Washington State Commission on Pesticide Registration
REQUEST FOR FUNDING

PROJECT # ______

Project Title: Development of a molecular assay for *Fusarium oxysporum* f. sp. *spinaciae*, and assessment of liming for management of Fusarium wilt in spinach seed crops in western Washington.

1) Applicant (user group) Name and Address:	**3) Project Contact:**
Puget Sound Seed Growers' Association (PSSGA): 18488 Best Road, Mount Vernon, WA 98371	Kirby Johnson (360) 466-3485/466-3181 homes@laconnerrealty.com

2) Details of Project:	**4) Research Lead:**
Crop/Site: Spinach seed crop Chemical: Lime Pest Management Issue: Fusarium wilt Pest: *Fusarium oxysporum* f. sp. *spinaciae*	Lindsey J. du Toit: Washington State University NWREC, Mount Vernon, WA. (360) 848-6140 dutoit@wsu.edu

5) Project Category: Check all that describe the focus of your project.

Old Mandate _25_%

_X_ Efficacy Trial
___ Phytotoxicity Study
___ Residue Study

New Mandate_75_%

__x_ Integrated Pest Management
_____ Pesticide Resistance Study
_____ Other ___________________

___ GLP
_X_ non-GLP

6) Project Duration Start Date : 03/2007 End Date : 12/2007

7) Total Project Cost $26,359 **WSCPR Request** $14,905
Matching $9,954 (PSSGA) + $1,500 (in-kind)

Project Summary: Spinach seed crops in the PNW produce up to 50% of the US and 25% of the world supply of spinach seed. Depletion of virgin ground for spinach in western WA has resulted in Fusarium wilt becoming the limiting factor for high yielding spinach seed crops, necessitating rotation intervals of 8+ years. In Denmark, Fusarium wilt is not a problem in spinach seed crops despite 3-4-year rotations. Danish soils are alkaline and high in calcium from limestone deposits. Limestone applications helped control Fusarium wilt of tomato in acid soils in Florida. This proposal requests funding to evaluate, for a second year, the efficacy of limestone for suppressing spinach Fusarium wilt and reducing rotation intervals for seed crops in acid soils of western WA, and to develop a molecular soil assay to help identify appropriate fields for production of seed of cultivars with different levels of resistance to Fusarium wilt.

9) Signatures *I certify to the best of my knowledge that the information in this application is true and correct.*

____Kirby Johnson_______
Printed Name of Applicant

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Signature of Applicant

President, Puget Sound Seed Growers' Association
Title of Applicant

___13 September 2006_______________
Date Signed

10) *Send original application to:*
Washington State Commission on Pesticide Registration; 2621 Ringold Road, Eltopia, WA 99330

Problem Description

Spinach seed crops are grown on 1,500-3,000 acres in western Washington annually, at a farmgate value of $1,000-$1,200/acre. These crops produce up to 50% of the US and 20% of the world supply of spinach seed annually. The competitive global seed industry necessitates production of high quality, pathogen-free seed.

Fusarium wilt of spinach, caused by the soilborne fungus *Fusarium oxysporum* f. sp. *spinaciae*, is present in all areas of the US where spinach is grown. The fungus invades spinach roots and blocks the vascular system, causing wilting and, in seed crops, reductions in seed yield. The pathogen may be seedborne, potentially resulting in phytosanitary restrictions. Benomyl seed treatment helped prevent seed transmission of the fungus, and infection of seedlings from soilborne inoculum. However, the loss of benomyl registrations in the US has created a need for alternative seed treatments, and enhanced the importance of identifying fields with low populations of *F. oxysporum* f. sp. *spinaciae* for planting spinach seed crops.

Efforts to manage Fusarium wilt of spinach in western Washington include planting resistant cultivars, where possible, and using extensive crop rotations. Cultivars with partial resistance are available, but many carry little, if any, resistance. In addition, seed crops are grown on a contract basis, so growers have little choice of the cultivars they grow. Once introduced into soil, *F. oxysporum* f. sp. *spinaciae* can survive many years as a saprophyte on organic matter, and as a pathogen on spinach and related non-host crops (e.g., beet and chard). When spinach Fusarium wilt was first identified in western Washington in the 1960s, the disease was managed by growing seed crops in fields not previously planted to spinach. Subsequent depletion of virgin ground for spinach led to Fusarium wilt becoming the main factor limiting production of high yielding spinach seed crops in this region. Losses to Fusarium wilt now necessitate rotation intervals of 6-10 years for spinach lines with partial resistance, and 12-15 years for susceptible lines. Confounding this is the need to isolate seed crops to avoid unwanted cross pollination. Therefore, selection of fields for spinach seed crops is a complex and limiting factor for seed growers. Development of a molecular assay for *F. oxysporum* f. sp. *spinaciae* would enable growers to assay soils for populations of the pathogen, in order to identify more accurately appropriate fields for planting seed crops of cultivars with known levels of resistance to Fusarium wilt. The molecular assay could also be used to assess the incidence of the pathogen in spinach seed lots.

In contrast to western Washington, 12,000-15,000 acres of spinach seed crops are grown in Denmark annually with no Fusarium wilt, despite rotation intervals of only 3-5 years. Danish soils are typically alkaline (pH 7.5-8.5) and high in calcium because of extensive limestone deposits. This may account, in part, for the lack of Fusarium wilt. Research in Florida in the 1970s demonstrated that the increase in soil calcium and pH resulting from limestone (calcium carbonate) applications suppressed Fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici*. In those studies, limestone elevated soil and plant calcium levels, enhancing crop resistance to the pathogen. Liming also raised soil pH, which reduced aggressiveness of the fungus by making some micronutrients (particularly Zn) unavailable. Manipulation of soil calcium and pH by liming remains to be investigated for management of Fusarium wilt in spinach seed crops in the acid soils of western Washington. Although dolomitic lime (calcium carbonate + magnesium carbonate) is commonly used in this region to raise soil pH above 6.0, effects of higher rates of liming on Fusarium wilt and the nutrient status of spinach seed crops needs to be investigated. The 2006 trial showed very promising results with a significant increase in suppression of spinach Fusarium wilt in plots treated with agricultural lime at rates ranging from 0 to 4.2 tons/acre.

Ranking and Prioritization

General Criterion (I, II, other):

I. Results are expected to lead to development of a more effective regional IPM program for spinach seed crops in western Washington.

Category A: Protection of Human Health (I, II, III, IV, other):

Category B: Protection of the Environment (I, II, III, IV):

Category C: Significance to the Local or Regional Economy (I, II, III, IV, other):

I. Existence of an emergency situation with no effective alternative, i.e., depletion of virgin ground for spinach in western Washington, which has been integral for effective management of Fusarium wilt in seed crops of susceptible cultivars.
II. Develop an integrated pest management tactic, i.e., a) liming to reduce rotation intervals for spinach seed crops; and b) develop a molecular seed and soil assay to help select appropriate fields for planting seed crops of spinach cultivars with different levels of resistance to Fusarium wilt, and to identify infected seed lots.

Project Description

1. Personnel:

Lindsey J. du Toit, WSU Vegetable Seed Pathologist. dutoit@wsu.edu. WSU-NWREC, 16650 State Route 536, Mount Vernon, WA 98273-4768. Tel: (360) 848-6140. Fax: (360) 848-6159.

2. Project Objectives:

I. Assess the potential efficacy of limestone applications to enable spinach seed growers in western Washington to reduce crop rotation intervals from 6-15 years, which is currently necessary to avoid losses to Fusarium wilt.
II. Develop a molecular assay for *F. oxysporum* f. sp. *spinaciae* that can be used to: a) determine populations of the pathogen in soils in order to identify appropriate fields for planting seed crops of cultivars with known levels of resistance to *F. oxysporum* f. sp. *spinaciae*; and b) assay seed lots for the pathogen to assess the need for seed treatment (e.g., hot water or chlorine).

3. Project Description and Procedures:

I. A field trial consisting of a randomized complete block design with 5 replications of a 5 x 2 factorial treatment design will be established in Skagit Co. in spring 2007, in a field with acid soil that had a spinach seed crop 4-5 years prior. Treatments include 5 rates of agricultural limestone incorporated ~5 weeks prior to planting (0, 2, 4, 6, and 8 tons/acre = subplots), and 2 spinach cultivars (partially resistant and highly susceptible to Fusarium wilt = main plots). Soil will be sampled before liming to determine pH and nutrient status. Soil will be also be sampled from each plot at planting, and again ~1 and 3 months after planting, for pH and nutrient assays by a commercial lab. Plots will be monitored for spinach stand counts, incidence of Fusarium wilt and other soilborne diseases, crop growth (biomass), and nutrient deficiency symptoms. Plant samples will be collected mid-summer and assayed for nutrient status. Seed will be harvested, threshed, cleaned, and sized; and germination and health assays completed. Relationships between liming rate, soil pH, Fusarium wilt, and crop nutrient status will be assessed statistically.

II. PhD student, Leigh Ann Harrison, will start this project in January 2007 while in Pullman for classes. A real-time polymerase chain reaction (PCR) assay for *F. oxysporum* f. sp. *spinaciae* will be developed with assistance from Drs. Pat Okubara and Kurt Schroeder (USDA). The assay will differentiate this fungus from other *formae speciales*, including Fusarium wilt pathogens of onion, pea, radish, tulip, daffodils, gladiolus and lilies. The assay will be evaluated for quantifying the spinach pathogen in soil and spinach seed. Soil will be sampled from a number of fields in western Washington that have no history of spinach seed production (sandy to silty loam types). Each soil will be inoculated with the pathogen at 0, 10^2, 10^4, 10^6, and 10^8 cfu/g soil (verified by dilution plating). Seed of three cultivars (highly susceptible, moderately susceptible, and partially resistant to Fusarium wilt) will be planted in each soil in flats in a greenhouse (4 replications). Seed will be treated with mefenoxam to control *Pythium* spp. Plants will be monitored for ~12 weeks for stand counts and incidence of wilt. Isolations will be carried out to verify the causal agent. Incidence of wilt vs. populations of *F. oxysporum* f. sp. *spinaciae* will be assessed statistically to determine sensitivity of the assay. In addition, soil will be sampled from 5-10 fields previously cropped to spinach, and assayed for the Fusarium wilt pathogen using the real-time PCR assay. Soil dilution assays will be carried out to quantify *F. oxysporum* in each soil and correlate fungal population with incidence of Fusarium wilt. If the assay is adequately sensitive across a range of soil types, expenses will be estimated for offering the assay as a diagnostic service to growers. The molecular assay will also be evaluated and refined for a seed assay.

5. Timeframe:

Mar 2007	Set up field trial after location of spinach seed crops is determined by seed crop pinning on 03/01/07. Soil samples collected & assayed for pH.
Late-Mar	Lime applied & incorporated at appropriate rates.
Late Apr	Hybrid spinach seed crop planted (male + moderate & susceptible females).
Jun-Aug	Plants monitored for wilt, biomass, & nutrient deficiency symptoms; soil (pre- & post-plant) & plant samples collected & assayed.
Aug-Sep	Seed harvested, dried, threshed, cleaned, sized, & weighed.
Sep-Nov	Seed germination & health assays completed.
Dec	Results submitted to *Plant Disease Management Reports.*
Jan 2008	Results presented at PSSGA/Western WA Hort. Assoc. meeting in SeaTac.

Project Budget

Expenditure	WSCPR (Request)	Matching (CASH or IN-KIND)			TOTAL COST
		Source: PSSGA	Source: Grower & seed co.	Source: Grower, seed & chemical co.'s	
		Amount (CASH)	Amount (IN-KIND)	Amount (IN-KIND TIME)	
Salaries[1]	$ 6,556	$ 4,370			$10,926
Employee Benefits[1]	$ 2,399	$ 1,634			$ 4,033
Temporary or hourly workers[1]	$ 800	$ 800		$ 500	$ 2,100
Travel[2]	$ 150	$ 150			$ 300
Equipment					
Other: seed, pesticides, crop maintenance[3]	$ 500		$ 500	$ 500	$ 1,500
Other: field & lab supplies, soil & plant assays[4]	$ 4,500	$ 3,000			$ 7,500
Total	$14,905	$ 9,954	$ 500	$ 1,000	$26,359

1 0.25 FTE of Ag. Research Tech. III (ART III) + 160 hours of time-slip for lime treatments, soil & plant sampling, disease assessments, harvest, & germ & health assays. Salary ($10,926) + wages ($1,600) + benefits at 35% for ART III ($3,825) + 13% for time-slip ($208). Time donated by seed co. reps & grower for planting & crop maintenance ($500).

2 Travel between WSU-NWREC & field site(s) for field trial & collecting soil samples for the molecular assay; presentation at 2008 WWHA/PSSGA meeting in SeaTac, WA.

3 Agricultural lime for field & greenhouse trials. Spinach stock seed donated for hybrid seed crop (~1 acre); seed co. field rep time, field crew labor, & crop maintenance donated by seed co. & grower.

4 Expenses for soil & plant assays for nutrient status & pH by a commercial lab (60 plots/sampling time); real-time PCR assay supplies (development & evaluation of primers, soil & seed assays); supplies for field (flags, stakes, sample bags, etc.), lab (Petri plates, agar media, molecular reagents, etc.), & greenhouse trials (potting media, flats, fertilizer, etc.), & seed germ/health assays. (The PhD student is supported, in part, by the Robert MacDonald Vegetable Seed Memorial Fund at $8,000/year).

Has this budget been reviewed for accuracy? Yes **By Whom?** M.L. Bricker

Projected Expenditures (by quarter)

Time Period	Jan-Mar 2007	Apr-Jun 2007	Jul-Sep 2007	Oct-Dec 2007	Jan-Mar 2008	Apr-Jun 2008
WSCPR Funds	$2,700	$5,000	$4,000	$3,205		
Total Funds	$5,000	$8,079	$8,080	$5,000	$200	

Has this project been funded previously by WSCPR? Yes
If so, for how long and with what progress? Since 2006. Seed health assays and germination assays are in progress (to be completed by November 2006).