

DEVELOPMENT OF CYANOGEN FOR SOIL FUMIGATION

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Introduction

The CSIRO Stored Grain Research Laboratory (SGRL) has developed and patented cyanogen (C_2N_2) as a fumigant to replace methyl bromide (MeBr) in a variety of applications (Desmarchelier & Ren 1996). Cyanogen appears to have very promising fumigant properties for soil application such as excellent penetration in moist soils, high toxicity to insects, nematodes, fungi and weeds, and easy application through the irrigation system or by direct injection into soil. All of the degradation products of C_2N_2 naturally occur in the soil environment.

This report covers the results from our recent laboratory and greenhouse experiments and aims at assessing the potential of C_2N_2 for its use as a multi-functional soil fumigant.

Laboratory Studies

Penetration of C_2N_2 through soils

The procedure for studying sorption was first to condition the soil moisture content to 27% (Gingin sand) and 52% (Pemberton loam), as determined by the oven drying method. Second, the soil sample, loosely packed, was weighed and then transferred to a 700mL PVC column (7 cm ϕ \times 18 cm h) equipped with sampling ports on the wall of column. Cyanogen (60mg/L) was injected at bottom of column and the concentrations at different levels were measured by gas chromatography (GC).

Cyanogen diffused and penetrated through the soils faster and farther than MeBr. Cyanogen was more rapidly and strongly sorbed by all soils compared to MeBr. The higher partitioning of C_2N_2 into soils than MeBr, means less emission of C_2N_2 to air. Cyanogen was stable in soil for 3-5 hr, after which it was broken down to naturally occurring soil components.

Laboratory bioassays on insects

Tests were conducted in 200mL glass bottles equipped with an airtight cap that allowed gas injection through a septum. The insect cage, containing about 50-60 whitefringed beetle larvae (*Graphognathus leucoloma*) was placed into the bottle and then covered with soil (30% full). Fumigants were injected into separate bottles with airtight syringe. Controls sets consisted of 50 larvae in sealed bottles containing the soil sample.

Table 1: Toxicity to 1st-instar whitefringed weevil larvae, *Graphognathus leucoloma*, at 25±2°C, with soil (30% fill) and 5 hours exposure.

L(CXt) mg h/L	C ₂ N ₂	MeBr
L(CXt)50	30	100
L(CXt)95	50	135

Laboratory bioassays on nematodes

A small bottle (8mL) containing nematodes in 2mL of water, was placed in an Erlenmeyer flask filled with 30% sandy soil and fumigated by injecting C₂N₂ gas (5mg/L). Alternatively, C₂N₂ in aqueous solution (0.2mL) was injected into the flask. The flask was incubated at room temperature (25°C). The species of nematode tested was infective juveniles of *Steinernema carpocapsae* strain BW. After 5h of incubation, the flasks were opened in the fume hood for aerating them. Mortality of the larvae was assessed, under a microscope, at 24h after the application of fumigant.

Nematodes died quickly after exposure to C₂N₂, as shown in Table 2. For example, a nominal application of 5mg/L killed 404/404 nematodes of *Steinernema carpocapsae*, as against a control mortality of only 5/462.

Table 2: Toxicity of C₂N₂ to nematodes (*Steinernema carpocapsae*) at 25±2°C, in the presence of soil (30% fill) and 5 hours exposure.

L(CXt) mg h/L	C ₂ N ₂	MeBr
L(CXt)50	25	75
L(CXt)95	40	100

Laboratory bioassays on fungi

Eight pieces of paper (6×6 mm) containing a pathogen were fumigated in empty flasks (275mL) and in flasks 50% full of wet soil (Gingin sand) at 1, 5, 10 and 20mg/L of C₂N₂. The flasks were then incubated at 25±2°C for 6 hours and 24 hours. After fumigation, the flasks were opened and fumigated papers were placed on a growth medium (potato dextrose agar) in Petri dishes. The pathogens incubated at 25±2°C.

Table 3: Doses in mg/L of C₂N₂ required for control of soil pathogens at 25±2°C and exposure time of 6 or 24 hours.

Pathogen	C ₂ N ₂ (mg/L) without soil		C ₂ N ₂ (mg/L) with soil (50% full)	
	6 hrs	24 hrs	6 hrs	24 hrs
<i>Schlerotium rolfsi</i>	5	1	20	5
<i>Pythium sulcatum</i>	5	1	20	5
<i>Rhizoctonia solani</i>	5	1	20	10
<i>Fusarium acuminatum</i>	1	1	10	5
<i>Phytophthora cactorum</i>	5	1	20	5
<i>Phytophthora cryptogea</i>	1	1	10	5
<i>Bipolaris soroikiniana</i>	5	1	20	10

Greenhouse trials

Greenhouse trials on strawberry runners for evaluation of phytotoxicity

The soil (Gingin sand, 18% m.c.) was fumigated in a sealed container at 25, 50 and 100mg/L for 24 hours. After fumigation, the treated soil was divided into two lots. Strawberry runners were directly planted into first lot of treated soil without aeration and in the second lot after passive aeration for 24 hours. All treatments were applied in four replicates.

The strawberry runners were not affected by the fumigated soil after 24 hours passive aeration at all tested doses of C_2N_2 . The strawberry runners were reversibly affected by the fumigated soil without aeration only at the highest dosage rate of 100mg/L.

Greenhouse trials for control of branched broomrape (*Orobanche ramosa*) seeds

Glass Petri dishes containing broomrape seeds (>1000) were placed at 3 levels of depth in a pot (2.5 L capacity) filled with dry or moist sandy soil. Cyanogen was injected into the soil from bottom of pot at the rate of 25, 50 and 100mg/L. After 24 hours fumigation, the soil was aired and the broomrape seeds were collected for assessment of germination.

The broomrape seeds were 100% controlled at 25mg/L of C_2N_2 in moist soil. However, C_2N_2 could not kill the broomrape seeds in dry soil even at 100mg/L, indicating the benefit of moistened soil.

Environmental impact and OH&S considerations

Cyanogen is not listed as a greenhouse gas or ozone-depleting substance.

The threshold limit value (TLV) for C_2N_2 of 10 ppm (v/v) is higher than methyl bromide (5 ppm). The chemistry of C_2N_2 is also well understood. It is a colourless gas with a boiling point of 21.2°C. It has an almond-like odour, which becomes acrid and pungent at high concentrations, making it detectable to the user should a leak occur.

Unlike most fumigants, C_2N_2 is readily soluble in water, with 1 volume of water dissolving 4 volumes of C_2N_2 . In aqueous solutions C_2N_2 is slowly hydrolysed to form oxalic acid and ammonia. At low pH, C_2N_2 reacts to form derivatives of formic acid and hydrogen cyanide (HCN). Hydrogen cyanide is found in nature in some vegetable substances, e.g., bitter almonds, apple seeds, cherries and sorghum. It is usually combined in glycoside molecules and is released when broken down by enzymes during metabolism. The NEPM (1999) guideline maximum soil concentrations for HCN in residential soil are 250mg/kg free HCN and 500mg/kg if complexed. For commercial or industrial soil, they are 1250mg/kg free HCN or 2500mg/kg complexed. Data from our laboratory studies showed 150mg/kg of C_2N_2 can control all target organisms: insects and nematodes at <80mg/kg; soil borne pathogens and soil fungi at <120mg/kg. Assuming a 100% conversion to HCN of applied C_2N_2 (150mg/kg), the level of HCN residue formed will be 78mg/kg, which is much lower than the guideline for maximum concentration in residential soil.

Conclusion

These results indicate that the C₂N₂ appears to have great potential as soil fumigant to replace methyl bromide. Formulations and application methods are being investigated to develop good agricultural practices for this fumigant.

References

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