EFFICACY OF ETHANEDINITILE AT LOW TEMPERATURE AGAINST CEREAL PATHOGENS: A SITUATION REPORT

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Drought causes periodic shortfalls in feed availability for the Australian intensive livestock industry. Importation of feed into Australia presents is one solution but presents quarantine concerns as many serious pests and pathogens carried on feed grains are not established in Australia. Ethanedinitrile (EDN) is being investigated by CSIRO Australia as an alternative to methyl bromide for a range of other uses(Ren et al, 2002, Rosskopf, 2007), including soil and timber fumigation (Wright et al 2002, Waterford, 2004). The feasibility of using EDN to sterilise imported commodities of quarantine risks was tested (Waterford, 2004) and was successful in devitalising barley, maize, sorghum and wheat and is being evaluated as part of an import treatment protocol for devitalising feed grain imports into Australia to provide an acceptable method for managing these concerns.

This paper reports preliminary assessment of efficacy at temperatures between 3-22ºC against target pathogens of concern including *Tilletia indica* Mitra (Karnal bunt), *Peronosclerospora sorghi* Weston & Uppal (sorghum downy mildew), *Tilletia controversa* Kühn (dwarf bunt) and *Ustilago maydis* (DC.) Corda (boil smut), which is being conducted in collaboration with the USDA ARS.

EDN has been patented by the CSIRO (Desmarchelier and Ren 1996) as a new fumigant effective against insects and micro-organisms. It has a threshold limit value (TLV) of 10 ppm, which compares favourably with 5 ppm for methyl bromide.

Treatments included: 1.) naked spores; 2.) bunted seed, when present as a propagule in the pathogen life cycle; and 3.) spores dusted on maize.

The EDN was generated in the laboratory in a fume hood by slowly injecting saturated KCN into hot (95^{o}C) $CuSO_4$. The air in an inverted bell, fitted with a gas sampling septum, was first withdrawn filling the bell with the hot $CuSO_4$ The generated gas was transferred into a Tedlar® gas sampling bag. After cooling percent purity was analysed using a Thermal Conductivity Detector (TCD) fitted to an SRI model 8610C gas chromatograph using a 3 foot 1/8 inch column packed with Porapak Q 80/100 mesh, run at 100ºC with a carrier gas (He) 20 mL-1. Purity measured ranged between 78 to 89 % which reflected the actual temperature of the $CuSO_4$ solution.

Three replicates of each were put into open Ependorf tubes and placed into open desiccators of measured volume, allowed to equilibrate to 75% relative humidity overnight. The lids then closed to seal, and injected with EDN through a gas septum port, having first withdrawn an equivalent volume of air to prevent desiccator lids

from popping. In the case of P. sorghi, homogenised infected leaf material with oospores was placed into small Nitex® bags made of 20 µm pore-size polyester screen and placed into racks in the desiccator.

Application of EDN was at 120 mgL^{-1} and held at 5, 17 and 22°C. Exposure times were 10, 25, 60, 120, 250, 500, 1000, 1750, 3000, 4500, 7000 minutes. At completion of exposure spores of treated material and untreated controls were plated out for assessment of efficacy. In the case of sorghum downy mildew, seed of a susceptible variety of sorghum was inoculated with treatment and control spores and planted out as a bioassay of efficacy.

Target concentrations in each desiccator were calculated from percent purity of EDN and desiccator volume. Concentrations during exposure were measured by taking samples and analysing them with a Flame Ionisation Detector (FID) using the same column and GC. Concentrations for the longer exposures were topped up from time to time to maintain the concentration as near to 120 mg/L as possible. The mgh/L dosage, Ct product achieved, was calculated from the FID results for each exposure.

Treated material and untreated controls spores of *T. indica*, *T. controversa* and *U. maydis* were seeded onto water agar medium to assess viability based on spore germination. Oospores of *S. sorghi* germinate poorly, if at all, on artificial medium hence a bioassay on susceptible plants was used. Treated oospores where mixed into the upper 5 cm layer of soil in a 2 X 2 inch plastic pot and planted with seeds of a highly susceptible sorghum cultivar and placed in a growth chamber for disease development.

In general, though data indicate EDN was more toxic at higher temperatures, good control was obtained at the lowest temperature of 3ºC. T. Overall, *T. indica*, with its large teliospore, was the most tolerant of the smut fungi.

Figures 1 to 3 present the response of the three smut fungi (*T. indica*, *T. controversa* and *U. maydis)* to treatments at a range of doses for three temperatures. These data indicate that naked teliospores were more easily controlled than spores still contained within the fungal structure or sorus of *T. indica* and *T. controversa*. Spores that were dusted onto corn were the most difficult to control. This would indicate that surface interactions on the corn seeds and penetration of EDN into the fungal structures reduce the effective dose. All three smut species treated were controlled to a high level at all temperatures and at dosages likely to be useful in treatment schedules.

No vital stains were shown to be effective with oospores of *P. sorghi*. However, the treated oospores mixed into soil and planted with seeds of susceptible sorghum also proved problematic as an assessment of efficacy. Trace infection was observed in the untreated controls, and in one replicate of the 1 hr treatment at 17°C at a dose of 120mgh/L. No other infection was recorded in the remaining 44 treatments however, cross-contamination of the treated oospores cannot be ruled out. Most likely, given that initial inspection of the infected material indicated a high number of spores, is that the newly acquired oospores may have been exhibiting yearly season dormancy, which would explain the low levels on infection in the inoculated control plants and near absence of infection in any of the treatments (Pratt, 1978).

Acknowledgements

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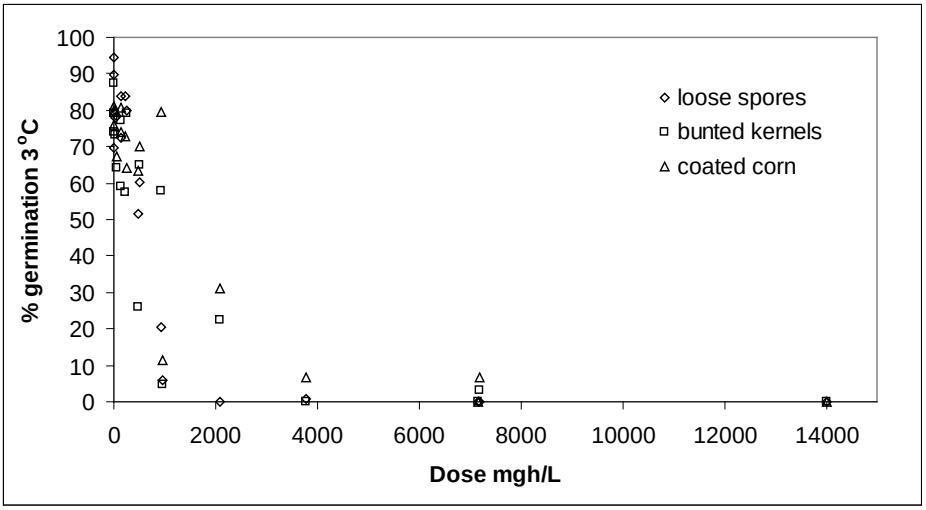
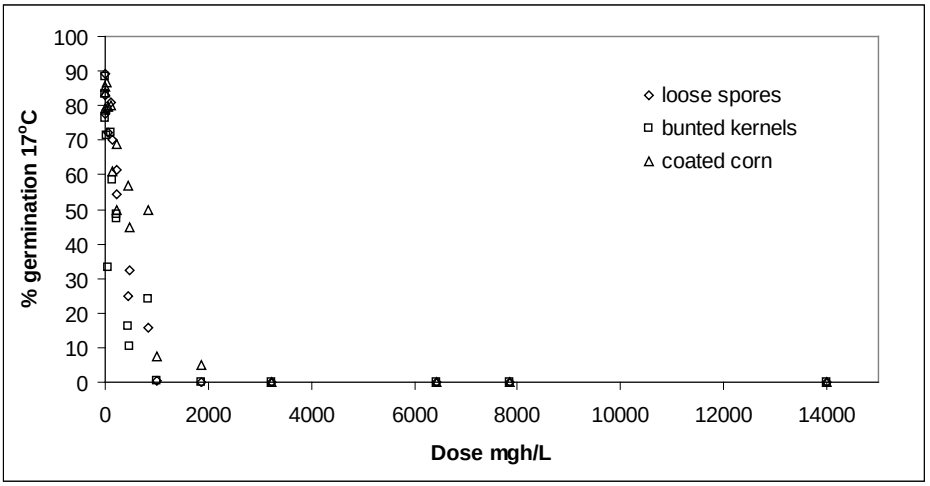




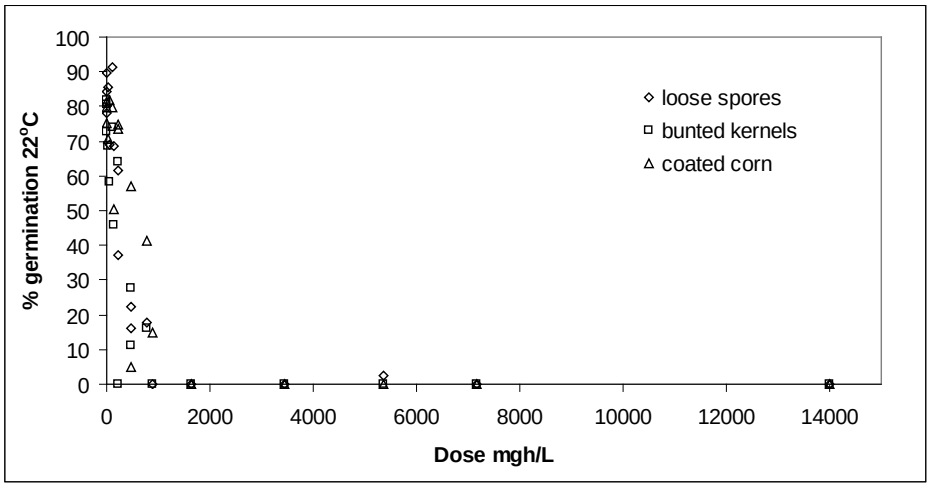


Figure 1. Efficacy of ethanedinitrile (C_2N_2) at 120 mg/L against teleospores of *Tilletia controversa* treated as loose spores, bunted kernels and spores dusted onto corn for 10, 25, 60, 120, 250, 500, 1000, 1750, 3000, 4500, 7000 minutes of exposure at 3, 17 and 22°C

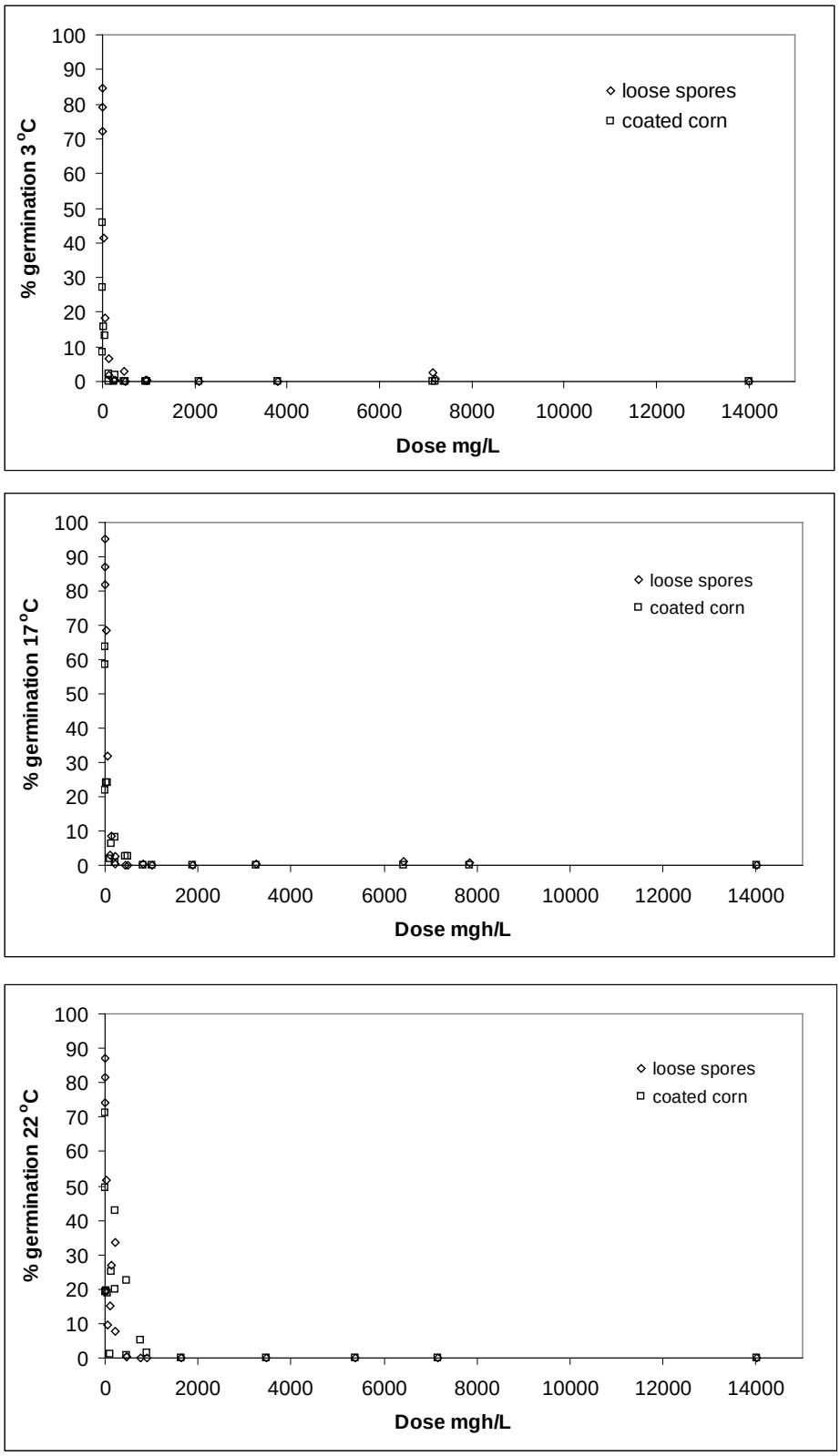
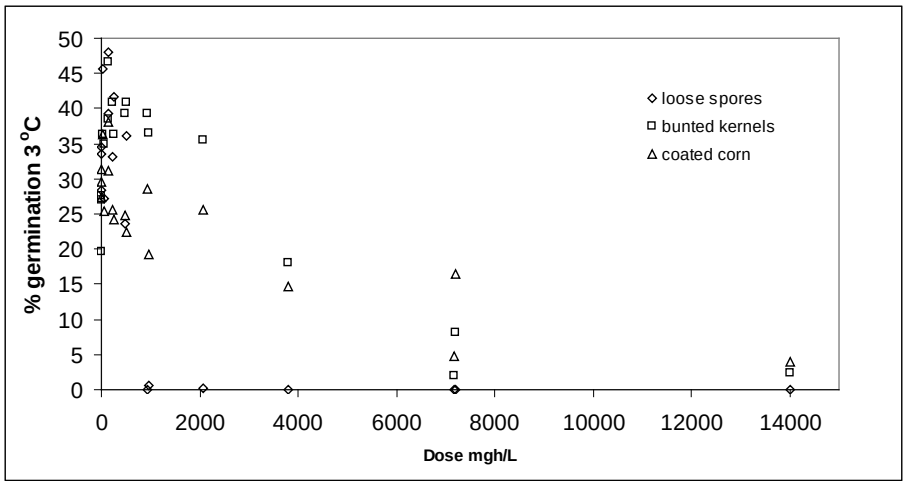
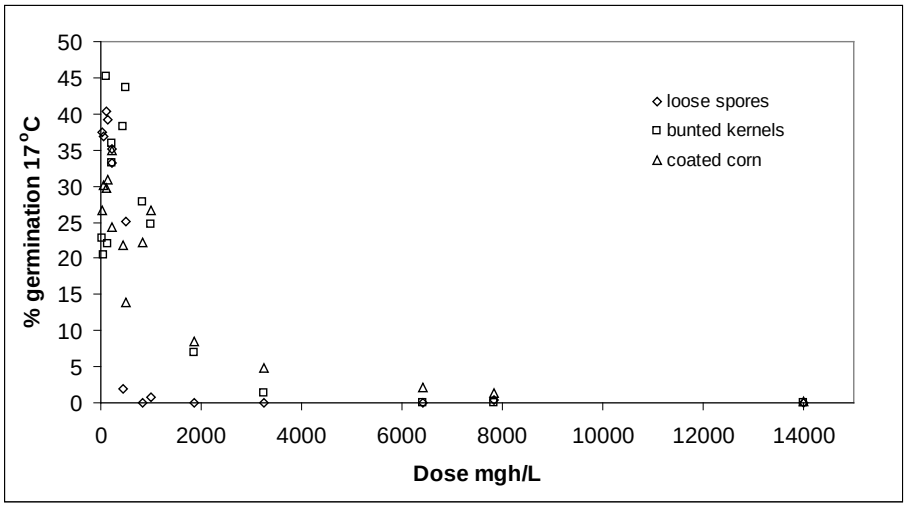


Figure 2. Efficacy of ethanedinitrile (C_2N_2) at 120 mg/L against teleospores of *Ustilago maydis* treated as loose spores and spores dusted onto corn for 10, 25, 60, 120, 250, 500, 1000, 1750, 3000, 4500, 7000 minutes of exposure at 3, 17 and 22°C





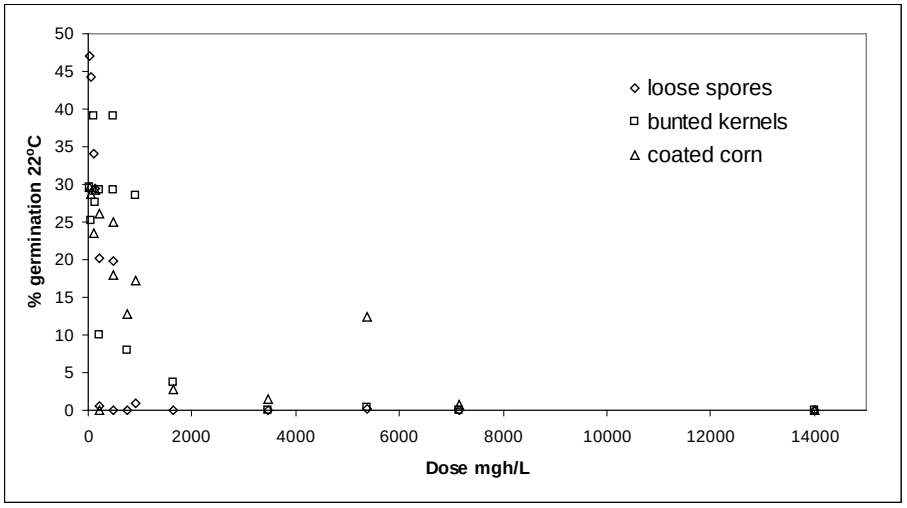


Figure 3. Efficacy of ethanedinitrile (C_2N_2) at 120 mg/L against teleospores of *Tilletia indica* treated as loose spores, bunted kernals and spores dusted onto corn for 10, 25, 60, 120, 250, 500, 1000, 1750, 3000, 4500, 7000 minutes of exposure at 3, 17 and 22°C