ETHANEDINITRILE (C_2N_2) – A NOVEL SOIL FUMIGANT FOR INSECT, NEMATODE, PATHOGEN & WEED CONTROL

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Introduction

The CSIRO Entomology has developed and patented cyanogen - ethanedinitrile (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 occur naturally in the soil environment. This report covers the results from our recent laboratory, greenhouse and field trials 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 × 18 cm h) equipped with sampling ports on the wall of column. Etanedinitrile (60mg/L) was injected at bottom of column and the concentrations at different levels were measured by gas chromatography (GC).

When injected into soil, C_2N_2 diffused and penetrated through the soils faster and farther than MeBr and was more rapidly and strongly sorbed by all soils compared to MeBr. This higher partitioning of C_2N_2 into soils than means less emission of C_2N_2 to air. ethanedinitrile 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 an 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±2C°, with soil (30% fill) and 5 hours exposure.

L(CXt) mg h/L	C_2N_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 C_2N_2 gas (5mg/L). Alternatively, C_2N_2 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. Mortality of the larvae was assessed, under a microscope, at 24h after the application of fumigant. Nematodes died quickly after exposure to C_2N_2, 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 C_2N_2 to nematodes (*Steinernema carpocapsae*) at 25±2C°, in the presence of soil (30% fill) and 5 hours exposure.

L(CXt) mg h/L	C_2N_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 C_2N_2. 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 were incubated at 25±2°C.

Table 3: Doses in mg/L of C_2N_2 required for control of soil pathogens at 25±2°C and exposure time of 6 or 24 hours**.**

Pathogen	C_2N_2 (mg/L) without soil		C_2N_2 (mg/L) with soil (50% full)	
	6 hrs	24 hrs	6 hrs	24 hrs
Schlerotium rolfsi	5	1	20	5
Pythium sulcatum	5	1	20	5
Rhizoctonia solani	5	1	20	10
Fusarium acuminatum	1	1	10	5
Phytophthora cactorum	5	1	20	5
Phytophthora cryptogea	1	1	10	5
Bipolaris soroikiniana	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 did not kill the broomrape seeds in dry soil at 100mg/L, indicating the benefit of moistened soil.

Trial 1: Control of Branched broomrape (Mannum, South Australia)

A commercial scale field trial on application of C_2N_2 for broomrape control (sandy soil) was conducted in Mannum, SA on 15-16 July 2003. The trial was conducted in collaboration with the SA DPI (Dr John Virtue, Leader of Broomrape program) and K & B Adam (fumigator). The trial was designed to determine:

- Control of broomrapes
- Control of weeds
- OH&S during application
- Any interaction between fumigant and wheat (phytotoxicity)
- The movement of C_2N_2 in soil
- The residues of C_2N_2 and HCN in soil

Results

- During application the levels of C_2N_2 in the environment were 0.1-0.5ppm (near by plastic covered plots) and 0.5-2ppm (near by without plastic covered plots), much lower than the TLV of 10ppm, and no detectable levels of HCN in the air.
- C_2N_2 penetrated the soil very quickly (<5 min after application, C_2N_2 was evenly distributed through the soil from the surface to a depth of 25cm).
- C_2N_2 and HCN residues in soil were 75-120ppm and 1-5ppm in plastic covered plots, and 45-85ppm and 0.5-3ppm in plots without plastic covers respectively, 20 hours after application. Both C_2N_2 and HCN residues had declined to indistinguishable levels within 48 hours.
- Broomrape and weeds were well controlled by C_2N_2 at 25 g/m^2 in plastic covered plots, similar to results achieved C_2N_2 at 50 g/m^2 in without plastic covered plots and methyl bromide at 50 g/m^2 in plastic covered plots.
- Telone-35 and sprayed herbicide show a high level of herbicide/fumigant residual phytotoxictity. Four weeks after treatment wheat planted was almost killed and 2-3 months post treatment broomrape and other weeds were well established when soil was wetted.
- The phytotoxicity studies were also conducted in a green house scale trial. The results indicate that plant-back after C_2N_2 treatment could be as short as two days. This would be a significant advantage in commercial use of C_2N_2.

Trial 2: Control of wintergrasses and pathogens in strawberry runner beds (Toolangi, Victoria Australia)

A recent commercial scale field trial to control pathogens and winter growing grasses in strawberry runner beds was conducted in Toolangi, Victoria (Table 4). Three test pathogens

(*Phytophthora cactorum, Sclerotium rolfsii, Rhizoctonia fragariae*) placed in the C_2N_2 treated beds were controlled. The vegetative growth present (wintergrasses) were controlled in the 25 g/m^2 plastic covered plot (Table 5). Rapid degradation of ethanedinitrile was also confirmed as these plots were replanted and proved safe to the replanted crop.

Table 4. Details of broomrape and strawberry runner field trials

Site		Mannum, SA	Toolangi, VIC
Date		15-16 July. 2003	30 May 2003
Soil type		Sand	Heavy loam
		Canola & wheat	Strawberry runner field
		(30º13' S, 135º49' E)	(37º32' S, 145º28' E)
Moisture		Irrigated	Rained
Temperature: Surface		19-55°C	12.5-26.4°C
	Soil (10 cm)	20-30°C	15.3-17.7°C
RH (Soil)		50-100%	65-100%
Dosage:	C_2N_2 (g/m^2)	50 g/m^2, covered & uncovered	50 g/m^2, covered & uncovered
		25 g/m^2, uncovered	25 g/m^2, uncovered
	Telone C-35	50 g/m^2 , covered	50 g/m^2 , covered
	Methyl bromide	50 g/m^2 , covered	
	Methyl bromide		50 g/m^2 , covered
	: chloropicrin (70:30)		
	Methyl iodide: chloropicrin (30:70)		50 g/m^2 , covered
			25 g/m^2, covered
Plot size		20m × 1m (28 plots)	35m × 2.7m (18 plots)
Target organisms		Branched broomrape and weeds	Wintergrasses, *Phytophthora cactorum, Sclerotium rolfsii, Rhizoctonia fragariae*

Table 5. Weed assessments Toolangi, Vic trial, 2 months post fumigation.

	Rate		Total Weeds		Monocots		Dicots	
Treatment	(g/sq m)	Covered	Mean No.*	% of controls	Mean No.*	% of controls	Mean No.*	% of controls
C_2N_2	25	no	969.2	153.2	806.2	238.2	163.0	55.4
C_2N_2	50	yes	7.8	1.0	6.8	1.1	1.0	0.7
C_2N_2	50	no	693.1	109.5	602.4	178.0	90.7	30.8
Methyl Bromide	50	yes	3.1	0.4	0.0	0.0	3.1	0.8
Methyl Iodide	50	yes	2.5	0.4	1.6	0.4	0.9	0.6
Methyl Iodide	25	yes	100.0	43.1	97.9	73.0	2.1	0.5
Telone C35	50	yes	168.8	72.4	147.9	112.1	20.8	15.2
Untreated	0		632.8		338.5		294.3	

* No. per quadrat (0.4 x 0.4 m), mean of 4 counts

Environmental impact and OH&S considerations

Ethanedinitrile 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) a 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 for maximum soil concentrations for HCN in residential soil is 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 indicate that 150mg/kg of C_2N_2 is sufficient to control all target organisms. Assuming a 100% conversion to HCN of applied C_2N_2 (150mg/kg), the level of HCN residue formed will be 78mg/kg much lower than the guideline for maximum concentration in residential soil. Studies on the actual conversion to HCN in soils indicate that approximately 2% is converted to HCN

Conclusion

These results indicate that the C_2N_2 appears to have a great potential as a soil fumigant to replace methyl bromide for a range of targets including insects, nematodes, pathogens and weeds. Formulations and application methods are being investigated to develop good agricultural practices for this fumigant.