Abstract. We proposed to test a UC Davis-patented technology that uses selected radiofrequencies (RF) to determine if it can disinfect spinach without any deleterious effects on seed germination. In theory, the process could have resulted in improved fungal pathogen control in spinach, most critically for *Verticillium dahliae,* but also for other seed-borne pathogens on spinach: *Stemphylium botryosum* f. sp. s*pinacia; Cladosporium variabile;* and *Peronospora farinosa* f. sp. *spinaciae*. Although RF results in heating, the RF technology differs from hot water or hot humid air treatments in that the seeds are heated from within, and consequently, in theory, treatment times could potentially be shorter, more uniform, and importantly, not require drying after treatment. RF technology also differs from heat treatments in that RF may generate short-term electric effects that can also be fungitoxic. In addition, this particular RF technology utilizes specific wavelengths of RF that were selected to inactivate a variety of pathogens without affecting plant material. Unfortunately, we were unable to find RF conditions that disinfected without adversely affecting spinach seed germination, and concluded that the standard hot water treatment provided more predictable results than the RF conditions that we tried.

Project title- Testing of RF Biocidics radiofrequency disinfection of Verticillium-infected spinach and lettuce seed

Project Investigator(s):

Lynn Epstein, Professor of Plant Pathology, UC Davis

Manuel Lagunas-Solar, Professor Emeritus, UC Davis, and RF Biocidics, Vacaville, CA

Summary: RF Biocidics is a fast-growing company in Vacaville CA that uses radiofrequency (RF) as a disinfection (pasteurization) and disinfestation of food (primarily dried fruits and nuts) and other products. In theory, the process could be used for disinfection of spinach seeds that are used for leaf production. In practice, we were unable to develop a suitable protocol that disinfected but retained seed germinability.

CALIFORNIA LEAFY GREENS RESEARCH REPORT

Project title- Testing of RF Biocidics radiofrequency disinfection of Verticillium-infected spinach and lettuce seed

Principal investigator(s): Lynn Epstein, Professor of Plant Pathology, UC Davis

Cooperating personnel: Manuel Lagunas-Solar, RF Biocidics and Professor Emeritus, UC Davis; Steven Koike, UCCE Monterey County Farm Advisor; Sukhwinder Kaur, SRA, Dept. of Plant Pathology, UC Davis

Objective: To determine radiofrequency (RF) conditions with maximum efficacy for disinfection of spinach and lettuce seed without any reduction in seed germination

Procedures

Five lots of spinach seed that were thought to be infected were obtained from either a seed company, Farm advisor Steven Koike or UC Davis Professor Krishna Subbarao. Uninfected seed were kindly donated by TSL.

Seeds were surface sterilized before all assays (Hernandez & duToit 2006). For each replication, 2 g of seeds were placed in a mesh tea strainer, and surface-sterilized in 1.2% sodium hypchlorite (i.e., 0.2X of commercial bleach) for 1 minute and then rinsed in sterilized, deionized water three times. Seeds were air dried on sterilized paper towel in a laminar flow hood before assaying. To assess germination, seeds were transferred aseptically onto moist sterile, germination papers (#38, regular weight, 3 3/8" circles, Anchor paper company, St Paul, MN) in 100mm-diameter sterilized, Petri dishes. Dishes were incubated under lights and percentage germination was recorded after 4 days.

To assess *Verticillium dahliae* infection, seeds were transferred aseptically onto polygalacturonate agar (Morgan et al. 1992, Epstein et al. 2004) and incubated in the dark for five to seven days. These plates were examined under the dissecting microscope for typical *V. dahliae* conidiophore and microsclerotia production.

To assess infection by other fungi, seeds were transferred aseptically onto potato dextrose agar, incubated under lights for five to seven days, and then examined under the dissecting scope for any other fungal growth.

For most radiofrequency (RF) trials, 10 g aliquots of spinach seeds were put in a nylon mesh bag, buried in larger container of uncontaminated seed, treated with the RF Biocidics instrument at various watts for various time periods, and then assayed. Untreated seed were used as controls.

Experiments with lettuce were indicated in the proposal as time and resources permitted; all resources were used on the spinach.

Results and Discussion

The uninfected control seed lots (data not shown) had 0% *V. dahliae* and 90 and 98% germination. For the infected seed, the percentage spinach seed germination, the percentage infected with *Verticillium dahliae* and the percentage infected with fungi other than *V. dahliae* are shown in Table 1. There was no significant correlation between germination, incidence of *V. dahliae* or incidence of other fungi.

Table 1. Five spinach seed lots used in the radiofrequency (RF) studies.

Lot	Seed germination	Infected with *Verticillium dahliae*	Infected with fungi other than *V. dahliae*
		% ± SE	
cv. Novico	96 ± 2	32 ± 5	99 ± 1
cv. Kauai	17 ± 2	30 ± 4	41 ± 8
41SK-A	95 ± 2	22 ± 3	42 ± 4
SK-B	72 ± 4	47 ± 2	36 ± 6
SK-C	78 ± 6	19 ± 3	28 ± 2

Table 2. Typical results of a Biocidics RF trial with spinach seed.

Final temp, °F	U(nwetted), or M(isted) before RF	Total RF time, min.	Time, 1st RF, min	Watts, 1st RF,	Watts, 2nd RF	Time, 2nd RF min.	Seed germin-ation, %	*V. dahliae*, %
106	U	2	2	500	NA	NA	73	43
118	U	2.5	2.5	500	NA	NA	10	53
131	M	1.5	1.5	500	NA	NA	0	0
136	U	6.33	2.33	500	100	4	0	0
140	U	7.5	2.5	500	180	5	7	10
140	U	7	3	500	125	4	0	3
153	M	2	2	500	NA	NA	3	0
163	U	5	5	500	NA	NA	0	0
163	M	2.5	2.5	500	NA	NA	0	0
194	U	6	6	500	NA	NA	3	0
Control	NA	NA	NA	NA	NA	NA	72	63

[a]For those marked with W, spinach seeds were lightly misted with isopropanol immediately before RF treatment.
[a]NA, not applicable

Overall, the RF Biocidics instrumentation was suitable for relatively large conditions in which we had no kill of *V. dahliae* (117.5 ºF for 2.5 minutes) in spinach and a variety of conditions in which we had undetectable survival of *V. dahliae* but reduced germination (e.g., 136.4 ºF for 6.3 minutes).

Heat, generally applied as hot water, is a classical method of seed disinfection (Taylor and Harman 1990), and can have some value (du Toit and Hernandez-Perez 2005b). However, there are often issues with decreased seed germination, the potential to disseminate pathogens in the water, and the need to dry seed post-treatment. Recently, ThermoSeed, a patented humid hot-air treatment from Europe has been reported to produce excellent results for disinfection of V*erticillium*, *Stemphylium* and *Cladosporium* in spinach seed (Sanchez-Sava and Forsberg, 2009). Here we tested another disinfection process that utilizes radiofrequency (RF), which partly operates by heat.

RF uses oscillating electric fields to generate a combination of heat and short-term electric (not radioactive) effects that can be very efficient in killing pathogens and pests without damage to plant material (Lagunas-Solar et al. 2006, Marra et al. 2009, Wang et al. 2010). RF

Biocidics (http://www.alliedminds.com/Portfolio/RF_Biocidics/Technology.htm) is a joint private and UC Davis venture to commercialize RF technology that was patented at UC Davis. The technology utilizes specific wavelengths of RF that presumably selectively inactivate a variety of pathogens without affecting the plant material. Much shorter exposure times are typically required than with hot air or hot water because the RF-treated tissue is heated from inside the seed. However, because RF heating depends upon the chemical composition of the product, particularly the water content, we were concerned that different batches of seeds would require slightly different conditions. Because the RF instrumentation allows control over wattage, but not directly over temperature, we also were concerned that one batch could be over-heated and another batch under-heated. Finally, the protocol would have to include a step of rapid cooling to remove the heat from within the seeds. In collaboration with RF Biocidics personnel, we were unable to develop a feasible protocol for seed treatment that would disinfect without adversely affecting seed germination. To conclude, while RF may be an excellent strategy for post-harvest treatments for nuts and dried fruit for consumption for example, the current apparatus does not appear to be useful for disinfection of seeds in which the embryo must remain viable for germination, and there is a narrow gap between the requisite temperature and time for pathogen disinfection and seed lethality.

While we thought that the RF strategy was possible, we investigated various thermal denaturation models so that we would be able to better predict target conditions for the RF. We primarily utilized the probabilistic Weibull model, which is used in the food industry, but was used to predict survival of *Verticillium dahliae* microsclerotia during soil solarization (Marshall 2007). If ThermoSeed or other heat processes are used by the industry for seed disinfection, we believe that the Weibull model could be very useful in protocol optimization.

Literature cited

du Toit, L. J., Hernandez-Perez, P. 2005b. Efficacy of hot water and chlorine for eradication of *Cladosporium variabile*, *Stemphylium botryosum*, and *Verticillium dahliae* from spinach seed. Plant Disease 89*:*1305-1312.

Epstein, L., Beede, R., Kaur, S., and Ferguson, L. 2004. Rootstock effects on pistachio trees grown in *Verticillium dahliae*-infested soil. Phytopathology 94:388-395.

Hernandez-Perez, P., and du Toit, L. J. 2006. Seed borne *Cladosprorium variable* and *Stemphylium botryosum* in spinach. Plant Dis. 90:137-145.

Lagunas-Solar M.C., N.X. Zeng, T.K. Essert, T.D. Truong, and C. Piña U. 2006. Applications of Radiofrequency Power in Foods, Agriculture, and Environment. California Agriculture 60:192-199.

Marra F, Zhang L, Lyng JG. 2009. Radio frequency treatment of foods: Review of recent advances. Journal of Food Engineering 91: 497-508.

Marshall, MN. 2007. Development and validation of predictive models of soil heating and pathogen inactivation during soil solarization. University of California at Davis Dissertation.

Morgan, D.P., L. Epstein, and L. Ferguson. 1992. Verticillium wilt resistance in pistachio rootstock cultivars: assays and an assessment of two interspecific hybrids. Plant Disease 76:310-313.

Sanchez-Sava V., Forsberg G. 2009. ThermoSeed – a novel seed disinfection technology. Proc. T.E. Morelock International Spinach Conf. Fayetteville, AR (Abstr.)

Taylor A.G., Harman, G.E. 1990. Concepts and technologies of selected seed treatments. Annual Review of Phytopathology 28: 321-339.

Wang S, Tiwari G, Jiao S, Johnson JA, Tang J. 2010. Developing postharvest disinfestation treatments for legumes using radio frequency energy. Biosystems Engineering, 105:3 4 1 – 3 49.