THE USE OF *VERTICILLIUM DAHLIAE* AND *STREPTOMYCES* SPP. AS INDICATORS OF SOIL DISINFESTATION.

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In Ontario, early dying of potato and tomato, caused by pathogenic nematodes and the fungus *Verticillium dahliae,* has become a limiting factor in production. Potato scab, incited by *Streptomyces* bacteria, is more sporadic in incidence, but it can also render the crop unmarketable as the deep pitted lesions preclude tuber storage. Control measures often rely on soil fumigation. The use of such highly toxic biocides however, is becoming less acceptable to society and fumigants continue to be targeted for removal from the marketplace. Possible alternatives to chemical control advocated include the use organic of amendments and biological control agents. Evaluating the efficacy of organic pest control howeer, has proven to be more difficult than that encountered with chemicals. Screening of pesticides generally involved mixing a target organism with concentrations of chemical in the presence of a growth medium. Efficacy, as based on inhibition of either cell density increase or spore germination, or mycelial growth, etc. could be determined in hours, or days. Screening procedures for biocontrol agents however, often require weeks or months. During this time natural mortality of the test agent can become a problem. If the assays are carried out in soil separating the target agent from the multitude of soil inhabitants also becomes a difficult task. The objective of this study was to develop simple, reliable, quantitative assays for measurement of inoculum reduction of soilborne pathogens in field soils following application of biological control agents or soil amendments.

Two pathogens were used as model agents, *V. dahliae* and *Streptomyces* bacteria. The two parasites were chosen because they survive in soil for very long periods and are frequent targets of fumigation. *V. dahliae* resting structures, microsclerotia, (MS) were produced in the laboratory on a semi-solid agar medium as described by Hawke and Lazarovits (1). The MS were sorted to size and those between 75-105 μm placed in nylon mesh bags (73 μm mesh) mixed with acid washed silica sand to prevent clumping. For laboratory tests, the bags were placed directly into the soils in plastic boxes or were suspended above the soil in the headspace on a plastic grid. For field studies, the bags were glued (Crazy glue) to plastic marker stakes and then lowered into the soil to a specific depth. The bags were recovered at periodic intervals and 25 MS were individually transferred with a needle to grid square on an agar plate containing a semi selective medium. At least 50 MS were tested for germination and colony growth from each treatment. Lethal effect was reflected by the number of MS failing to germinate. Sublethal effects were evaluated from reductions in colony diameters.

MS preparations could be stored in the laboratory with little loss of viability for at least one year. Most high nitrogen containing amendments tested completely reduced MS viability within 10-21 days after incorporation at 24C and 50% water holding capacity (Fig 1). The MS were also killed in the headspace but the effect was delayed by a few days.

This does indicate that at least some of the toxic agents were volatile. Not all amendments were equally effective in all soils. For instance, blood meal which killed all MS in the Alliston soil, was ineffective in the Simcoe soil (Fig 1). Similarly, meat and bone meal worked equally well at the three concentrations tested in Simcoe soils, but in Alliston soil there was a clear relationship between concentration and efficacy (Fig 2). When soils amended with poultry feather meal were incubated at three different temperatures the reduction in MS viability was similar at 10, 17 and 25 C in Simcoe soil. In amended Alliston soil however, MS died faster at the higher temperatures than at the cooler ones (Fig 3). Amendments that caused reductions in MS viability in the laboratory assays were also effective under field conditions. This was confirmed by simulataneous tests of MS reductions of specific treatments under both laboratory and field conditions. Laboratory experiments in general, accurately predicted the potential efficacy of an amendment to control a disease and most often the results tended to underestimated field efficacy.

We also developed a rapid and reproducible procedure for determining total populations of Streptomyces spp. in soil. Quantitation involves the following steps: air drying and sieving of soils, shaking of 10 g quantities in plastic bags containing water with 0.1% agar, homogenization with a Stomacher homogenizer, plating of serial dilutions on a semi-selective media containing 35 g of NaCl liter^{-1}, incubation for at least 5 days at 25 C, and enumeration of sporulating *Streptomyces* colonies. Pathogenic isolates were distinguished from saprophytes by transferring bacteria from isolated colonies on dilution plates into liquid medium (in 96 well microtitre plates) that induces thaxtomin production. Thaxtomin, a toxin recognized as a virulence marker in *Streptomyces*, was identified by its yellow color. In soils collected from Ontario potato fields with a known history of scab the population of *Streptomyces* generally varied from about 10^4 - 10^5 colony forming units (CFU) per gram of soil. Streptomyces were present in greatest numbers in the 5-20 cm depth, but at depths lower than 25 cm, the population declined to 10^2 CFU/g soil and at 40 cm, few or no bacteria were detected. In soils with known levels of high disease incidence, approximately 10-25% of the total population produced toxin and were considered pathogens. Such strains were not detected in soils with no known history of scab. Drying of soil for extended periods did not alter recoverable populations. Amendments that resulted in reductions of Streptomyces populations under laboratory conditions had an identical effect in the field. The amendments that controlled Verticillium also controlled Streptomyces but some that controlled Streptomyces, did not effect Verticillium MS. Treatments that reduced MS viability and Streptomyces populations also reduced disease incidence in tests carried out in the growth chambers, in the green house and in the field.

The assay procedures described here provide a rapid and simple method for testing a large number of biological products and agents in a specific soil for their effects on reducing the populations of two soilborne pathogens that are normally very difficult to eradicate. The results generated can predict whether the changes occurring in the populations will lead to a reduction in disease incidence under field conditions.

1. Hawke, M. A. and G. Lazarovits. 1994 Phytopathology, 84:883-890

Fig 1. Effect of blood meal (BM), feather meal (FeM) or soymeal (SM) amendment on soil pH and the germination of *V. dahliae* microsclerotia (MS) in two types of soils under laboratory conditions.

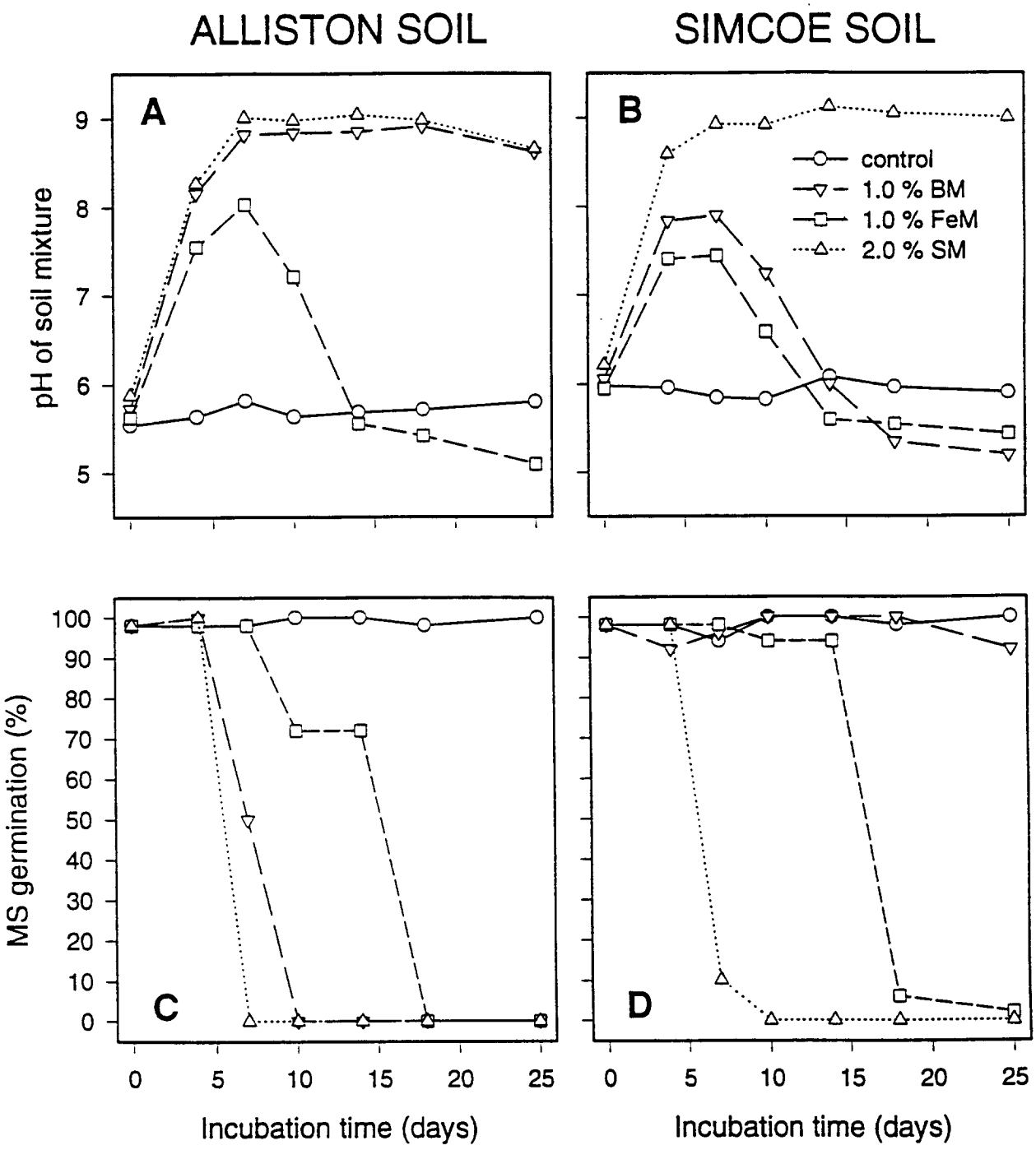

Fig 2. Effect of meat and bonemeal (MBM) amendment on the germination of *V. dahliae* microsclerotia (MS) buried in two types of soils.

Values represents mean (-SEM), n=3.

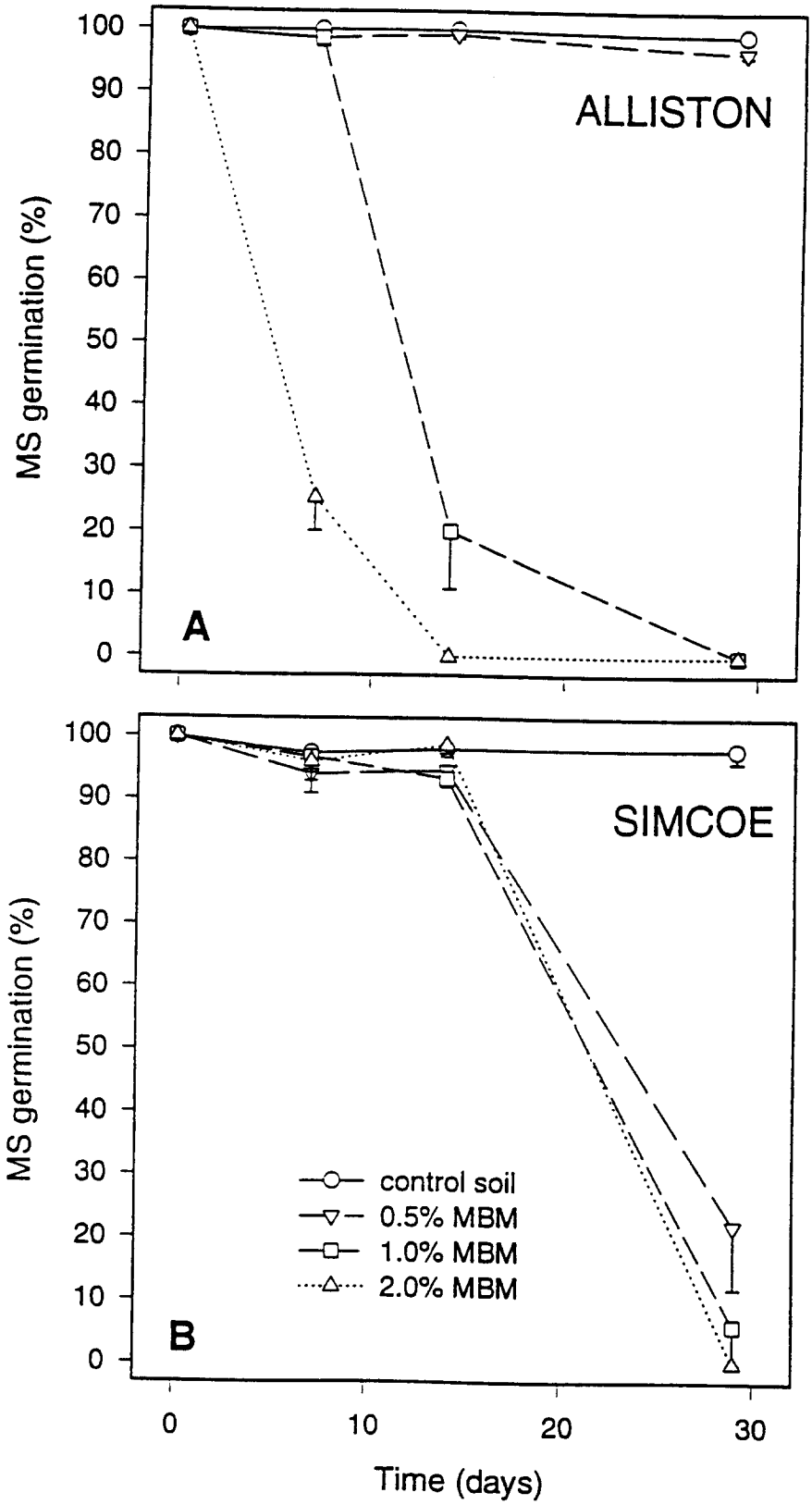

Fig 3. Effect of feather meal (FeM) amendment (1% w/w) at various ambient temperatures on soil pH and germination of soil-placed *V. dahliae* microsclerotia (MS) in two types of soils under laboratory conditions.