TECHNICAL NOTE

Efficacy of Ethanedinitrile against Pinewood Nematode in Woodchips under Field Conditions

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Abstract

As the ban on methyl bromide widens, the need for an effective phytosanitary fumigant alternative grows. Currently available alternatives, phosphine and sulfuryl fluoride, lack efficacy against the pinewood nematode. Ethanedinitrile is a highly efficacious fumigant with chemical properties similar to methyl bromide. Ethanedinitrile was tested against pinewood nematodes in a large-scale field setting with southern yellow pine wood chips at dose rates of 75 and 120 g/m^3 for 24 hours. All treatments resulted in complete control of pinewood nematodes whereas a nontreated control confirmed the presence of live nematodes. These preliminary results confirm the efficacy of ethanedinitrile against pinewood nematodes in pine wood chips under field conditions.

The United States is the largest industrial timber producer in the world, with almost 20 to 25 percent of the world's production prior to the 2009 recession and 15.9 billion ft^3 harvested in 2017 (Howard and Liang 2019). Overall, forest products comprise about 1.5 percent of the total US economy and contribute about 5 percent of the total manufacturing output in the country. Furthermore, the timber industry is one of the top three contributors to most southern state economies (Alvarez 2018). In 2017, 80 percent of US lumber production was from softwoods, which are primarily grown in the southern United States. After harvest, timber is often transformed into lumber, wood pulp, or wood chips. Approximately 87.5 percent of softwood timber is processed prior to export (Export Market Analysis for Selected Texas Commodities 2020).

Due to the minimally processed nature of forest products, the international trade of this commodity has great potential to lead to the introduction of nonnative invasive pest species (Westphal et al. 2008; Work et al. 2005). This risk has great potential to lead to serious negative impacts on the local biodiversity and economy, with economic costs estimated at hundreds of billions of dollars to economies globally (Lopian and Stephen 2013). Once introduced, invasive pest species often have few, if any, competitors and predators among highly susceptible hosts, resulting in unchecked reproduction and spread.

The introduction of invasive pests through international trade of wood-based products has risen as forest insect pests continue to spread (Brockerhoff et al. 2006). Among the major pests of concern is the pinewood nematode (PWN) (*Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle 1970), the causal agent of pine wilt disease. Pine wilt disease has severely damaged exotic pine (*Pinus* spp.) trees in Europe and Asia; however, North American pine trees are naturally resistant (Dwinell and Nickle 1989, Bonifácio et al. 2013). Damage caused by PWN in Japan, China, and Korea has been extensive, with more than 2 million hectares affected by this disease (Yun et al. 2012).

To mitigate the risk of invasion by PWN and other invasive pests, many countries have implemented strict phytosanitary measures for wood chips exported from North America. Treatment prior to export must be carried out using heat treatment or fumigation with methyl bromide (MB). The use of heat treatment is typically uneconomical

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for commodities such as wood chips; therefore, fumigation with MB is the primary treatment option. Due to its ozone-depleting properties, the use of MB is being phased out globally under the Montreal Protocol and is restricted in the United States under the Clean Air Act (UNEP 2006, Hagstrum et al. 2012). Currently, only quarantine and preshipment (QPS) uses of MB are authorized in the United States; however, to reduce global MB usage, the European Union does not accept product treated with MB. In addition, the phaseout of MB has led to an increase in input costs (Goodhue et al. 2005).

Few fumigant alternatives are currently available for the treatment of wood chips. Sulfuryl fluoride (SF) has been used as a fumigant for more than 50 years but is generally only effective at temperatures above typical fumigation conditions (>20°C) (Buckley 2010, Ren et al. 2011). When applied to blocks and wood chips, SF was ineffective against PWN at all dose rates during 24-hour treatment times but was effective when treated for 48 hours with high dose rates (Seabright et al. 2020). In addition, SF is a potent greenhouse gas with an estimated lifetime of 36 ± 11 years in the atmosphere (Mühle et al. 2009). The global warming potential of SF is reported to be similar to that of CFC-11 (trichlorofluoromethane), which is already banned under the Montreal Protocol (Papadimitriou et al. 2008).

Another alternative control measure, phosphine (PH_3), has also been used as an alternative fumigant. While PH_3 is generally an effective alternative, its use requires an extremely lengthy treatment period (>5 days) to control some insect egg and pupal life stages (Ren 2013). In addition, some insects have been found to be resistant to PH_3 (Opit et al. 2012). The volatile nature of gasses often requires lengthy treatment with PH_3 and additional fumigant to be applied to the commodity to maintain the appropriate concentrations necessary to effectively control pests. Furthermore, treatment of PWN-infested wood chips with PH_3 over 25 days was not 100 percent effective, which is required by the importing country to accept the commodity (Leesch et al. 1989).

Ethanedinitrile (EDN) is a broad-spectrum fumigant with efficacy against a wide range of insect pests, including PWN (Douda et al. 2015, Seabright et al. 2020, Uzunovic et al. 2022). Due to its highly volatile nature, EDN can penetrate both across and with the grain of timber quicker and further than MB (Ren et al. 2011, Park et al. 2014). EDN is also highly sorptive, with less than 1 percent of the applied concentration remaining following 10-, 16-, 20-, and 24-hour treatments (Hall et al. 2017, Najar-Rodriguez et al. 2020, Park et al. 2021, Uzunovic et al. 2022). These data indicate that EDN can be safely released into the atmosphere without the need for recapture technology, as it is not an ozone-depleting substance nor a greenhouse gas. EDN was found to be an economically viable fumigant with minimal impact to the environment by the Solar Impulse Foundation (Anonymous 2020).

While laboratory-scale studies have confirmed the efficacy of EDN against PWN in wood chips, its use in a commercial setting has not been reported. The objective of this study was to examine the efficacy of EDN in wood chips against PWN on a large, commercial scale with artificially and naturally infested wood chip samples.

Materials and Methods

Application site and set up

Two experiments were conducted in Savannah, Georgia, with Experiment 1 occurring at East Coast Terminal Company (136 Marine Terminal Drive, Savannah, Georgia 31404. GPS: 32°04′45.66″N, 81°03′49.00″W) on July 29, 2020. Air temperature and relative humidity in each container was 29.4 ± 0.2°C and 88.1 ± 1.0 percent, respectively. A second confirmatory experiment (Experiment 2) was conducted at Atlantic Marine Warehouse Company (2495 Tremont Road, Savannah, Georgia 31405. GPS: 32°03′52.2″N 81°08′01.4″W) on November 9, 2020. During Experiment 2, air temperature and relative humidity were 27.2 ± 0.9°C and 85.1 ± 0.6 percent, respectively.

Containers used for these experiments were 12.1-m ''high cube'' shipping containers with a holding capacity of 76 m^3. Typically, 26 metric tons of wood chips will fit into one container. The average loading factors for wood chip–loaded containers used during Experiments 1 and 2 were 67 and 57 percent, respectively. Per commercial fumigation standards, a tarp was placed inside each container, covering the floor and bottom half of each wall to assist in minimizing gas escape. Prior to wood chip loading, each container was weighed.

The application tube (Festo Corporation, 1377 Motor Parkway, Islandia, NY 11749) was placed in the top left corner of the back of the container approximately 45.5 cm inside the container and 45.5 cm below the container ceiling. Temperature and relative humidity were monitored in each container using a HOBO data logger (Onset Computer Corporation, 470 MacArthur Blvd., Bourne, MA 02532) placed in the top left corner of the back of the container, close to the application tube.

Treatments for Experiment 1 were a nontreated control, and 75 and 120 g/m^3 EDN. One container was used for each treatment. For Experiment 2, a dose rate of 75 g/m^3 was used to treat one container and confirm the efficacy of the lower dose rate against PWN in wood chips. No nontreated control was evaluated for Experiment 2.

Sample preparation and placement

Wood chips sourced from the southern yellow pine species, loblolly pine (*Pinus taeda*), were sized 0.45 cm by 4.5 cm, 2 percent maximum bark, and had a density of 170 kg/m^3. The moisture content of wood chips for these experiments was determined by weighing 1000 g of wood chips, drying in an oven for 16 hours, reweighing wood chips to calculate moisture loss, which was 51 percent on an ovendry basis. Prior to each experiment, samples were sent to the lab for confirmation of the presence of live PWN, which revealed an average of 1 PWN per gram of wood chip. In addition to wood chip samples, three water samples were included with different types of nematodes in each sample. Water samples included (1) a mixture of live PWN recovered from the naturally infested wood chips 1 day prior to EDN application; (2) a mixture of live soil nematodes including root-knot (*Meloidogyne incognita*), lesion (*Pratylenchus* spp.), lance (*Hoplolaimus galeatus*), spiral (*Helicotylenchus* spp.), and free-living nematodes (*Rhabditis* sp., *Oscheius* sp., *Cruznema tripartitum*) sourced from field crops; and (3) live lance nematodes sourced from turfgrass. These nematode samples were extracted in the lab prior to the application and placed in 50-mL falcon tubes with water. Immediately prior to application and after wood chip loading, each sample was uncapped and placed in each container near the doors during Experiment 2. These water samples allowed for immediate preliminary results of the trial without further nematode extractions. Nematodes in wood chips were extracted by soaking the wood chip in water for 24 hours, followed by counting live, mobile nematodes using a pie-pan method.

Six mesh bags filled with wood chip samples collected from the same wood chips to be treated (2 kg and 4 kg for Experiments 1 and 2, respectively) were placed in each container. Samples were placed in the center of each container during wood chip loading at 2.01-m and 0.43-m intervals, laterally and vertically, respectively, with the first sample placed on the container floor (Figs. 1a and 1b). This sampling scheme ensured that each sixth of the container was sampled. As the container was filled with wood chips, samples were placed accordingly until the container was deemed full. The filled container was then reweighed. During Experiment 1, the equipment used for wood chip loading broke beyond repair; therefore, only two wood chip samples were placed in the front of that container.

EDN application

Tubing used for application was placed inside the container and secured to the container wall 64 cm inside the door and below the ceiling. Following placement of all tubing, container doors were closed and further sealed with spray adhesive and duct tape. A cylinder of EDN (Lucebni Zavody Draslovka, Havlickova 605, 280 02 Kolin, Czech Republic) was placed on a scale and connected to the application tubing and a small cylinder of nitrogen (Airgas USA, LLC., 259 Radnor Chester Rd., Radnor, Pennsylvania 19087). EDN was deployed into each container, individually, with 5.7 and 9.2 kg deployed to achieve the 75 and 120 g/m^3 dose rates, respectively. The application tubing was taped at the end to prevent gas escape.

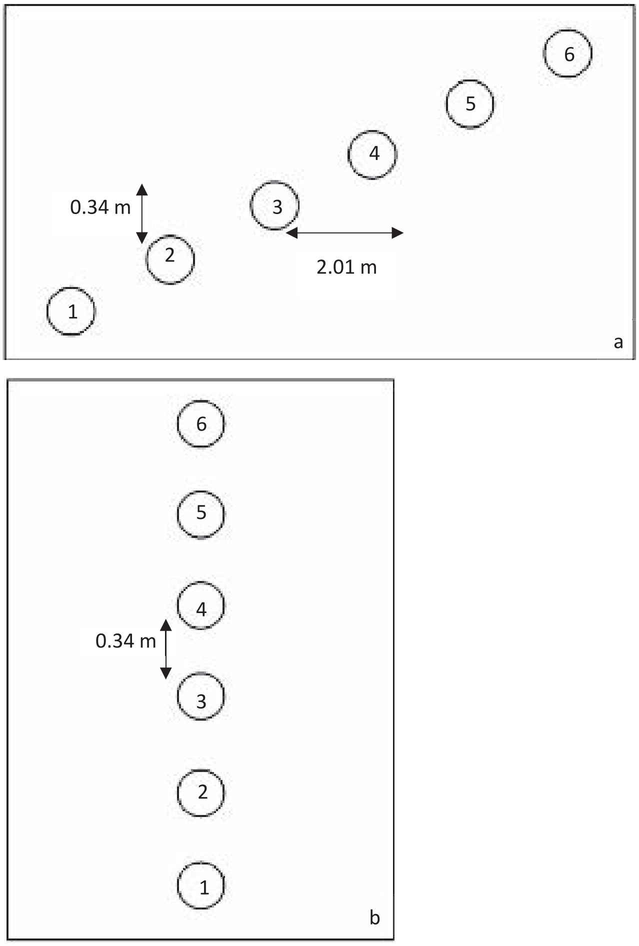

Figure 1.—Side (a) and front (b) view of PWN mesh bag sample placement.

Treatment time for both experiments was 24 hours. Immediately following 24 hours, door seals were broken, and doors opened to begin aeration for 60 minutes. Fan extractors were placed inside each container to aid in aeration. Once 60 minutes had elapsed, the buffer zone was deemed safe for reentry, and samples were collected to be sent to the lab for PWN mortality assessment.

Results and Discussion

Preapplication samples for both experiments confirmed the presence of live PWN infesting the wood chips (Tables 1 and 2). During Experiment 1, two of the preapplication samples were free of PWN; however, nonparasitic nematode species were present in both samples. Samples with PWN contained 10 to 12 PWN/g wood. During Experiment 2, preapplication samples averaged 1 PWN/g wood.

Postapplication wood chip samples in Experiment 1 revealed no live PWN or free-living nematodes at the low and high EDN application rates. In addition, all water samples in EDN-treated containers resulted in 100 percent mortality of PWN, lance nematode, and other soil

Table 1.—Incidence of PWN in southern pine chips and water samples before and after exposure to 75 or 120 g/m^{-3} EDN.

Pinewood nematode count: Experiment 1 (g PWN/g woodchips)						
	Postapplication (mesh bags)			Postapplication (water samples)		
Preapplication	Control	75 g/m^3	120 g/m^3	Control	75 g/m^3	120 g/m^3
0[a]	10	0	0	0	0	0
10	12	0	0	14	0	0
10	—	0	0	—	—	0[b]
12	—	0	0	—	—	—
0	—	0	0	—	—	—
10	—	0	0	—	—	—

[a] Two of the six preapplication samples did not have PWN, but free-living nematodes were present in these samples.

[b] Represents water sample containing a mixture of soil nematodes.

Table 2.—Incidence of PWN in southern pine chips before and after 24 hours of exposure to 75g/m^3 EDN.

Pinewood nematode count: Experiment 2 (PWN/g wood chips)	
Preapplication	Postapplication (mesh bags)
1	0
—	0
—	0
—	0
—	0
—	0

nematodes. Live nematodes were observed in all samples collected from the container that did not receive treatment. The same trend was revealed during Experiment 2, with the EDN-treated container resulting in complete control of PWN.

These results agree with those of Seabright et al. (2020), who reported 100 percent control of PWN in pine wood chips when EDN was applied at 30 g/m^3 for 24 hours. In the same study, EDN was found to effectively penetrate larger blocks of pine wood to control PWN. When applied at 40 g/m^3 for 24 hours, EDN provided 100 percent control of PWN in 75 by 75 by 150-mm wood blocks and 1.5-m-long logs by 19.1- to 39.1-cm diameter with the bark intact, while sulfuryl fluoride and phosphine only provided control in the smaller wood chips. Uzunovic et al. (2022) tested the efficacy of 50 and 100 g/m^3 EDN against PWN in 17-cm long pine logs and 2.5 by 3.8 by 0.64-cm pine wood blocks at 10°C and 20°C for 1-, 3-, 6-, 12-, 18-, and 24-hour treatment time. Their study found that EDN was 100 percent effective at all temperatures, treatment times, and rates for wood blocks. Both EDN rates provided complete control of PWN at 20°C when applied to pine logs for 12 and 24 hours and at 10°C when applied for 3, 12, 18, and 24 hours. EDN also provided control of PWN in 2-cm-thick log blocks with >13-cm diameter when applied at rates of 97g/m^3 and above (Chung et al. 2007). Previous research by Malkova et al. (2016) reported 100 percent control of PWN when treated with 50g/m^3 EDN for 6, 12, and 18 hours. Furthermore, EDN applied at 100, 120, and 150 g/m^3 at temperatures of 21 to 33, 6 to 12, and –1 to 3°C, respectively, resulted in complete mortality of PWN (Lee et al. 2017).

These findings and those of previous research indicate that EDN is a suitable alternative to MB for the control of PWN in pine wood chips of these specifications. As international timber trade continues, so too will the risk of invasive species both domestically and internationally. EDN provides effective control of invasive pests for QPS purposes.

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