.

This summer (2003), in collaboration with Drs. Scott Nissen and Samuel Essah, we will test the effect of (-)-catechin, an environmentally-benign herbicide, as a weed control strategy in SLV potato fields. Based on the results of these experiments (Summer 2003), we will plan in depth studies.

Budget

1. Salary (including fringe) for one research assistant/graduate student	$26,773
2. Supplies	$6,089
TOTAL:	**$ 32,862**

Budget Justification

Research assistant/graduate student: To maintain a vigorous output of ideas, data and to coordinate the different projects proposed in this application, we request support for a research assistant/graduate student salary over the course of the project. We need a dedicated person with natural product/chemistry/physiology experience who will complement the biochemistry expertise at Dr. Vivanco's laboratory.

Supplies: Funds are requested for chemicals, supplies, limited small equipment and maintenance of equipment used as part of this project.

References

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