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Efficacy of Ethanedinitile(C_2N_2) against Some Cereal Pathogens

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Abstract: Ethanedinitrile (EDN) is being investigated by CSIRO Australia as an alternative to methyl bromide for a range of uses, including soil and timber fumigation.

EDN is an effective devitalisation agent of cereals and is being evaluated for devitalising feed grain imports into Australia. Part of this process involves assessment of efficacy against target pathogens of concern including *Tilletia indica* Mitra (Karnal bunt), *Peronosclerospora sorghi* Weston & Uppal (sorghum downy mildew), *Tilletia controversa* K hn (dwarf bunt) and *Ustilago maydis* (*DC.*) *Corda* (boil smut), which is being conducted in collaboration with the USDA ARS.

This fumigant was tested on: 1) naked spores; 2) bunted seed, when this is a propagule in the life cycle of the pathogen; 3) spores dusted on maize. It was applied at 120 mg/L over a period ranging from a few minutes to 5 days at 5, 17 and 22℃.

The naked teliospores of the three smut fungi were more easily controlled than spores still contained within the fungal structure. (sorus) or those spores that were dusted onto maize seed, which were the most difficult to control. Oospores of *S. sorghi* germinate poorly, if at all, on artificial medium. Results of the bioassay showed trace infection of sorghum in the untreated controls, and in one plant that was treated for 1 hr at 17℃.

Introduction

The Australian intensive livestock industry periodically suffers shortfalls in cost effective feedstuff during droughts. Importation of feed into Australia presents quarantine concerns as many serious pests and pathogens carried on feed grains are not established in Australia.

Ethanedinitrile (EDN) is being investigated by the CSIRO Australia as an alternative to methyl bromide for a range of uses, including soil (Ren et al, 2002 [1]; Rosskopf, 2007[2]) and timber fumigation (Wright et al 2002[3], Waterford, 2004[4]). The feasibility of using EDN to sterilise imported commodities of quarantine risks was tested (Waterford, 2004[5]) and was successful in devitalising barley, maize, sorghum and wheat. This present study reports results from assessments of efficacy against target pathogens of quarantine concern including *Tilletia indica* Mitra (Karnal bunt), *Peronosclerospora sorghi* Weston & Uppal (sorghum downy mildew), *Tilletia controversa* K hn (dwarf bunt) and *Ustilago maydis* (*DC.*) *Corda* (boil smut), which is being conducted by the CSIRO in collaboration with the USDA ARS.

EDN is a colourless gas with an almond-like odour; its chemical and physical properties are listed in Table 1. EDN has been patented by the CSIRO (Desmarchelier and Ren 1996[6]) 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.

Table 1. Chemical and physical properties of cyanogen compared to other fumigants

Formula	CH_3Br	C_2N_2
Molecular weight	95	52
Boiling point @1 atm	3.6℃	-21.17℃
Specific gravity (gas), air =1.0	3.3	1.82
Flammability limits in air, v/v%	13.5-14.5	6-32
Solubility in water, v/v%	3.4	Highly soluble
Conversion factor mg/L to ppm, v/v@1 atm	260	480

Materials and Methods

The efficacy of EDN was tested on naked spores, bunted seed, when this is a propagule in the life cycle of the pathogen and spores dusted on maize. Three replicates of each were put into open Ependorf tubes and placed into open des-

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iccators of measured volume, allowed to equilibrate to the 75% relative humidity overnight. The lids then closed to lids 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 m pore-size polyester screen and placed into racks in the desiccator.

EDN was applied at 120 mg/L and held at 5, 17 and 22℃. Times of exposure were 10, 25, 60, 120, 250, 500, 1000, 1750, 3000, 4500, 7000 minutes. After treatment 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.

The EDN was generated in the laboratory in a fume hood by slowly injecting saturated KCN into hot (95℃) $CuSO_4$. 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 then transferred by syringe into a Tedlar gas sampling bag and more EDN generated until sufficient for the days doses was made. After cooling to room temperature 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 100℃ with a carrier gas (He) 20 mL – 1. Purity was measured from 78 to 89 % which reflected the temperature of the $CuSO_4$.

The quantity of EDN needed to achieve target concentrations in each desiccator was calculated based on percent purity from the TCD analysis and desiccator volume. Exposure concentrations were then measured by taking samples with a gastight syringe through a gas sampling septum 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 mg · h/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. Treated oospores where mixed into the upper 5 cm layer of soil in a 2 × 2 inch plastic pot and planted with seeds of a highly susceptible sorghum cultivar and placed in a growth chamber for disease development.

Results and Discussion

Figures 1 to 3 present the response of treated spores to the range of doses and temperatures. These data indicate that naked teliospores of the three smut fungi (*T. indica*, *T. controversa* and *U. maydis*) 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 at 22℃ were controlled to a high level at dosages less than 2 000 mg · h/L. As this is the likely treatment temperature of the commodity and the proposed dosage would be greater than this experimental treatment, using EDN should provide good control of these pathogens.

In general the data indicate that EDN was more toxic at higher temperatures. Overall, *T. indica*, with its large teliospore, was the most tolerant of the smut fungi.

Oospores of *S. sorghi* germinate poorly, if at all, on artificial medium hence this was not a feasible method to check efficacy for this pathogen. 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℃ at a dose of 120mg · h/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[7]).

Acknowledgements

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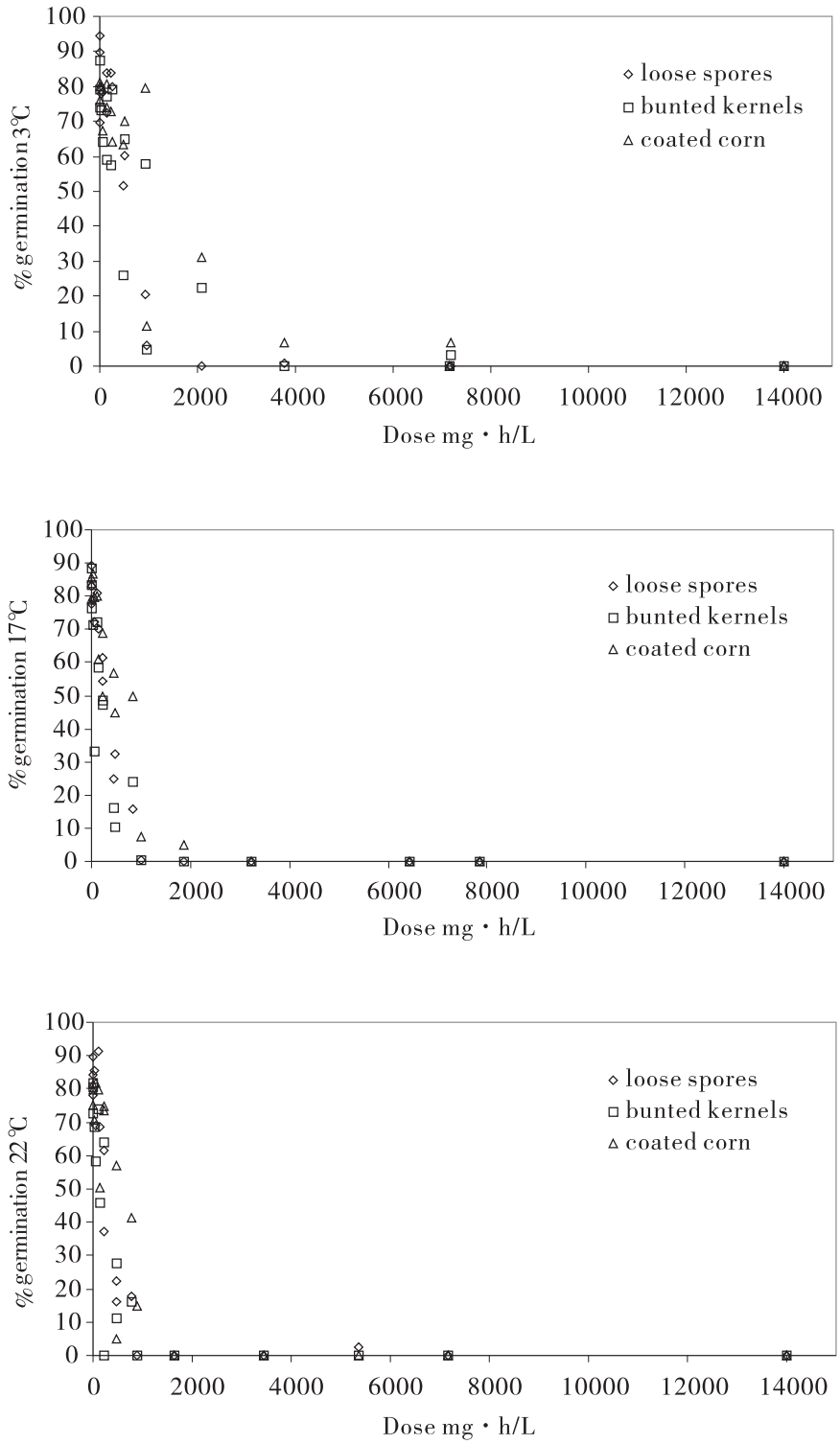

Fig. 1 Efficacy of ethanedinitrile (C_2N_2) at 120 mg/L against teleospores of *Tilletia controversa* treated as loose spores, bunted kernels and spores dusted onto corn for 10, 25, 60, 120, 250, 500, 1000, 1750, 3000, 4500, 7000 minutes of exposure at 3, 17 and 22℃

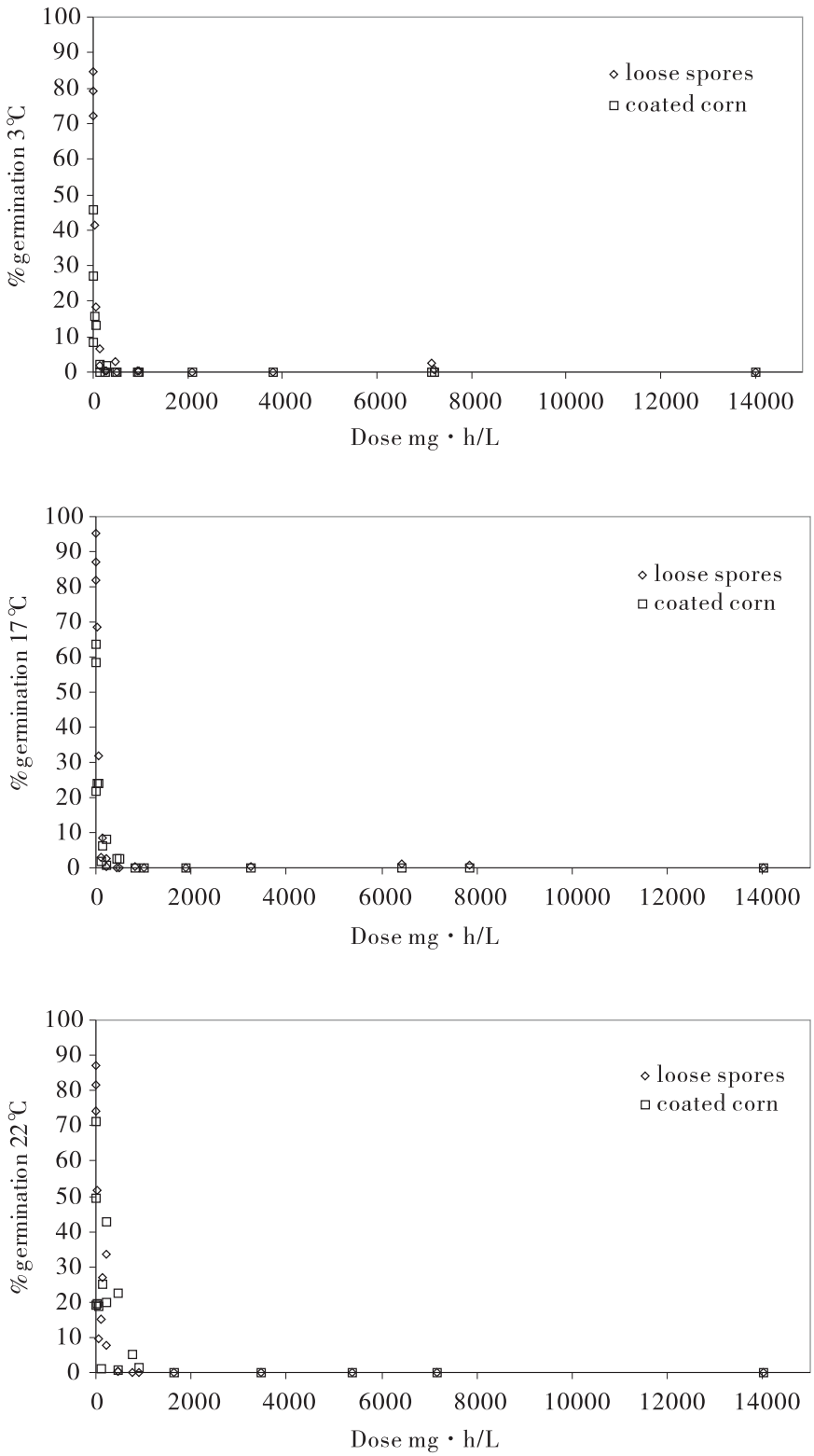

Fig. 2 Efficacy of ethanedinitrile (C_2N_2) at 120 mg/L against teleospores of *Ustilago maydis* treated as loose spores and spores dusted onto corn for 10, 25, 60, 120, 250, 500, 1000, 1750, 3000, 4500, 7000 minutes of exposure at 3, 17 and 22℃

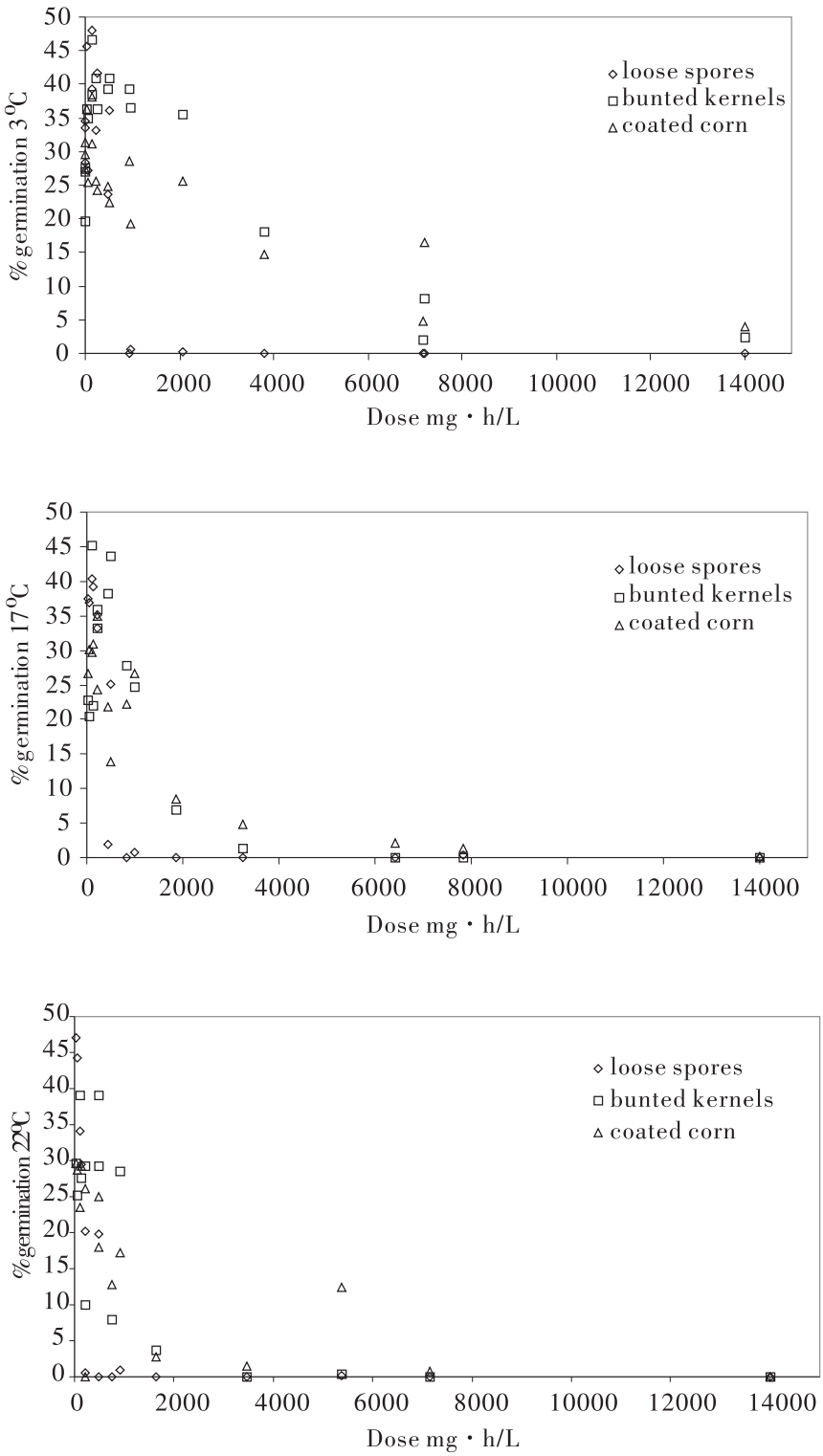

Fig. 3 Efficacy of ethanedinitrile (C_2N_2) at 120 mg/L against teleospores of *Tilletia indica* treated as loose spores, bunted kernals and spores dusted onto corn for 10, 25, 60, 120, 250, 500, 1000, 1750, 3000, 4500, 7000 minutes of exposure at 3, 17 and 22℃

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