APPLICATION TECHNIQUES INFLUENCE THE EFFICACY OF ETHANEDINITRILE (C_2N_2) FOR SOIL DISINFESTATION

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Introduction

Laboratory and glasshouse studies conducted by CSIRO on ethanedinitrile (cyanogen, C_2N_2) have demonstrated its potential as an alternative to methyl bromide (MB) for soil disinfestation. In laboratory trials, C_2N_2 diffused and penetrated soils in loosely packed columns faster and further than MB. Furthermore, C_2N_2 was sorbed by soil particles more rapidly and strongly than MB, thus minimizing atmospheric emissions. C_2N_2 was stable in soil for 3-5 hours, with separate glasshouse trials showing that the required plant-back time for strawberries was as short as 24 hours, provided soil was aerated prior to planting. In laboratory bioassays, C_2N_2 controlled a range of soil-borne pathogens, insects and nematodes (Ren *et al*., 2002). The strong potential of C_2N_2 for soil disinfestation led CSIRO to patent the product in 1996 (Desmarchelier & Ren, 1996).

Collaborative research was initiated in early 2003 between CSIRO, K&B Adams Pty Ltd fumigant contractors, and the Victorian Department of Primary Industries, aimed at: (1) developing practical methods and machinery for applying C_2N_2 to field soils and (2) assessing the efficacy of C_2N_2 for soil disinfestation in the field.

Application of ethanedinitrile to field microplots

A microplot field study was conducted at Bayswater, Victoria (37º50'S, 145º15'E) in a silty-clay soil. Fumigants were applied through a single injection point in the middle of the microplots (1m × 1m) at a rate of 30g/m². Fumigant treatments included: 98% MB (injected at the soil surface under 35µm low-density polyethylene as 'hot gas'); C_2N_2 (injected at a depth of 20cm under LDPE); C_2N_2 (injected at a depth of 20cm under no LDPE); C_2N_2 (injected at the soil surface under LDPE); and untreated soil sealed with LDPE. Prior to fumigation, muslin bags containing inoculum or seed of various soil-borne pathogens or weeds (see Table 1) were buried in the microplots at depths of 10 and 20cm, at distances of 25 and 50cm from the injection point. Cracked Dräger tubes, specific for C_2N_2, were buried next to the muslin bags in C_2N_2 treatments and recovered 1 day after fumigation. Five days after fumigation inoculum/seed was retrieved and plated onto selective media or germinated to determine viability. Soils were sampled 2 weeks after fumigation and nematode counts made using the Baermann technique. Also, soil populations of various microflora (Table 4) were determined using cultural procedures. Treatments were replicated three times.

C_2N_2 was most efficacious at killing pathogens and weeds when injected into soil at a depth of 20cm under LDPE (Table 1). In this treatment, C_2N_2 killed indicator pathogens and weeds at a distance of 25cm from the injection point as effectively as MB but failed to kill them at 50cm, even though low concentrations of C_2N_2 were detected at this distance (Table 2). In contrast, C_2N_2 was ineffective at killing indicator pathogens and

weeds when injected at the soil surface or when left uncovered. In this case lateral movement of C_2N_2 in uncovered plots was restricted to 25cm. These results suggest that the greatest challenge with applying C_2N_2 in the field is to retain it long enough in soils to allow adequate exposure times for target pests.

At 2-weeks after fumigation, C_2N_2 controlled parasitic nematodes to similar levels as MB, but populations of free-living nematodes were greater in C_2N_2 plots (Table 3). Although MB reduced levels of soil fungi more than C_2N_2, there were higher populations of soil bacteria in C_2N_2 plots (Table 4). The elevated recolonisation in C_2N_2 fumigated soils by some components of the biota might mean it has less of an impact on soil health and function than MB, and possibly enhances the increased growth response of plants. Future studies will investigate the changes in: (1) the diversity of soil biota using DGGE, and (2) soil chemistry (particularly soil N), following soil disinfestation with C_2N_2.

Application of ethanedinitrile in strawberry runner field trials

Based on the microplot results, K&B Adams designed a new fumigation rig to apply C_2N_2 in the field, using a tyne spacing of 25cm. The prototype rig has the capacity to seal treated soils with LDPE or with a roller. An ongoing field trial has been established at Toolangi, Victoria (37º32' S, 145º28' E) on a clay soil to investigate soil disinfestation with C_2N_2 (applied with the prototype rig and sealed with LDPE) compared with other fumigants for strawberry runner production. So far C_2N_2 (sealed with LDPE) has reduced the number and diversity of winter weeds emerging in treated plots to similar levels as MB (Table 5). Furthermore, C_2N_2 (and all other fumigants) totally killed buried inoculum of *Phytophthora cactorum*, *Rhizoctonia fragariae* and *Sclerotium rolfsii* (sclerotia) to a depth of at least 30cm.

A concurrent trial has also been established comparing sealing techniques (LDPE and rolling) and application rates of C_2N_2 (25 and 50 g/m²). Following application, concentrations of C_2N_2 at different soil depths were measured over a 24-hour period using GC. Results showed that soils sealed with the roller did not retain high concentrations of C_2N_2 compared to those sealed with LDPE (Fig 1).

Conclusions

C_2N_2 continues to show promise as an alternative to MB for soil disinfestations of pathogens, nematodes and weeds. In general, methods that sealed C_2N_2 for longer periods in soils enhanced its efficacy. The challenge is to refine application equipment and sealing methods to optimize the retention of C_2N_2 in soils. This might include water sealing techniques, 'in line' applications, new formulations or split applications made with other fumigants.

References

Desmarchelier, J.M.; Ren, Y.L. 1996. Cyanogen as a fumigant and application method. International Patent Appellation IPPCT/AUS 95/00409.

Ren, Y.L.; Sarwar, M.; and Wright, E.J. 2002. Development of cyanogen for soil fumigation. Ann. Int. Res. Conf. MB Alt. Em. Red. 63/1-4.

Table 1. Percentage viability of buried inoculum of soil borne-pathogens or weed seeds exposed to various applications of C_2N_2. Methyl bromide and untreated soil formed the controls. All fumigants were applied at a rate of 30 g/m². Inoculum consisted of the following pathogens grown on vermiculite or millet seed: *Pythium ultimum* (P. u); *Phytophthora cactorum* (P. c); *Fusarium oxysporum* (F. o); *Rhizoctonia fragariae* (R. f); *Rhizoctonia solani* (R. s); and slerotia of *Rhizoctonia solani* (R. s (s)). Weed seeds (shaded in gray) included: *Lolium perenne* (L. p); *Brassica napus* (B. n) and *Trifolium repens* (T. r). Values followed by different letters in each column are significantly different, where $p \leq 0.05$.

Treatment	Distance from injection point (cm)	Depth buried (cm)	Percentage Viability of Inoculum or Seed								
			P. u	P. c	F. o	R. f	R. s	R. s (s)	L. p	B. n	T. r
98% MB (injected at soil surface under LDPE)	25	10	0a	0a	0a	0a	0a	-	0a	0a	30bc
	25	20	7ab	0a	0a	0a	0a	-	0a	0a	10a
	50	10	3a	0a	0a	0a	0a	-	0a	0a	18ab
	50	20	20b	0a	0a	0a	0a	-	0a	0a	18ab
C_2N_2 (injected at a depth of 20cm under LDPE)	25	10	7ab	0a	0a	0a	0a	0a	5a	0a	28bc
	25	20	10ab	0a	0a	0a	0a	0a	10a	0a	33c
	50	10	100c	45c	100c	100b	100d	100b	100b	90ef	93de
	50	20	100c	100d	100c	100b	100d	100b	98b	80d	98e
C_2N_2 (injected at 20 cm, no LDPE)	25	10	7ab	23b	100c	100b	0a	-	92b	90ef	96e
	25	20	3a	53c	100c	100b	10b	-	98b	87e	96e
	50	10	100c	100d	100c	100b	100d	-	100b	95f	90de
	50	20	100c	100d	100c	100b	100d	-	92b	87e	81d
C_2N_2 (injected at soil surface under LDPE)	25	10	87c	0a	47b	100b	90c	-	95b	45b	92de
	25	20	100c	97d	100c	100b	100d	-	100b	92ef	95e
	50	10	100c	43c	43b	100b	100d	-	95b	87e	95e
	50	20	100c	100d	100c	100b	100d	-	100b	88e	93de
Untreated	25	10	100c	100d	100c	100b	100d	100a	97b	88e	90de
	25	20	100c	100d	100c	100b	100d	100a	92b	97f	82de
	50	10	100c	100d	100c	100b	100d	100a	92b	92ef	93de
	50	20	100c	100d	100c	100b	100d	100a	97b	65c	98e

Table 2. Average concentrations of C_2N_2 as determined by Dräger tubes buried in soil at various depths and distances from the injection point.

Distance from injection point (cm)	Depth (cm)	Concentration of C_2N_2 (ppm)	
		Sealed with LDPE	Unsealed
25	10	25.5	13.7
25	20	7.5	15.2
50	10	1.4	0.0
50	20	0.4	0.0

Table 3. Numbers of nematodes retrieved from 200mL soil samples fumigated with methyl bromide or C_2N_2, or left untreated. Parasitic nematodes included *Tylenchus* and *Helicotylenchus* spp. Values followed by different letters in each column are significantly different, where $p \leq 0.05$.

Treatment	Parasitic Nematodes	Free-Living Nematodes	Total Nematodes
98% MB (injected at soil surface under LDPE)	0 a	1112 a	1112 a
C_2N_2 (injected at a depth of 20cm under LDPE)	55 a	2504 b	2559 b
Untreated	694 b	728 a	1480 ab

Table 4. Populations of microflora in soil determined 2 weeks after fumigation using the soil dilution method. Values are $\log_{10}$ colony forming units per gram of dry soil. Populations of bacteria in one of the C_2N_2 treatments were too high to determine from the dilutions used. Values followed by different letters in each column are significantly different, where $p \leq 0.05$.

Treatment	Total Fungi	Total Bacteria	Gram + Bacteria	Gram - Bacteria	Pseudo-monads	Actino-mycetes
98% MB (injected at soil surface under LDPE)	2.69 c	8.29 b	8.28 b	6.82 b	6.22 a	5.80 ab
C_2N_2 (injected at a depth of 20cm under LDPE)	3.79 b	>9.43	ND	>8.43	>8.43	5.95 ab
C_2N_2 (injected at 20 cm, no LDPE)	4.23 b	7.80 a	7.55 a	7.36 c	7.14 c	3.89 a
C_2N_2 (injected at soil surface under LDPE)	4.88 a	7.74 a	7.66 a	6.93 b	7.14 c	6.65 ab
Untreated	5.16 a	7.74 a	7.73 a	6.12 a	5.78 a	6.99 b

Table 5. Emergence of winter weeds in plots fumigated with various treatments in a strawberry runner trial at Toolangi, Victoria. *Poa annua* (winter grass, the monocot total) and *Spergula arvensis* (corn spurry) were the dominant weeds on the site (65 and 28% abundance, respectively). Values followed by different letters in each column are significantly different, where $p \leq 0.05$.

Treatment	Total Weeds (No./m²)	Total Monocot Weeds (No./m²)	Total Dicot Weeds (No./m²)	Weed Diversity (species/m²)	*Spergula arvensis* (No./m²)
Untreated	633 a	339 a	294 a	7.00 a	254 a
MB:Pic (70:30) 50 g/m²	3 d	0 d	3 b	1.33 b	2 b
C_2N_2 50 g/m²	8 cd	7 bcd	1 b	1.00 b	1 b
MI:Pic (30:70) 50 g/m²	3 d	2 cd	1 b	1.33 b	0 b
MI:Pic (30:70) 25 g/m²	100 bc	98 abc	2 b	1.67 b	1 b
Telone C-35 50 g/m²	169 ab	148 ab	21 b	3.00 b	3 b

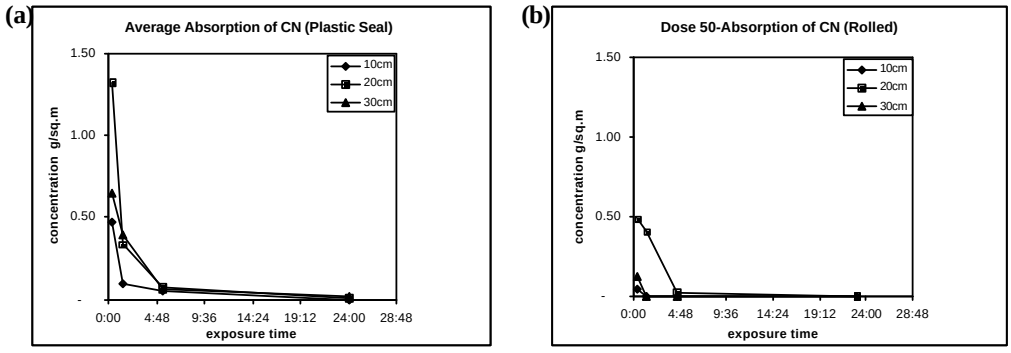

Figure 1. Concentrations of ethanedinitrile in soil at various depths (10, 20 and 30 cm) and times after fumigation, in a strawberry runner trial at Toolangi Victoria. Ethanedinitrile was applied by a prototype rig at a rate of 50 g/m² and sealed with (a) LDPE or (b) by a roller.