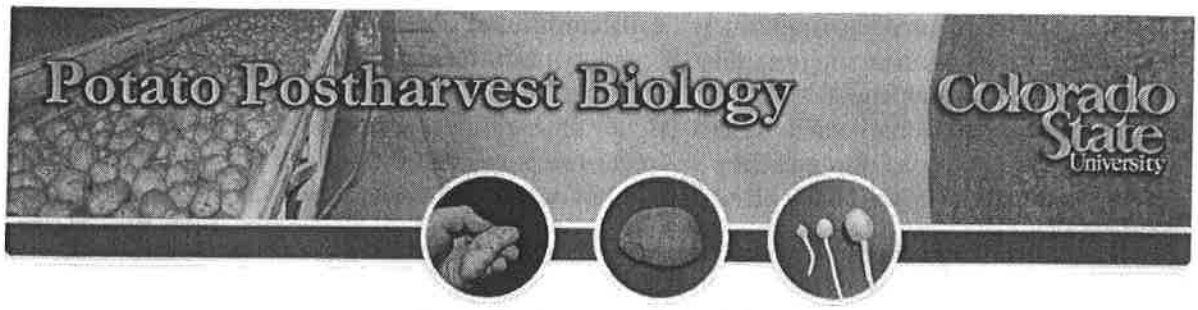

Proposals for 2015-2016

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Potato Postharvest Physiology
San Luis Valley Research Center, Department of Horticulture & LA

Funding Source: CCPGA Royalties

Title: Title: Management of powdery scab and developing molecular markers for powdery scab resistsance

Nature, scope, objectives of proposed research: Powdery scab disease caused by *Spongospora subterranea f. sp. subterranea* is one of the major concerns for potato producers in production regions throughout the world. This is a soil borne pathogen that infects root hairs, stolon epidermal cells, lenticels, eyes and wounds of developing tubers. Infected tubers and roots may have white gall-like growths, which later develop into brown powdery scab tuber lesions as they mature (Harrison et al. 1997).

Powdery scab symptoms cause significant economic losses in both fresh and seed markets. Depending on the severity of symptoms, tubers could become non-marketable or grade quality may be reduced in fresh and seed markets. Seed lots infected with powdery scab may or may not pass inspection depending on the regulations of the certifying agency and the degree of infection. Infected tubers are also more susceptible to secondary infections, such as fusarium dry rot, bacterial soft rot and other pathogens during storage.

Christ (1993) observed that potato cultivars with smooth or light skin (i.e whites and reds) are more susceptible; whereas russet-skinned cultivars are less prone to powdery scab, although root galls are common. There are reports on partial russeting in some cultivars such as Rio Grande Russet. Partial russeting and irregular skin set can lead to disease, loss of water and susceptibility to skin bruise, effecting tuber quality (Lulai EC, 2002). A better understanding of russeting mechanisms will help us to develop new cultural tools for better skin set in new cultivars to enhance the native capacity of tubers for skin set.

We standardized and established a facility to test soil samples to detect and estimate powdery scab spores at postharvest lab. We conducted metabolic studies on how smooth skin cultivars differ from russet skin varieties. We could identify significant differences between them.

The potato genotypes with russet tuber skin are generally resistant to powdery scab.

Lipoxygenase and patatin are two key storage proteins that are known to offer resistance to several diseases and insects. The objective of this study was to find out the relationship of these proteins in stored tubers with potato tuber powdery scab resistance, especially in russet skinned potatoes.

The evaluation of potato germplasm with different tuber characteristics for several years in a green house environment suggests that the genotypes with russet skinned tubers (Mesa Russet, Centennial Russet and Russet Nugget) provided resistance to tuber powdery scab with negligible tuber DSI and 100% marketable tubers. Higher physiological levels of LOX protein in the skin region are directly related to powdery scab resistance and tuber russet skin (Figure 1). The total protein and patatin-lipase levels of tubers did not reveal their role in powdery scab resistance in these genotypes.

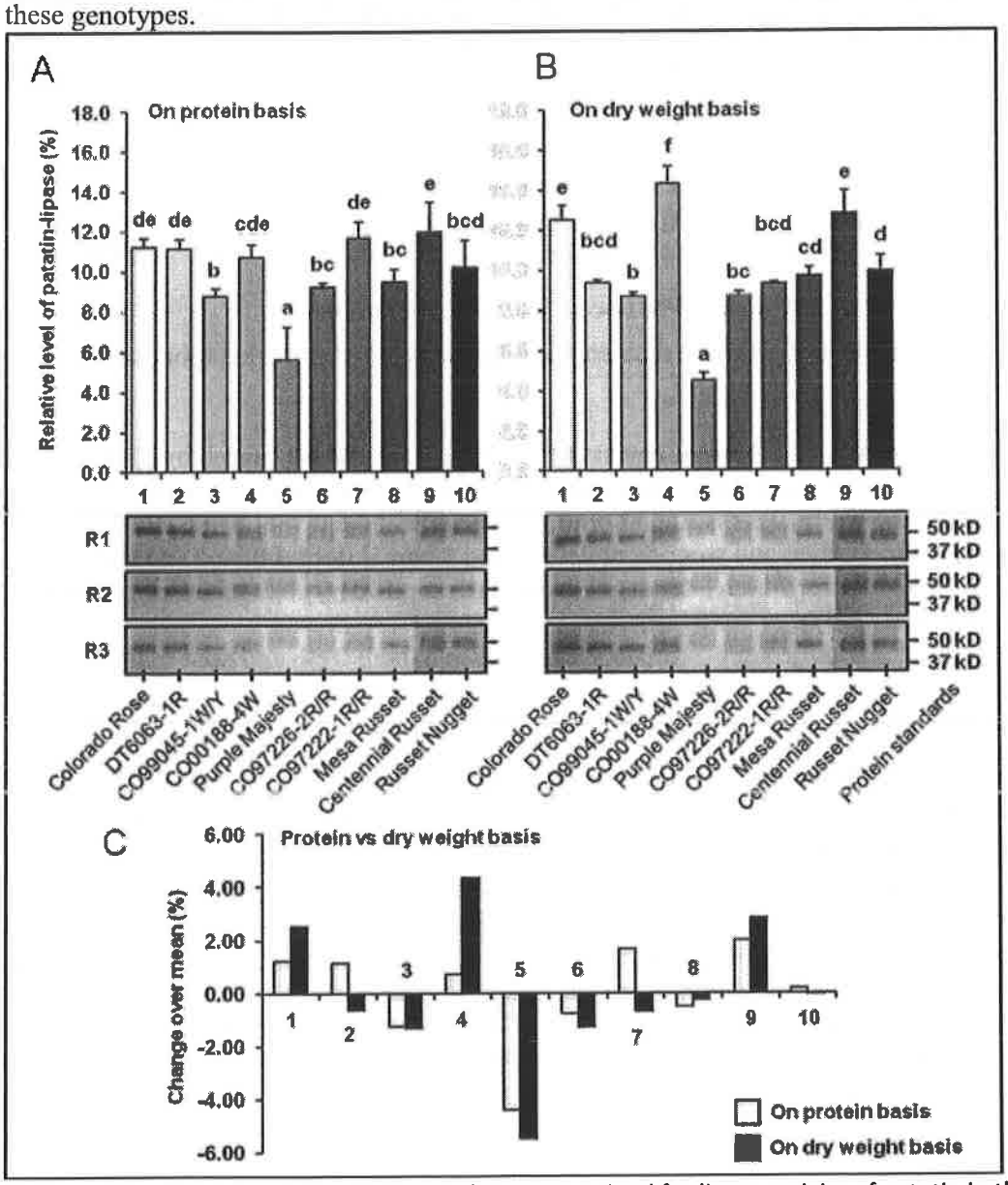


Figure 1: One dimensional SDS-PAGE gels that were stained for lipase activity of patatin in the tubers of various potato genotypes. (A) On protein basis: From each genotype, 5 µg of total tuber protein was used for the SDS-PAGE. (B) On dry weight basis: From each genotype, total protein present in 0.5 mg of tuber dry weight was used for the SDS-PAGE. (C) Changes over mean tuber patatin-lipase value of genotypes were compared between the protein and dry weight basis of analysis. ANOVA was performed

on the relative levels of patatin-lipase that were estimated from 3 gels. Mean values that are not significantly different from each other ($P > 0.05$) in each graph are represented by the same letter after the Fisher LSD multiple comparison test. Each bar in a graph represents the mean ± SD of 3 determinations.

Possible resistance mechanisms that were associated with higher physiological levels of LOX protein that include accumulation of phytoalexins, HR mediated cell death and the accumulation of suberin in the periderm of the tubers were discussed here. Physiological levels of LOX protein may be used as a marker for powdery scab resistance in initial screening of stored potato germplasm. Higher levels of LOX segregate with powdery scab resistance may further confirm present findings and the proposed role of LOX in powdery scab resistance in russet skinned potato tubers

Objectives:

1. To find out the relationship of LOX and other cell wall proteins in stored tubers with potato tuber powdery scab resistance, especially in russet skinned potatoes
2. Developing enzyme based marker to screen the germ plasm for resistance to powdery scab disease
3. Developing information on cultivars tolerance to powdery scab spore load in the soils.

Extension-outreach plan for reporting project information to growers

Results will be presented and reported to grower community and scientific community using following avenues

- Rocky Mountain Ag conference,
- Northern Colorado Potato Grower meeting
- Potato Association of America Annual meetings
- Field days,
- Open house,
- Field Tours,
- Annual Reports
- Spuditems, newsletter or fact sheet.
- And site visits to commercial storages and
- Web site (ppb.colostate.edu)

Potential for results to leverage additional outside funding

This is one of the important research areas identified by Specialty Crop Research Initiative grant advisory panel, which includes nutritionists, dieticians, potato trade organizations, Chefs and processing, fresh market, growers and related industry.

Funds requested- $25,000

Research Associate (50%):	$16,000.00
Equipment and laboratory supplies:	$4,000.00
Chemicals Supplies and Services:	$5,000.00

Title: Exploring alternatives to the chemical control of nematodes in potato in the San Luis Valley-Year 2

Most relevant funding source: CSU Cultivar Royalty Funds (from CCPGA).

Investigator name(s) and department(s): Principal Investigators: Drs. Jorge Vivanco (CSU-Horticulture) and Samuel Essah (CSU-Horticulture and SLV Research Center). Collaborator: Dr. Antoon Ploeg (University of California, Riverside – Nematology Department)

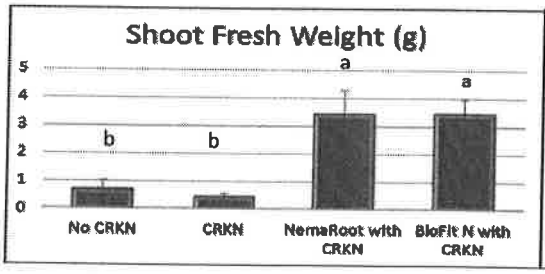
Background

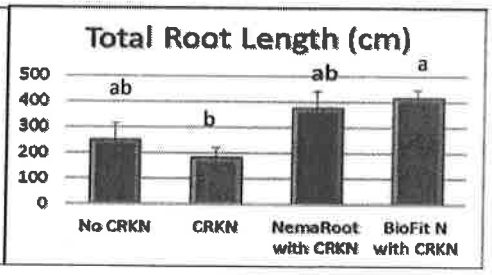
Apart from PVY virus which is transmitted by aphids, root knot nematodes (*Meloidogyne* spp) cause substantial economic damage. The Columbia root-knot nematode (*M. chitwoodi*) is of particular importance to potato as it is commonly found in potato fields and even low populations of this nematode can severely impact tuber quality. The most widespread method for nematode control is the use of Vydate. This chemical is highly toxic, and poses a serious risk to human health and the environment. The use of microorganisms antagonistic to nematodes or compounds produced by these organisms may provide additional opportunities to manage the damage caused by the root-knot nematode (Radwan et al., 2012). The fungus *Paecilomyces lilacinus* preys on *Meloidogyne* females and reduces the population density of nematodes in the soil (Khalil et al., 2012). There are several biological nematicides available in the market containing various formulations of *Paecilomyces lilacinus*. In addition, bacteria such as *Bacillus subtilis* have been reported to have nematicidal activity.

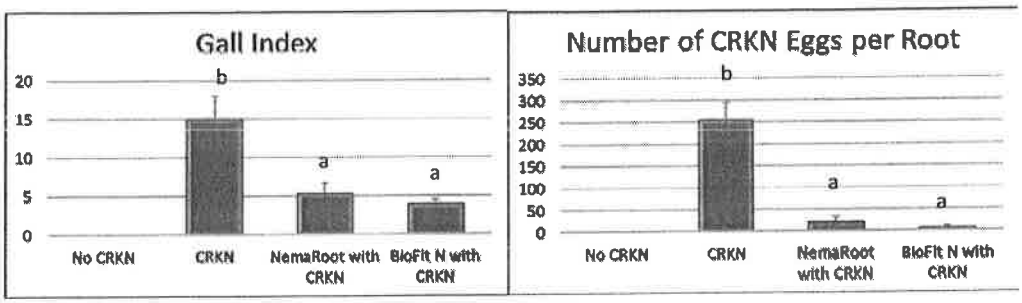
In year 1 of our studies, we tested the effect of two biological nematicides against *M. chitwoodi*. One of these nematicides contained *P. lilacinus* (Nemaroot) and the other had a combination of beneficial microbes including *Bacillus* (BioFitN). The results of our trials were successful and in this second year we propose to expand our studies in the field under realistic SLV management conditions (Aim 1). Additionally, SLV growers have expressed a need to isolate and characterize beneficial microbes from their own fields that have activity against nematodes (Aim 2).

Greenhouse Results – First Round of Funding

As proposed in Year 1, Dr. Antoon Ploeg trained personnel at Dr. Vivanco's lab at CSU. Based on that training we accomplished our greenhouse studies and are now prepared to perform long-term nematode studies at CSU to aid the SLV growers. The results of our greenhouse studies are summarized in the following figures in which potato plants were infected with nematode eggs (CRNK) and treated with the biological products.







Aims

The specific research aims of our proposal are:

1. Test the two commercial biological nematicides for effectiveness in controlling *M. chitwoodi* under field conditions.
2. Isolate beneficial microbes from San Luis Valley soils that could be natural antagonists of *M. chitwoodi*.

Research Team

Dr. Jorge Vivanco is a Professor of Horticulture at CSU. His laboratory works on interactions between root and the soil microbiology with particular focus on identifying beneficial microbes that could help plants deal with biological and environmental stresses. Dr. Samuel Essah is an Associate Professor of Horticulture located at the San Luis Valley Research Center of CSU. Professor Essah's program focuses on developing the best cultural management practices for potato cultivation. Professors Vivanco and Essah have a long-standing collaboration to bring and test new technologies related to potato cultivation to the SLV. Professor Antoon Ploeg (Nematologist from the University of California, Riverside) has been invited as a close collaborator of this project.

Experimental Methodology

Aim 1. Test one commercial biological nematicide for effectiveness in controlling *M. chitwoodi* under field conditions.

Specific potato fields in the San Luis Valley infested by nematodes will be identified by collaborating with our extensive network of collaborators/growers in the SLV. Soils from infected sites will be sampled early in the season and brought to the laboratory of Dr. Vivanco to monitor the starting levels of nematodes of those fields. We will find 2-3 commercial sites and in each of those sites we will conduct the following treatments:

1. Control: farmer's crop management practices
2. Treatment with Vydate at the beginning of the season followed by four applications of the biological nematicide
3. Four applications of the biological nematicide

Experimental Design: The study will be laid out as a randomized complete block design. Each treatment will be replicated four times.

In-Season Plant Sampling and Measurements: During tuber bulking, plants will be sampled from each plot to evaluate treatment effects on tuber set, mean tuber weight, and tuber bulking rate.

Tuber Yield and Quality Evaluation: Potato tubers will be harvested and evaluated at the end of the growing season for yield and tuber size distribution. Tubers from each plot will be weighed and graded for external (misshapes, knobs, growth cracks) and internal (hollow heart and brown center) defects. The harvested tubers will be separated into various size distribution groups based on weight (<4 oz, 4-6 oz, 6-8 oz, 8-10 oz, 10-12 oz, 12-16 oz, and >16 oz) to evaluate treatment effects on tuber size profile for each cultivar. Ten large (10-16 0z) tubers from each plot will be taken for hollow heart and brown center evaluation. Specific gravity will be measured using the weight-in-air/weight-in-water method.

Statistical Analysis: Yield data collected from the field will be subjected to analysis of variance to test for treatment effects. The least significant difference test (LSD) will be used to analyze the data to estimate differences between treatment means. Some treatment means will be separated using the *a priori* single degree of freedom contrast.

Aim 2. Isolate beneficial microbes from San Luis Valley soils that could be natural antagonists of *M. chitwoodi*.

This objective was proposed during the last funding cycle. However, due to the reduction of funds we could not conduct the studies that were originally proposed. Therefore, we are proposing it again in the 2015 funding cycle. It is imperative that we find these microbes and that these could eventually become part of a commercial product. The rationale behind this hypothesis is the fact that the SLV experiences very drastic environmental conditions in their soils; therefore, the beneficial microbes that survive under those conditions are more likely to be bioactive against nematodes.

This project will identify and characterize soil microbial populations from the SLV that could be natural antagonists of *M. chitwoodi*. For this purpose, we will rely on an extensive mapping of the SLV and determine areas that are consistently nematode free yet adjacent to nematode infected locations. We will place special attention to avoiding locations that have received either chemical or biological nematicides. Soils from those areas will be collected and brought to the laboratory of Dr. Vivanco at CSU to isolate native species of *Paecilomyces lilacinus* and *Bacillus subtilis*. Soil slurries will be prepared by diluting the soils in water and plating them in specific media. There are specific medium preparations and cocktails of antibiotics available to isolate specific genera such as *Paecilomyces and Bacillus*. We propose that these native beneficial microbes from the SLV might be better prepared to control the nematode populations that affect potato cultivation in the valley than commercial biological nematicides.

Plans to Validate the Technologies

We believe that the success of Aim 1 will create new IPM strategies in the SLV to deal with nematodes infestations. If successful in the isolations of beneficial microbes (Aim 2), we will propose in year 3 to test the effectiveness of these natural strains alone and in combination with commercial biological products under greenhouse conditions and subsequently in the field. These combined results will constitute preliminary data to apply for external USDA funding and/or seek corporate sponsorship. Dr. Vivanco and Essah have recently completed one round of corporate funding from BASF; therefore we are confident that these studies will lead to sponsorship by corporations.

Extension-Outreach Activities

The progress of our activities in year 1 was presented at the 2015 Potato and Grain Conference. In addition, Drs. Essah, Vivanco and Ploeg visited the fields of several farmers in the SLV in July 2014 to talk about nematode damage and potential control strategies. This summer we will invite again Dr. Ploeg and organize a special event about nematodes at the SLV Research Center.

How this Project will Enhance Colorado Potato Grower Competitiveness

The identification of alternatives to Vydate for nematode control in the San Luis Valley will reduce the use of these toxic chemical nematicides. Vydate decreases microbial populations containing beneficial microbes in the soil, thus impoverishing soil health in potato fields. The use of alternative methods to control nematodes will improve the soil health of potato fields which is usually accompanied by increased tuber quality.

Budget

Personnel ($11000). Partial salaries are requested for a technician and student-hourly aids to assist in the tasks proposed here.

Materials and Supplies ($10,000). These expenses include chemicals, media, and disposables for the isolation of nematodes and beneficial microbes from soils.

Travel ($7000). Travel expenses related to visiting (4-5 visits) the SLV for soil collection purposes and to participate in the outreach/extension activities proposed here. This cost also includes the visits of Dr. Ploeg to the SLV and to CSU.

Total: $28,000 for 1 year

References

Dong LQ, Zhang KQ (2006) Microbial control of plant parasitic nematodes: a five party interaction. Plant and Soil 288:31-45

Radwan MA, Farrag SAA, Abu-Elamayem MM, Ahmed NS (2012) Biological control of the root-knot nematode, *Meloidogyne incognita* on tomato using bioproducts of microbial origin. Applied Soil Ecology 56: 58-62

Khalil M, Allam A, Barakat AT (2012) Nematicidal activity of some biopesticide agents and microorganisms against root-knot nematode on tomato plants under greenhouse conditions. Journal of Plant Protection Research 52: 47-52

Tian B, Yang J, Zhang K-Q (2007) Bacteria used in the biological control of plant-parasitic nematodes: populations, mechanisms of action, and future prospects. FEMS Microbial Ecology 61:197-213