METHYL BROMIDE ALTERNATIVES FOR USE IN WALNUT PRODUCTION SYSTEMS

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The goal of this project is to test, optimize and foster stable adoption of integrated pest management approaches for managing soil-borne diseases of Walnut without methyl bromide (MB). Specific objectives include; 1.) Improved Pre Plant Fumigation Strategies for Walnut Orchards and their economic assessment;   2.) Demonstrate the utility of predictive/diagnostic microbial detection technologies in evaluating MB alternatives.

Improved Pre Plant Fumigation Strategies for Walnut Orchards: The field site being prepared for these studies is located in northern Kings County. The site is a 40 acre walnut orchard consisting of 50 year-old Hartley and Franquette cultivars which were removed during the winter of 2006-2007. Removal was performed by blading out the base of the trees to reduce residual large roots. The upper part of the tree and the stumps were chipped. The grower then repeatedly ripped the field to a depth of about 4 feet. The exposed roots were then hand collected and chipped. Following root removal, the field was laser leveled, bordered every 40 feet, and irrigated in preparation for planting corn.

Nematode status: Four composite samples, each consisting of 10 sub samples taken at a depth of 24", were found to contain 136, 363, 233, 281 *P. vulnus* per 250 cc of soil using sieve/mist chamber extraction.

Soil Type: The subject property is a deep, well drained Nord fine sandy loam formed from alluvial parent material. Permeability is moderate. Water holding capacity is high. Effective rooting depth is greater than 60".

Treatments: Using six replications per treatment and plot sizes approximately 75' x 160', the following five treatments will be examined.
1. Untreated Control
2. Methyl bromide broadcast at 400 lb/ac and tarped.
3. Telone C35 at 49 gal/ac broadcast or Telone II at 33.7 gal/ac broadcast shanked at 20".
4. Telone II at 33.7 gal/ac. broadcast and shanked at a depth of 20", then, depending on moisture content, followed by 250 lb. Chloropicrin broadcast shanked at 28".
5. Methyl iodide at 235 lb/ac broadcast and shanked at a depth of 20", followed by Telone or Chloropicrin broadcast and shanked at a depth of 28".

The actual fumigant rates used in these trials will vary as a function of soil moisture content.   If soil moisture levels are greater than 12% the application rates of some treatments will be adjusted accordingly. Soil moisture measurements will be collected at each foot to the five-foot depth from across each field with attention to soil textural differences. This effort will assist the decision of how much to rip the soil prior to treatment. Samples of soil from each appropriate site will characterize soil moisture content on a dry weight basis using the microwave oven method.

Treatment evaluation: Trunk circumferences will be measured during the fall of each year from all trees in each treatment for 5 years post planting. Starting 3 yrs post planting marketable crop yields will be determined for all treatments. Nematode populations will be examined approximately 60 d after the fumigation at one-foot increments from each rep

down to five foot depth. Subsequently, samples from 0 to 2-feet will be composited from 5 trees from each replication during June and November on each of the next 5 years.   We also will track *A. tumefaciens* populations at 10 and 25cm 90days after the beginning of the re-entry period and then quarterly for the next 2 yrs.

Diagnostic microbial detection technologies in evaluating MB alternatives. The success of any given fumigation protocol is typically evaluated by the post fumigation plant growth response and/or the incidence of a target plant disease or pest such as phytopathogenic nematodes. Perhaps a more direct and efficient evaluation of fumigation success would involve the direct monitoring of the etiological agents of the target diseases.

By exploiting the power of DNA array technology it is currently possible to detect a large number of specific microbial pathogens in a culture independent and highly parallel fashion (Sessitsch et al. 2006). Using these same technologies we will be able to characterize the soil-borne microbial community as a function of any soil treatment applied.

By directly monitoring the presence of the pathogenic agent, the grower would avoid the time and expense of planting a field where the fumigation procedure showed limited effectiveness.   Finally, this approach will facilitate a rapid and direct comparison of the effectiveness of the many MB alternatives being examined in the area-wide project to control specific phytopathogenic agents.

In addition, by exploiting an effective detection protocol, growers will be able to avoid the time and expense of fumigating a field or greenhouse where the targeted pathogen(s) do not exist. This ability to provide predictive capabilities of the microorganisms present would dramatically reduce the application of all fumigants.   Any of these scenarios will only be possible with the development and use of a rapid, reliable, sensitive and cost effective diagnostic tool that reveals the presence of target phytopathogenic agents.

One of the many agricultural applications of this technology can be found in the report by Lievens et al. (2003) who developed species specific primers for the ITS region which facilitated the culture independent detection of the following tomato vascular wilt pathogens; *Fusarium oxysporum f. sp. lycopersici*, and *Verticillium albo-atrum and V. dahliae.* Direct DNA extraction techniques coupled with PCR amplification and hybridization to DNA arrays containing ITS probes facilitated detection of these important pathogens in symptomless plants, water and raw soil samples within 24 hrs. Similarly, others have developed DNA arrays that facilitated the detection of both human and plant pathogenic nematodes, oomycetes, and bacteria (Levesque, et al. 1998, Uehara, et al. 1999, Fessehaie et al. 2003, Rudi et al. 2002).

Here we propose to develop and use DNA arrays to detect soil-borne target pathogens as a function of soil fumigation treatments. We will start with the development of arrays to detect *Phytophthora* spp, *Fusarium* spp, and *Verticillium dahliae/alboatrum* using the micro heterogeneity in the ribosomal internal transcribed spacer regions, *Agrobacterium* using T-DNA specific regions, and root knot nematode (*Meloidogyne* spp) specific sequences.

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