THE EFFECTS OF ACROLEIN ON THE MICROBIOLOGY AND KEY BIOCHEMICAL ACTIVITIES OF THE SOIL

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In the continuing efforts to develop alternatives to methyl-bromide, -enal compounds with pesticidal effects similar to that of methyl-bromide, were tested at Auburn University for efficacy. Several compounds from this group have potential as pesticides and acrolein was chosen for further investigation and development. Acrolein is currently labeled as an aquatic herbicide for use in irrigation canals, and recently, reports of nematicidal activity and agricultural use to control weeds have been published. All reports to date have focused on the efficacy of the compound on agricultural pests and no work has been done to elucidate the effects of acrolein on the soil microflora. When considering chemical alternatives to methyl bromide it is essential that the effects of the alternative compound on the soil microflora be considered. Dangerous soil microbiological voids and selection for plant pathogenic micro-organisms is a common problem often associated with biocidal compounds like methyl bromide and other potential alternatives. In efforts to avoid or at least be aware of potential pitfalls with acrolein, greenhouse and laboratory trials were conducted in 2006 and 2007 at Auburn University to study the effects of soil-applied acrolein on the microflora and several key biochemical processes of the soil. Soil microbial survey included soil plating using media selective for fungi, bacteria, and actinomycetes for general identification and enumeration. Soil enzymatic activities measured included: catalase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, chitobiase, phosphotase, protease, sulfatase, and urease.

Materials and Methods:

Soil Biochemistry- A greenhouse experiment was conducted using a sandy loam soil (pH 6.5; organic matter <1%; C.E.C.< 10meq./100 g soil) from a central Alabama cotton field infested with various plant pathogens. The soil was mixed 50:50 with sand, divided in 1 K aliquots, placed in into 10 cm diameter PVC pots, and placed on a greenhouse bench. Acrolein treatments at rates: 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, and 200 mg were drench-applied in a final volume of 100 ml water (controls were treated with 100 ml water only). Immediately following treatment, pots were covered with a 2 mil thick HDPE plastic bag sealed with a rubber band, and were arranged in a randomized complete block design (7 replications per treatment). Pots were uncovered 10 days after treatment, 100 cm^3 soil samples were taken for nematode analysis (salad bowl technique), and 5 cucumber seeds (*Cucumis sativus* 'Marketmore 76') were planted per pot. Cucumbers were allowed to grow for 52 days at which point they were taken out of the pots for plant growth assessments and final nematode counts. Plant growth parameters recorded were: number of plants, height of shoots (cm), weight of fresh shoots (g), weight of fresh roots (g), root condition rating (1-5, 1= best & 5= worst), gall rating, and galls per gram of root. At both nematode samplings, 50 cm^3 of soil was taken, air-dried, and stored frozen for soil enzymatic analyses. Enzymatic analyses used were modifications of procedures found in

common soil microbiology text books (Alef & Nannipieri, Methods in Applied Soil Microbiology and Biochemistry 1995).

Soil Microbiology- Acrolein treatments and experimental setup was the same as for the biochemistry experiment except the soil was not mixed with sand. Pots were uncovered 13 days after treatment and 50 cm^3 soil samples were taken from the 0, 20, 40, 60, 100, 140, 160, and 200 mg/ K treated pots for general microbial analyses (fungi, bacteria, & actinomycetes). Pots were replaced in the greenhouse and morning glory was planted and counted weekly to serve as a bioassay for seedling diseases. For general microbial enumeration, soil dilutions were made and plated using media selective for fungi (Ohio agar), bacteria (Thornton's standardized agar), and actinomycetes (Benedict agar).
-Data were analyzed using ANOVA and Fisher's LSD in SAS statistical software; all results presented are significant to the P=0.05 level.

Results:

Soil Biochemistry- As acrolein rates increased, most of the enzymatic activities measured decreased. This is indicative of a biocidal. Soil from pots treated with acrolein at ≥ 100 mg/ K soil showed an increase in soil protease activity in the initial soil sampling that was not noted in the final soil sampling. With the exception of sulfatase, enzymatic activities of the final soil samples remained less than those in the controls and the activities tended to decrease as rates of acrolein increased.
Root-knot nematodes extracted from the soil were reduced in all pots treated with acrolein and none were extracted from treatments ≥ 20 mg acrolein pre-planting and > 40 mg acrolein at the final sampling. Microbivorous nematode populations in the soil remained present throughout this trial with some treatments having greater populations than the controls. All plant assessments showed improvements as acrolein rates increased; root galling decreased as acrolein rates increased.

Soil Microbiology- Bacterial colonies decreased as rates of acrolein increased to 200 mg/ K soil. In contrast, the number of actinomycetes and fungi increased as rates of acrolein increased. This suggests that acrolein is not a biocide; rather that acrolein is a selective pesticide when applied to the soil. Furthermore, while the number of fungal colonies increased, diversity decreased to nearly one fungal genus: *Trichoderma*. This genus is known for species with antagonistic effects on plant pathogens. The *Trichoderma* data from the soil plating follow a very similar trend as the data taken on visual rating of *Trichoderma* presence on the surface of the soil following acrolein treatments.
As the rate of acrolein applied to the soil increased, morning glory germination and survival increased indicating that acrolein may reduce seedling diseases.

Discussion:

Unlike typical biocides, acrolein exhibited properties of a selective pesticide when applied to the soil. Acrolein stimulated *Trichoderma* spp., a desirable group of micro-organisms. These activities coincided with improved plant growth and nematode control. Results suggest that acrolein may be used to control pests and improve plant growth without creating dangerous microbiological voids in the soil.

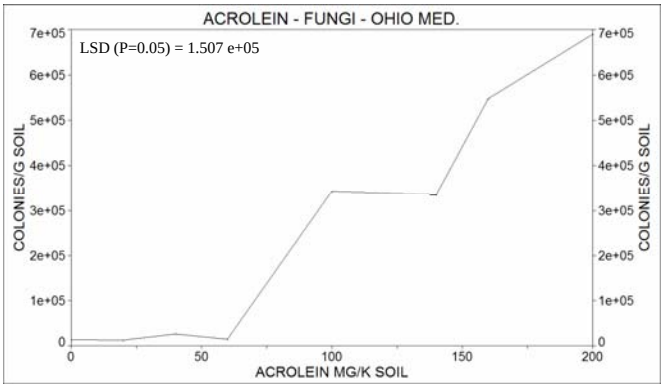

Figure 1: Colony counts on Ohio agar. As acrolein rates increased, the number of fungi increased. This increase was primarily due to *Trichoderma* spp.

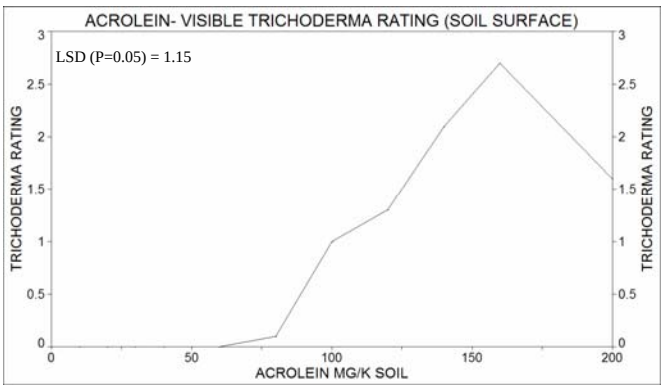

Figure 2: Ratings of *Trichoderma* on the surface of treated soil.

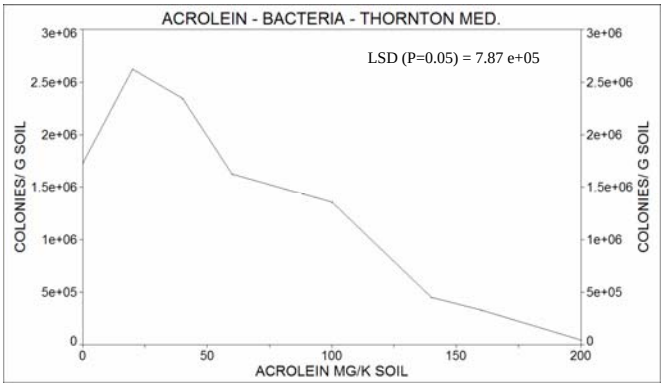

Figure 3: Colony counts on Thornton's agar. As rates of acrolein increased, the number of bacteria decreased.

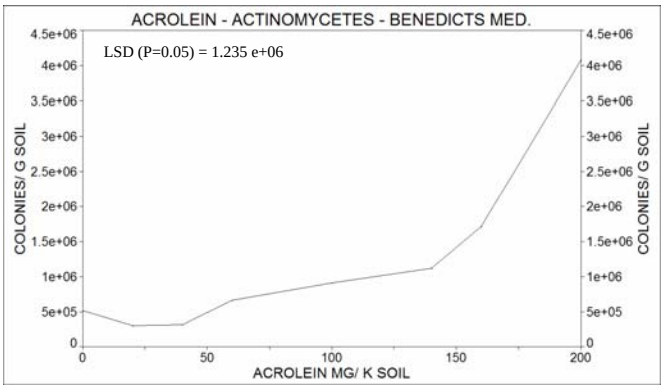

Figure 4: Colony counts on Benedict agar. As rates of acrolein increased, so did the number of actinomycetes.