Efficacy of ethanedinitrile (EDN) as a fumigant for export logs

A Report Prepared for Stakeholders in Methyl Bromide Reduction

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INTRODUCTION

Pine, *Pinus radiata* D. Don, logs exported from New Zealand must undergo phytosanitary treatment to ensure that no live insects are transported to the receiving market countries (MPI 2019a). Fumigation with methyl bromide (MB) is one of the primary phytosanitary treatments used, especially for logs exported to China and India (MPI 2019b).

In a 2010 ruling, New Zealand Environmental Authority (EPA, then Environmental Risk Management Authority, ERMA) requires the recapture or destruction of MB remaining in the headspace of completed fumigation after October 2020 (ERMA 2011). Stakeholders in Methyl Bromide Reduction, Inc. (STIMBR, www.stimbr.org.nz), embarked on a research program to find both recapture or destruction technologies and alternatives to MB for the phytosanitary treatment of export pine logs. The STIMBR research program was carried out through the auspices of the Crown Research Institutes for Forestry Research (Scion) and Plant & Food Research (PFR), University of Canterbury, and other entities with the scientific capabilities to develop alternatives to MB or recapture and destruction technologies and to develop tools to manage emissions to meet the October 2020 deadline.

To identify potential MB alternatives, STIMBR, Scion and PFR conducted a comprehensive literature review of all known quarantine treatment methods and technologies, including all available fumigants, heat and cold treatments using a wide range of technologies, irradiation, systems approach, and others. The review (Armstrong et al. 2014) found that fumigation with ethanedinitrile[1] (EDN) was the only treatment and fumigant option that had any potential for success as a phytosanitary treatment for the log export industry.

Pursuant to the review by Armstrong et al. (2014), STIMBR funded research to provide baseline data on the efficacy of EDN against the life stages of specific forest insects of phytosanitary interest, including burnt pine longhorn beetle, *Arhopalus ferus* (Mulsant) (Coleoptera: Cerambycidae); golden-haired bark beetle, *Hylurgus ligniperda* (F.) (Coleoptera: Scolytidae); and black pine bark beetle, *Hylastes ater* (Paykull) (Coleoptera: Scolytidae). The efficacy data (Najar-Rodriquez et al. 2015, 2018) showed that *H. ater* pupae were the most EDN-tolerant species and life stage. Najar-Rodriquez et al. (2018) recommended further testing to confirm the efficacy of EDN against *H. ater* pupae on a commercial scale (referred to as "confirmatory tests").

[1] Ethanedinitrile is an internationally recognized fumigant owned and produced by Lučební Závody Draslovka a.s. Kolín, Czech Republic, herein referred to as Draslovka.

STIMBR and PFR initiated the EDN confirmatory tests in February 2019 and completed six confirmatory tests by end of May 2019. Control (untreated) log stacks were also used on each occasion. Reported herein are the results of the EDN confirmatory tests on the survival of *H. ater* pupae.

MATERIALS AND METHODS

Terminology:

Replication vs. fumigation

We use the term, replication, for convenience to denote the repetition of a fumigation schedule, e.g., 120 g/m^3 for 24 h at 15°C or above. Fumigations are not true replications because many of the factors involved, e.g., small variations in log lengths, variations in log diameters, amount of bark remaining on the logs, total log weights and volumes, or the interstitial spaces under the tarpaulin, cannot be precisely repeated like the factors and measurements repeated in replicated laboratory experiments.

Simulation of commercial conditions

Our confirmatory tests simulated to every extent possible the log stack fumigations carried out under commercial conditions at the New Zealand ports from which logs are exported. Adding culverts to house the infested logs to the log stacks that we fumigated is not a commercial condition was necessary to protect the insect-infested logs from being crushed during the fumigation in order to obtain the efficacy data for EDN fumigation.

Fumigation site and site preparation

All confirmatory tests were carried out with freshly harvested pine logs that were placed on a private asphalt road (38°15'51.81°S 175°53'9.99°E) in a recently harvested commercial plantation owned by Oji Fibre Solutions adjacent to their Kinleith Pulp and Paper Mill, Tokoroa.

The asphalt road was approximately 9 m wide and had a pronounced crown at the center to facilitate water runoff. Because the crown would act as a fulcrum, the logs were placed on the east down-slope side of the road with about 0.5-m of the log stack bottom sitting on the soil verge adjoining the road. To prevent the fumigant from escaping through the soil during fumigation, a double-layer sheet of 285-g/m^2 woven polyethylene fumigation tarpaulin was placed on the ground to ensure that there was a substantial seal beneath the log stacks (commercial fumigations of logs at ports are done on solid cement or asphalt surfaces). The tarpaulin was supplied by Genera (Genera Biosecurity, Tauranga), a New Zealand-based international company that provides phytosanitary treatments for export logs and other commodities. The polyethylene tarpaulin was identical to the tarpaulins used to cover log stacks for MB fumigation.

Two 6.1-m sea containers were placed on site to house gas chromatography (GC) units and all equipment required to carry out the confirmatory tests and provide protection against inclement weather. To meet safety standards, both ends of the road were cordoned off in accordance with EPA requirements during fumigation and venting. No untrained personnel were allowed into the confirmatory test area while the tests were being conducted.

Logs and log stacks

The logs used in all confirmatory tests were A-grade commercial export logs that were grown in the various forests in the South Waikato region and were approximately 28 years in age. The logs were drawn from the supply chain by Hancock Forest Management, Tauranga. All logs were 3.75 m in length and varied in diameter within the specifications for export logs. Logs were trucked to the confirmatory test site by contracted cartage companies and off-loaded using equipment supplied by Alan Forbes Transport Limited, Tokoroa. Two or three standard commercial-sized log stacks (depending on the confirmatory test fumigation concentrations and times) were built before the start of each test using a commercial log loader. Therefore, there was one control log stack and one or two treatment log stacks in each confirmatory test (Diag. 1).

As the log stacks were built, sections of ribbed water culverts (442-mm-diameter by 3.0-m-long PE water culvert, type 0375-Farmboss, Hynds Pipe Systems Ltd., Auckland) were placed in parallel with the logs to provide space for laboratory-infested logs to be inserted at selected locations within the stacks (Diag. 1). The walls of the culverts were solid, and the only openings were the two ends[2].

During the process of building the large log stacks (400 tonnes each) for the control or treatment(s), two culverts were added to the top row of the first quarter of the stack, one culvert was added to the center of the second quarter of the stack, and two culverts were added to the third quarter of the stack forming a decussate cross (Diag. 1). In the case of the smaller stacks (100 tonnes), three culverts were placed in each stack to form an inverted 'v' (Diag. 1) as the stacks were built.

When the building of the log stacks with the culverts in place was completed, a selected number of infested logs and two gas-sampling lines were placed into the culverts. The end of one gas-sampling line was attached ≈10.0 cm inside the culvert opening with duct tape and another gas-sampling line was inserted into the middle (1.5 m) of the culvert and held in place with duct tape. After the infested logs and gas sampling tubes were placed in the log stacks, the control and treatment log stacks were covered with commercial tarp using the same equipment used at the ports for log fumigations with MB. All log stack covering with tarpaulins and removal of tarpaulins was carried out by Genera.

Insect handling and log infestation

All laboratory-infestations of logs to obtain *H. ater* pupae were done at PFR-Palmerston North using freshly cut pine logs (25.26 ± 0.10 cm length; 23.15 ± 0.30 cm diameter; 140-170% moisture content) sourced from the Whakarewarewa Forest, Rotorua (38°15'64" 176°26'94"). The surface of each log was cleaned with a brush and the log ends were coated with an industrial grade paraffin wax (Ceracell Beekeeping Supplies Ltd., Auckland) to reduce moisture loss during infestation. The logs were then infested with adult *H. ater* adults (8 to 18 weeks post-eclosion) as described by Najar-Rodriguez et al. (2018). The adult insects originated from laboratory-reared colonies that were kept at 20 ± 1°C, 50-60% RH and total darkness (Clare and George 2016) at the PFR-Mt. Albert, Auckland.

[2] A pilot-scale test with a MB fumigation of log stacks at the Port of Tauranga in 2018 (A. Adlam, Genera, pers. comm.) was done with both perforated (by drilling several hundred 25-mm holes into the walls of each culvert) and unperforated culverts to determine whether the culverts needed to be perforated to facilitate MB movement into the culvert. The results from the pilot-scale test showed that the fumigant concentrations in the perforated and unperforated culverts remained identical over time to the MB concentration in the interstitial spaces within the log stack. Because the results showed that the laboratory-infested logs in the culverts with solid walls received the same treatment as the logs in the stack, perforating the culvert walls was unnecessary. Additionally, the perforated culverts were found to be a safety hazard because they lost structural integrity and were subject to crushing by the surrounding logs. The loss of structural rigidity posed both a safety risk and a cause for potential to damage the infested logs in them. Hence, only culverts with solid walls were used in the EDN efficacy confirmatory tests.

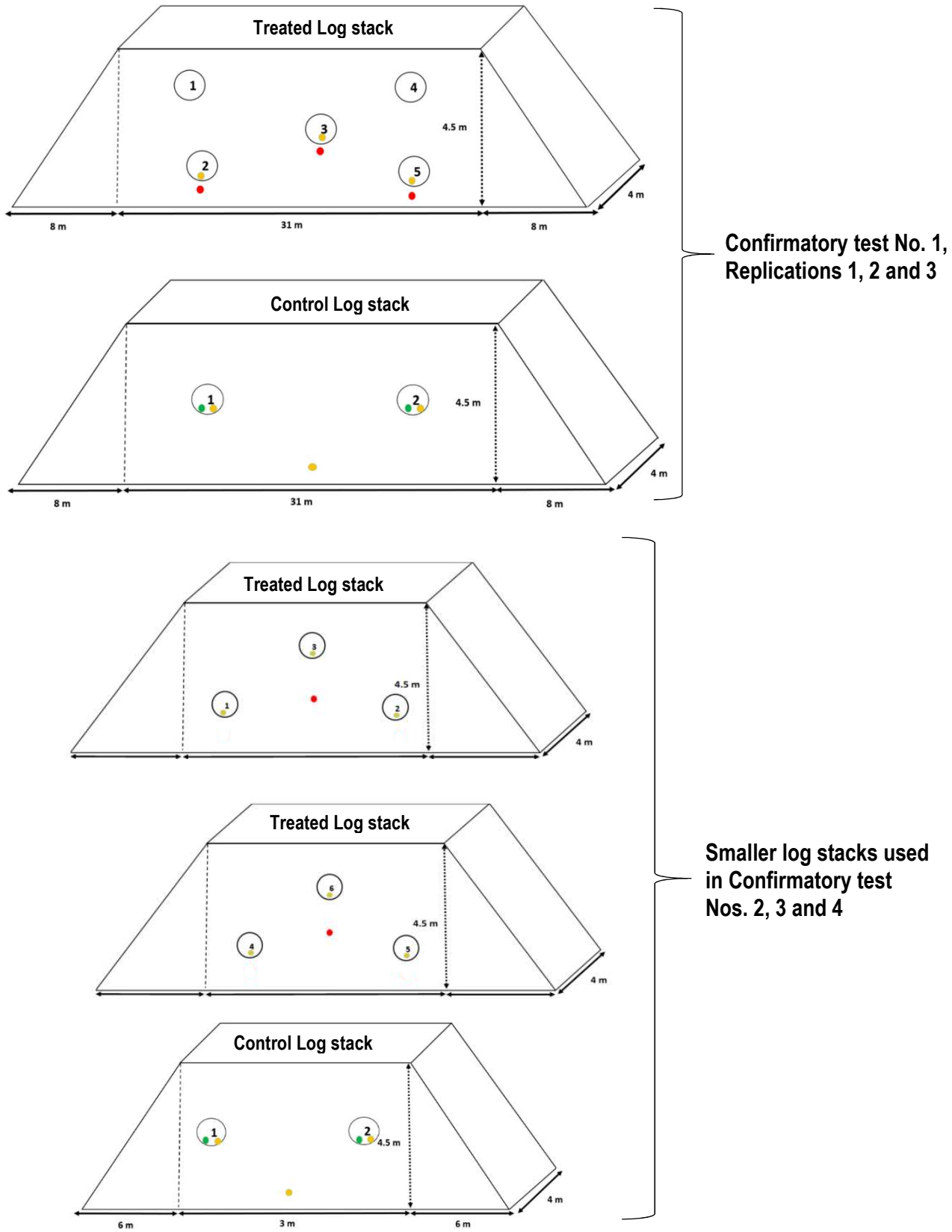

Diagram 1. Representative configuration of treated and control log stacks used in EDN confirmatory test No. 1, replications 1-3, and in confirmatory tests Nos. 2, 3 and 4. Circles show culvert placement, red dots indicate points of EDN injection, green and yellow dots indicate the placement of the Temprecord and/or Squirrel data loggers to monitor for temperature and RH.

For infestation, the logs were placed individually in black plastic rubbish bags with moist paper at the bottom to maintain humidity within the bag. Each log was infested with 20 sexed pairs of *H. ater* adults by placing them on the upper end surface of each log. The bags were then sealed by tying. The infested logs were kept in temperature-controlled rooms at 20 ± 1ºC, 62 ± 3.38% RH and total darkness for 8-10 weeks to allow *H. ater* pupae to develop. To avoid excessive accumulation of condensation and to limit fungal growth, the logs were aerated every other week by opening the bags for an hour. The logs were monitored every other week before the fumigations were planned by removing the bark from one or two logs to ascertain the life cycle progress until observations determined that the pupal stage was the predominant life stage in the logs. At that point, the logs were held at 11°C, if it was necessary to delay further development before the logs were used for confirmatory tests.

The infested logs were moved from the temperature-controlled rooms 72 h before fumigation to ambient temperature (Table 1) to equilibrate the log temperatures and acclimate the insects to the temperature at which the logs would be fumigated.

Table 1. Temperatures and RH at which infested logs[a] were held for 72-h to acclimatize the insects before they were used in confirmatory test fumigations.

Dates of acclimation[b]	Avg temperature (°C) [SE]	Avg. RH [SE]
01 – 03 February	21.07 [0.54]	68.30 [0.48]
16 – 18 February	19.60 [0.38]	64.59 [0.38]
02 – 04 March	20.01 [0.34]	54.16 [0.63]
16 – 18 March	21.70 [0.43]	67.87 [0.43]
31 March – 02 April	17.15 [0.64]	79.19 [1.68]
17 – 20 May	14.95 [0.60]	50.52 [1.66]

[a] Infested logs were used in both the control and treated log stacks.
[b] All acclimation periods (and subsequent fumigations) were in 2019.

After the logs completed the acclimatization period, they were transported from PFR-Palmerston North to the Tokoroa confirmatory test site in their respective plastic bags 24 h before the fumigation was initiated. On the day they were fumigated, the logs were removed from the bags, the wax at the ends of each log was removed using a handheld electric planer (Bosch PHO2000, Auckland, New Zealand), and any frass or dirt present on the surface of the logs was removed with a paint brush.

Immediately after the infested logs were cleaned and dewaxed, they were moved to the log stacks using a hand cart and placed into the culverts. Five to six infested logs were placed in each of two culverts in the control log stacks for all confirmatory tests. The number of infested logs in the treated log stacks varied with the confirmatory test replication and the stack sizes and weights. Confirmatory test No. 1, replications 1, 2 and 3 had two culverts with four or five infested logs per culvert in the control stack and five culverts with ten infested logs per culvert in the treated stack. All remaining confirmatory tests had two culverts in the control stack with four to five infested logs per culvert and three culverts in the treated stacks with five infested logs per culvert (Diag. 1). The infested logs were pushed end-to-end into the culvert using an iron bar. When the insertion of infested logs into the culvert was completed, the culvert ends were fitted with round, fine-mesh-cloth, pop-up food covers (Avanti Homewares, Sydney, Australia) that fit securely into the culvert ends and secured in place with duct tape. The fine mesh covers avoided any contamination of the infested logs by preventing access to individuals from local bark beetle populations (both *H. ligniperda* and *H. ater*) from flying into the

culverts in the control and treated log stacks while allowing the free movement of EDN into the culverts in the treated stacks.

In the treatment log stack(s) for all confirmatory tests, gas-sampling lines (6-mm diameter tubing, Ledathen Formula F45, Compressed Air Controls, Wellington) were extended from a custom-made (PFR Ruakura) pump-manifold multiplex gas-sampling system next to the GC located in the sea container to the west side of the treatment log stack. For each culvert in the treatment log stack, the end of one sampling line was secured to the edge of the culvert with duct tape and the end of a second sampling line was inserted to the middle of the culvert and secured with duct tape. (Diag. 1).The end of one gas-sampling line was secured to the space between logs at a selected point and used as an exhaust line for the gas-sampling system to return all EDN not used for GC analysis to the fumigated log stack (Diag.1).

Covering the log stacks

The treated and control log stacks were covered using standard commercial fumigation methods. Stack covers were standard industrial tarpaulins used for fumigations (e.g., MB) of logs for export. The tarpaulin arrived on site in a roll that was wrapped around a hollow steel pole designed to mount in a cover-rolling machine (CRM) and on a forklift spike. A forklift with a spike secured to the forks was engaged to the tarpaulin roll by inserting the spike through the hollow steel pole at the center of the roll. The roll was then elevated to an appropriate height to allow the tarp to be unrolled. The outer tarp end was attached to a steel cable that was placed over the top center length of the log stack and the opposite end of the cable was attached to a PTO winch on a second forklift. The stacks were covered by winching the cable to unroll the tarpaulin as it moved up and along the length of the stack. Genera personnel on each side of the stack held the edges of the tarp to guide its movement, to prevent the tarp from catching on log ends, and to ensure the tarpaulin edges were spread to the sides of the stack to provide adequate cover for fumigation. The process generally took 10-15 min for each stack to be covered.

After the tarpaulin was in place, the edges of the tarpaulin on all sides of the treated stack were held in place with continuous water-filled tubes called “water snakes”. The water snakes were wound twice around the tarpaulin edges at the base of the log stack to ensure that a proper seal was formed that would prevent the fumigant from escaping from the treated stack. Once the water snakes were in place, a visual inspection of the tarpaulin was done to ensure that no holes were created when the tarpaulin was being winched over the logs.

The control log stacks were covered identically to the treated stacks.

Fumigation with EDN

All fumigation of the log stacks under tarpaulin were conducted by Genera personnel and a Draslovka field representative who calculated the kilogram amount of EDN to inject into each treated stack. The amount of EDN injected into the stack was calculated by multiplying the stack weight (in tonnes) by a factor of 1.7 m^3. This is the standard method used for calculating the amount of MB to be injected into the log stack for the commercial phytosanitary fumigation of export logs (B. Edwards, Genera, pers. comm.). The load factors for the fumigated log stacks was approximately 58-59%. Load factors were calculated by multiplying the log weight (tonnes) by the factor of 1.7 m^3, then dividing weight of the logs (tonnes) by the product and multiplying the dividend by 100. For example, the treated log weight in Confirmatory test No. 1, replication 1 (Table 2) is 397.3 tonnes X 1.7 m^3 = 675.4 $\Rightarrow$ 397.3 ÷ 675.4 X 100 = 58.8%.

The EDN used for the fumigations was from standard steel (47 kg empty weight) EDN cylinders containing 50 kg (initial weight) EDN placed on an industrial scale to measure the combined cylinder and EDN weight. Before the treatment log stack was covered, a delivery (injection) tube (6.25 mm diameter in the first fumigation and 12.0 mm diameter in all subsequent fumigations) was connected to the regulator valve on the cylinder. For large log stacks, three lines were run to the stack to inject the fumigant whereas only one line was used to inject the fumigant into the small log stacks. After the stacks were covered, fumigations were initiated by opening the regulator valve and releasing EDN into the covered stack until the scale indicated the desired loss (kg) of weight. The EDN was injected into the log stack as a liquid and immediately vaporized.

Venting

When the fumigation was completed, the tarpaulins were removed from the control and treated stacks by Genera personnel. The tarpaulin removal allows residual fumigant to be released and is, therefore, referred to as "venting". Venting is a standard industry practice used internationally for commercial fumigations. Because some EDN would be released during venting, the control log stacks were always uncovered first.

The process of tarpaulin removal, or venting, was initiated by cutting the water snakes to release the water and then removing the empty water snakes. Genera personnel then pulled the edges of the tarp at the base of the stack outwards farther from the stack (a process called "fluffing") to facilitate the removal of the tarpaulin. The tarpaulin was removed by attaching one end of the tarpaulin to a steel pole mounted in the CRM that was mounted on a forklift. The tarpaulin was winched off the log stack by the CRM and re-rolled onto the steel pole.

The tarpaulin was removed in one-fourth increments at a time to avoid a sudden release of EDN into the atmosphere. Removal of the tarpaulin by quarters over time is a standard fumigation practice used for commercial fumigations of export logs with MB. Tarpaulin removal in the EDN confirmatory tests generally was accomplished over a period of 15-30 min.

Monitoring of log stacks during fumigations for temperature and RH

The temperature and RH data for each fumigation was recorded both from the inside (i.e., under the tarpaulin) and outside the control log stacks using a data logger (Temprecord International Limited ©, Auckland, New Zealand) per stack. The data loggers were placed on the edge of the culverts in the control stack as soon as the infested logs were placed in the culverts. The temperature and RH outside the stack was also monitored for each fumigation by placing a data logger close to the NIWA weather station that was positioned on the 20-m from one of the treated stack(s) (Diag. 1).

The temperature data from inside the treated stacks (as well as inside the control stacks) was recorded using remote squirrel meter/loggers (Grant 1250 series, CAMBEEP, Cambridge Building Energy & Environmental Portal, UK). The squirrel data loggers were placed inside the stacks (after the infested logs were placed in the culverts and before the stack was covered) with sensors lines in each culvert (i.e., three lines in three culverts). RH data was recorded with the same squirrel data loggers for all fumigations except for the replication 1 in Confirmatory Test No. 1 (Diag. 1).

Monitoring EDN concentrations in fumigated log stack

EDN concentrations under the tarpaulin of the fumigated log stacks were measured inside the culvert at set intervals by PFR scientists using a portable GC (SRI Instruments, Torrance, California, USA) that was fitted with a thermal conductivity detector (TCD) and a packed column (8600-PK2A 6' x 1/8" S.S. Molecular Sieve 5A). The oven and detector were held at 150°C and 300°C, respectively.

A 1-ml sample was taken from the gas port of the selected line in the multiplex gas-sampling system and injected onto the GC. Between each sample, the valve of the gas-sampling system was changed to the next position and the pump was run for 1 min before the next sample was collected to ensure that a representative sample was drawn from each line. The 1-min interval between sampling was determined previously by Hall et al. (2016) to be an adequate time to purge the lines of EDN between gas samples. EDN concentration was determined based on a five-point calibration curve that was developed for each confirmatory test replication using attenuated dilutions of EDN.

Mortality assessments

After the fumigations were completed, the infested logs in the control stack were removed first. The infested logs from the fumigated log stacks were removed after the stacks were adequately vented for worker safety. The control and treated infested logs were sealed in "mutton" cloth bags (Bunnings Warehouse, Palmerston North) and transported in separate vehicles back to the PFR laboratory at Palmerston North. Control and treated logs were held separately during all mortality observation processes (e.g., bark removal, insect collection, counting of insects), and the mortality observation processes were done by different teams to avoid any cross-contamination. All mortality observation processes were done with maximum care to prevent accidental death (e.g., crushing) of the insects.

While the control logs were held for 24 h in a separate room to prevent any potential for cross-contamination, the treated logs were vented for 24 h to ensure worker safety. After 24 h, bark removal began simultaneously in different rooms for both control and treated logs. Bark was removed using a screwdriver.

Although the EDN fumigations were targeted to kill *H. ater* pupae, all *H. ater* life stages that were found were collected and assessed for mortality. All the life stages were gently brushed off the bark onto a clean plastic cafeteria-style tray. Debris that remained on the log after the bark removal was also brushed off onto the tray. All the frass and debris from each log was also collected and placed in a self-sealing bag for further inspection, if needed. Bags containing the insects were individually emptied onto a clean tray and the insects were segregated by life stage and assessed as either live or dead based on whether they moved in response to tactile stimulation with a small paint brush (1.5 mm goat hair). After this first mortality observation, all collected insects (larvae and pupae) were held at 20 $\pm$ 2°C in darkness and reassessed for mortality at 48 h and 96 h after fumigation. Pupae were reassessed 10 d after fumigation to observe for adult emergence.

Confirmatory tests

A series of nine confirmatory tests were conducted from February through May 2019 to demonstrate the efficacy of EDN on a commercial scale using pine logs and commercial fumigation practices currently used for phytosanitary fumigations of export logs with MB at ports throughout New Zealand. Three fumigations with the most severe treatment (120 g/m^3 for 24 h) based on laboratory studies (Najar-Rodriguez et al. unpublished were done first. Six additional fumigations were done in which the

EDN concentration and/or fumigation time was decreased to determine whether EDN would remain efficacious under commercial conditions when using less severe or shorter fumigation schedules.

Confirmatory test No. 1 – replications 1, 2 and 3 of fumigations using 120 g/m^3 EDN for 24 h

The first three confirmatory fumigations with EDN were done using 120g/m^3 EDN for 24 h. The concentration and fumigation time were derived from the LC_{99} and $LC_{99.9}$ estimates of Najar-Rodriguez et al. (unpublished) and considered to be the most conservative (maximum possible) amount of fumigant and fumigation time needed to provide quarantine security against *H. ater* pupae. Additional confirmatory tests using lower EDN concentrations and/or fumigation times would follow if the three replications using 120 g/m^3 EDN for 24 h proved to be efficacious (i.e., resulted in complete mortality).

The log stack weights and volumes, the amounts of EDN applied to the treated stacks and the application and venting times for the three replications at 120 g/m^3 for 24 h are given in Table 2.

Table 2. Log stack weights and volumes, amounts of EDN applied, and application and venting times for confirmatory test No. 1 – three replications[a] of fumigations using 120 g/m^3 EDN for 24 h.

Replication No.	Log stack	Weight of logs (tons)	Log stack volume (m^3)[c]	Amount EDN applied (kg)	Application times[a] (start to finish) and total duration (min)	Venting times[a] and duration (min)
1	Treated	397.3	675	84	1330 to 1400 (30)	1405 to 1427 (22)
	Control	394.6[b]	671	0	—	—
2	Treated	396.3	674	82	1115 to 1140 (25)	1149 to 1156 (7)
	Control	394.6[b]	671	0	—	—
3	Treated	399.7	680	83	0958 to 1020 (22)	1025 to 1046 (21)
	Control	394.6[b]	671	0	—	—

[a] Replications 1, 2 and 3 were done on 04-05/02/19, 19-20/02/19 and 05-06/03/19, respectively. Fumigations were initiated on the first day and venting took place after 24 h on the second day. All times are given in 24-h notation.

[b] The same log stack was used for the control in all three confirmatory test replications. The treated stacks were built from new logs for each fumigation.

[c] Log stack volume was calculated based on the weight (tonnes) of the logs x 1.7 m^3 (the industry standard for calculating stack volume for methyl bromide fumigations.

Confirmatory test No. 2 – fumigations using 120 g/m^3 EDN for either 16 h or 20 h

After Confirmatory Test No. 1 showed that 120 g/m^3 EDN for 24 h was efficacious against *H. ater* pupae, two fumigations were done using either 120 g/m^3 for 16 h or 120 g/m^3 for 20 h to determine if reducing the fumigation time for the 120 g/m^3 concentration would result in survival of *H. ater* pupae. Further confirmatory tests would be indicated using lower concentrations if no survival occurred in

either the 16- or 20-h fumigations using 120 g/m^3. The log stack weights and volumes, the amounts of EDN applied to the treated stacks and the EDN application and tarpaulin removal times for the two fumigations at 120 g/m^3 for either 16 h or 20 h are given in Table 3.

Table 3. Log stack weights and volumes, amounts of EDN applied, and application and venting times for confirmatory tests[a] using either 120 g/m^3 EDN for 16 h or 120 g/m^3 EDN for 20 h.

Treatment time (h)	Log stack	Weight of logs (tons)	Log stack volume (m^3)[c]	Amount EDN applied (kg)	Application times[a] (start to finish) and total duration (min)	Venting times[a] and duration (min)
16	Treated	105.5	179	22	1748 to 1800 (12)	1017 to 1029 (12)
	Control	175.0[b]	175	0	-[d]-	-[d]-
20	Treated	105.6	180	22	1715 to 1730 (15)	1333 to 1348 (15)
	Control	175.0[b]	175	0	-[d]-	-[d]-

[a] Fumigations were done on 19-20/03/19. Fumigations were initiated on the first day and venting took place after 16 or 20 h on the second day. All times are given in 24-h notation.

[b] The same log stack was used as the control for both fumigations. The treated logs stacks were built from new logs for each fumigation.

[c] Log stack volume was calculated based on stack weight (tonnes) x 1.7 m^3 (the industry standard for calculating stack volume for methyl bromide fumigations).

[d] The control log stack was uncovered, and the infested logs were removed during the interim when the stack that was fumigated for 16 h was vented and before the stack that was fumigated for 20 h was vented. The stack fumigated for 20 h was furthest from the control stack (about 100 m distance and upwind).

Confirmatory test No. 3 – fumigations using either 80 g/m^3 or 100 g/m^3 EDN for 20 h

Two fumigations were done using either 80 g/m^3 or 100 g/m^3 for 20 h to determine if the amount of EDN used to treat the log stacks could be reduced from 120 g/m^3 to 80 g/m^3 or 100 g/m^3 while at the same time reducing the fumigation time from 24 h to 20 h without survival of *H. ater* pupae. Two replications of either 80 g/m^3 or 100 g/m^3 would be done if the both the 80- and 100-g/m^3 fumigations for 20 h provided complete control of *H. ater* pupae. The log stack weights and volumes, the amounts of EDN applied to the treated stacks and the EDN application and tarpaulin removal times for the two fumigations at either 80 g/m^3 or 100 g/m^3 for 20 h are given in Table 4.

Table 4. Log stack weights and volumes, amounts of EDN applied, and application and venting times for confirmatory tests[a] using either 80 g/m^3 EDN for 20 h or 100 g/m^3 EDN for 20 h.

Treatment time (h)	Log stack	Weight of logs (tons)	Log stack volume (m^3)[c]	Amount EDN applied (kg)	Application times[a] (start to finish) and total duration (min)	Venting times[a] and duration (min)
20	Treated	98.2	167	13.5	1201 to 1212 (11)	0825 to 0836 (11)
	Control	175.0[b]	175	0	-[d]-	-[d]-
20	Treated	98.4	167	17.0	1407 to 1420 (13)	1333 to 1348 (13)
	Control	175.0[b]	175	0	-[d]-	-[d]-

[a] Fumigations were done on 03-04/04/19. Fumigations were initiated on the first day and venting took place on the second day. All times are given in 24-h notation.

[b] The control log stack used for the confirmatory test No. 2 fumigations on 19-20/03/19 was retained and used again for the confirmatory test No. 3 fumigations.

[c] Log stack volume was calculated based on log weight (tonnes) x 1.7 m^3 (the industry standard for calculating stack volume for methyl bromide fumigations).

[d] The control log stack was uncovered, and the infested logs were removed during the interim when the stack that was fumigated with 100 g/m^3 was vented and before the stack that was fumigated with 80 g/m^3 was vented. The stack fumigated with 100 g/m^3 was furthest from the control stack (about 100 m and upwind).

Note: Log stacks were wet following rains, with only autumnal temperatures and no significant drying winds.

Confirmatory test No. 4 – replications 2 and 3 for fumigations using 100 g/m^3 EDN for 20 h

Two additional fumigations (replications 2 and 3) were done using 100 g/m^3 for 20 h to provide three replication data sets for EDN fumigations of log stacks at 100 g/m^3 for 20 h. The log stack weights and volumes, the amounts of EDN applied to the treated stacks and the EDN application and tarpaulin removal times for the two fumigations at 100 g/m^3 for 20 h are given in Table 5. (Note: at the time this report was provided to EPA on 10 May 2019, replications 2 and 3 of fumigations using 100 g/m^3 for 20 h were scheduled for 21-22 May 2019).

Table 5. Log stack weights and volumes, amounts of EDN applied, and application and venting times for confirmatory tests[a] using 100 g/m^3 EDN for 20 h.

Replication No.	Log stack	Weight of logs (tons)	Log stack volume (m^3)[c]	Amount EDN applied (kg)	Application times[a] (start to finish) and total duration (min)	Venting times[a] and duration (min)
2	Treated	96.8	164.6	16.6	1731 to 1734 (3)	1406 to 1413 (8)
	Control	103.3[b]	175.6	0	-[d]-	-[d]-
3	Treated	97.2	165.2	16.6	1532 to 1537 (5)	1210 to 1220 (10)
	Control	103.3[b]	175.6	0	-[d]-	-[d]-

[a] Fumigations were done on 21-22/05/19. Fumigations were initiated on the first day and venting took place on the second day. All times are given in 24-h notation.

[b] The control log stack used in confirmatory test No. 4 was the control for both replications 2 and 3 of fumigations using 100 g/m^3 EDN for 20 h.

[c] Log stack volume was calculated based on log weight (tonnes) x 1.7 m^3 (the industry standard for calculating stack volume for methyl bromide fumigations).

[d] The control log stack, which was upwind from the two treated log stacks, was uncovered and the infested logs were removed during the interim between the removal of the tarpaulin from the replication 2 and replication 3 log stacks.

Note: Log stacks were wet following rains and fog, with only autumnal temperatures and no significant drying winds.

Results

Concentrations of EDN in the log stacks during confirmatory test fumigations

The EDN concentrations in the fumigated log stack for the duration of the fumigation period for all confirmatory tests are provided in Tables 6 and 7 and Fig. 1 for confirmatory test No. 1; Tables 8, 9 and 10 and Fig. 2 for confirmatory test No. 2; Tables 11, 12 and 13 and Fig. 3 for confirmatory test No. 3; and Tables 14 and 15 and Fig. 5 for confirmatory test No. 4.

Confirmatory test No. 1 – replications 1, 2 and 3 for fumigations with 120 g/m³ EDN for 24 h

Table 6. EDN concentrations under the tarpaulin during confirmatory test No. 1 for three replications[a] of fumigations using 120 g/m³ EDN for 24 h.

Time after EDN application (h)	EDN concentrations under tarpaulin					
	Replication 1		Replication 2		Replication 3	
	ppm	g/m³	ppm	g/m³	ppm	g/m³
0	79,892.6	172.9	62,116.7	134.5	55,716.1	120.6
1	68,370.4	148.0	49,619.6	107.4	41,016.1	88.8
2	48,793.0	105.6	42,804.5	92.7	29,775.0	64.5
3	41,627.0	90.1	35,012.9	75.8	21,572.2	46.7
4	26,790.9	58.0	27,281.5	59.1	16,069.4	34.9
6	14,283.0	30.9	16,564.9	35.9	10,034.4	21.7
8	8,097.4	17.5	10,696.1	23.2	5,443.3	11.8
10	2,529.7	5.5	4,122.7	8.9	3,542.7	7.7
12	721.7	1.6	3,706.8	8.0	2,346.9	5.1
16	401.2	0.9	1,094.4	2.4	1,262.3	2.7
20	281.9	0.6	765.9	1.7	722.2	1.6
22	253.0	0.6	592.2	1.3	648.3	1.4
24	165.9	0.4	487.5	1.1	506.7	1.1

[a] Replications 1, 2 and 3 were conducted on 04-05/02/19, 19-20/02/19 and 05-06/03/19, respectively.

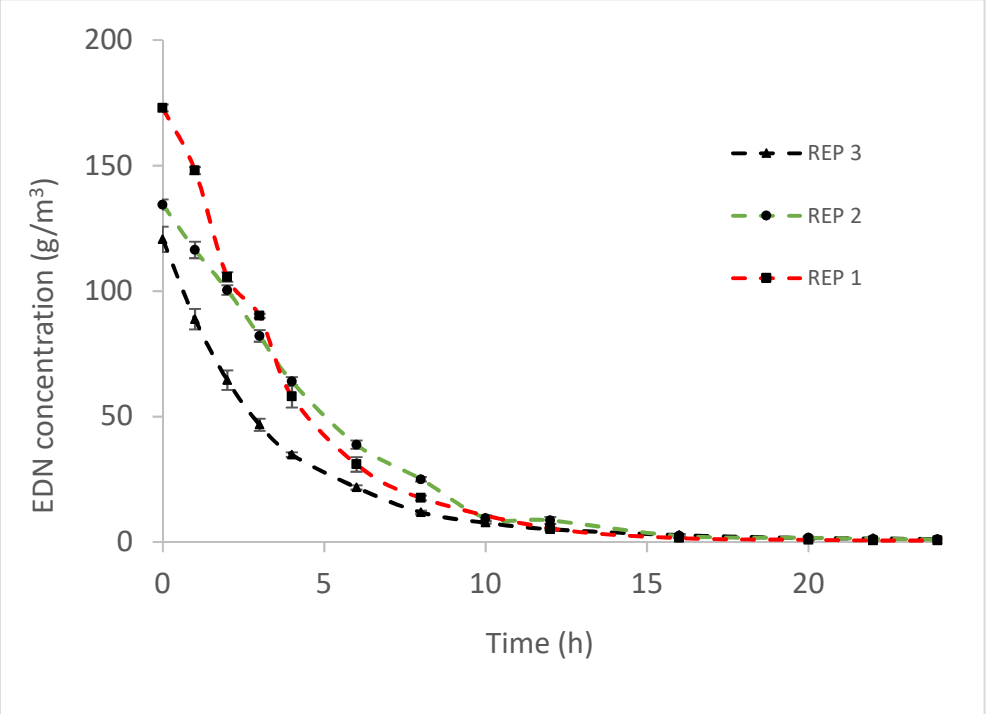

Fig. 1. Comparison of EDN concentrations in the treated log stack for three replications of fumigations using 120 g/m³ EDN for 24 h (confirmatory test No. 1). Replications 1, 2 and 3 were conducted on 04-05/02/19, 19-20/02/19 and 05-06/03/19, respectively.

Table 7. Comparison between the first and last EDN concentrations and the calculated percent EDN remaining under the tarpaulin at the completion of fumigations[a] using 120 g/m³ EDN for 24 h.

Rep.	Initial concentration		End concentration		Remaining concentration (%)
	ppm	g/m³	ppm	g/m³	
1	79,892.6	172.9	165.9	0.4	0.2
2	62,116.7	134.5	487.5	1.1	0.8
3	55,716.1	120.6	506.7	1.1	0.9

[a] Replications 1, 3 and 3 were conducted on 04-05/02/19, 19-20/02/19 and 05-06/03/19, respectively.

Confirmatory test No. 2 – fumigations using 120 g/m³ EDN for either 16 h or 20 h

Table 8. EDN concentrations under the tarpaulin during confirmatory test No. 2 for a fumigation[a] using 120 g/m³ EDN for 16 h.

Time after EDN application (h)	EDN concentrations under tarpaulin	
	ppm	g/m³
0	77,169.3	181.2
1	61,058.1	143.3
2	46,200.3	108.5
3	31,286.8	73.4
4	25,158.8	59.1
6	16,351.7	38.4
8	8,950.9	21.0
10	3,483.2	8.2
12	935.4	2.2
14	543.9	1.3
16	483.2[b]	1.1

[a] Fumigation was conducted on 19-20/03/19 (in combination with a fumigation using 120 g/m³ EDN for 20 h) and was not replicated.

b The same value (483.2 ppm) was found for 16 h and at 20 h in Tables 8 and 9, respectively (i.e., the values are correct as shown).

Table 9. EDN concentrations under the tarpaulin during confirmatory test No. 2 for a fumigation[a] using 120 g/m^3 EDN for 20 h.

Time after EDN application (h)	EDN concentrations under tarpaulin	
	ppm	g/m^3
0	58,381.1	137.0
1	52,435.4	123.1
2	43,169.3	101.3
3	34,117.6	80.1
4	28,195.1	66.2
6	20,670.5	48.5
8	14,528.6	34.1
10	7,000.7	16.4
12	2,356.6	5.5
16	1,506.5	3.5
18	882.4	2.1
20	483.2[b]	1.1

[a] Fumigation was conducted on 19-20/03/19 (in combination with a fumigation using 120 g/m^3 EDN for 16 h) and was not replicated.

[b] The same value (483.2 ppm) was found for 16 h and at 20 h in Tables 8 and 9, respectively (i.e., the values are correct as shown).

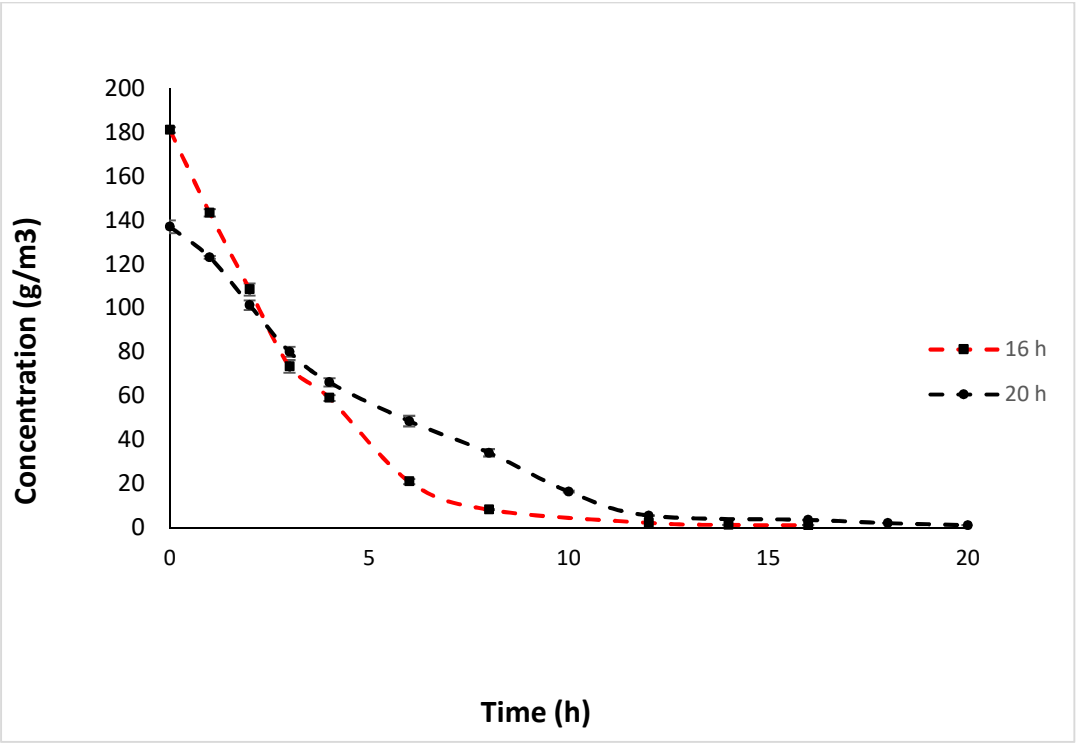

Fig 2. Comparison of EDN concentrations in the treated log stacks for fumigations using 120 g/m^3 EDN for either 16 h or 20 h. Fumigations were conducted on 19-20/03/19.

Table 10. Comparison between the beginning and end EDN concentrations and the calculated percent EDN remaining under the tarpaulin at the completion of fumigations[a] using 120 g/m^3 EDN for either 16 h or 20 h.

Treatment duration using 120 g/m^3 EDN	Initial concentration		End concentration		Remaining concentration (%)
	ppm	g/m^3	ppm	g/m^3	
16 h	77,169.1	181.2	483.2	1.1	0.6
20 h	58,381.1	137.2	483.2	1.1	0.8

[a] Fumigations were conducted on 19-20/03/19 and were not replicated.

Confirmatory test No. 3 – fumigations using either 80 g/m³ or 100 g/m³ EDN for 20 h

Table 11. EDN concentrations under the tarpaulin during confirmatory test No. 3 for a fumigation[a] using 80 g/m³ EDN for 20 h.

Time after EDN application (h)	EDN concentrations under tarpaulin	
	ppm	g/m³
0	79,134.4	171.3
1	66,797.6	144.6
2	54,874.2	118.8
3	50,370.8	109.0
4	44,403.1	96.1
6	25,195.6	54.5
8	16,631.0	36.0
10	5,869.6	12.7
12	2,595.2	5.6
16	983.0	2.1
20	751.7	1.6

[a] Fumigation conducted on 03-04/04/19 (in combination with a fumigation using 100 g/m³ EDN for 20 h) and was not replicated.

Table 12. EDN concentrations under the tarpaulin during confirmatory test No. 3 for a fumigation[a] using 100 g/m^3 EDN for 20 h.

Time after EDN application (h)	EDN concentrations under tarpaulin	
	ppm	g/m^3
0	72,416.7	156.8
1	67,290.8	145.7
2	57,071.4	123.5
3	48,695.6	105.4
4	44,204.1	95.7
6	30,526.2	66.1
8	14,279.6	30.9
10	8,731.5	18.9
12	5,074.8	11.0
16	3,659.9	7.9
20	2,855.4	6.2

[a] Fumigation conducted on 03-04/04/19 (in combination with a fumigation using 80 g/m^3 EDN for 20 h). Fumigation using 100 g/m^3 EDN for 20 h was replicated two more times on 21-22/05/19.

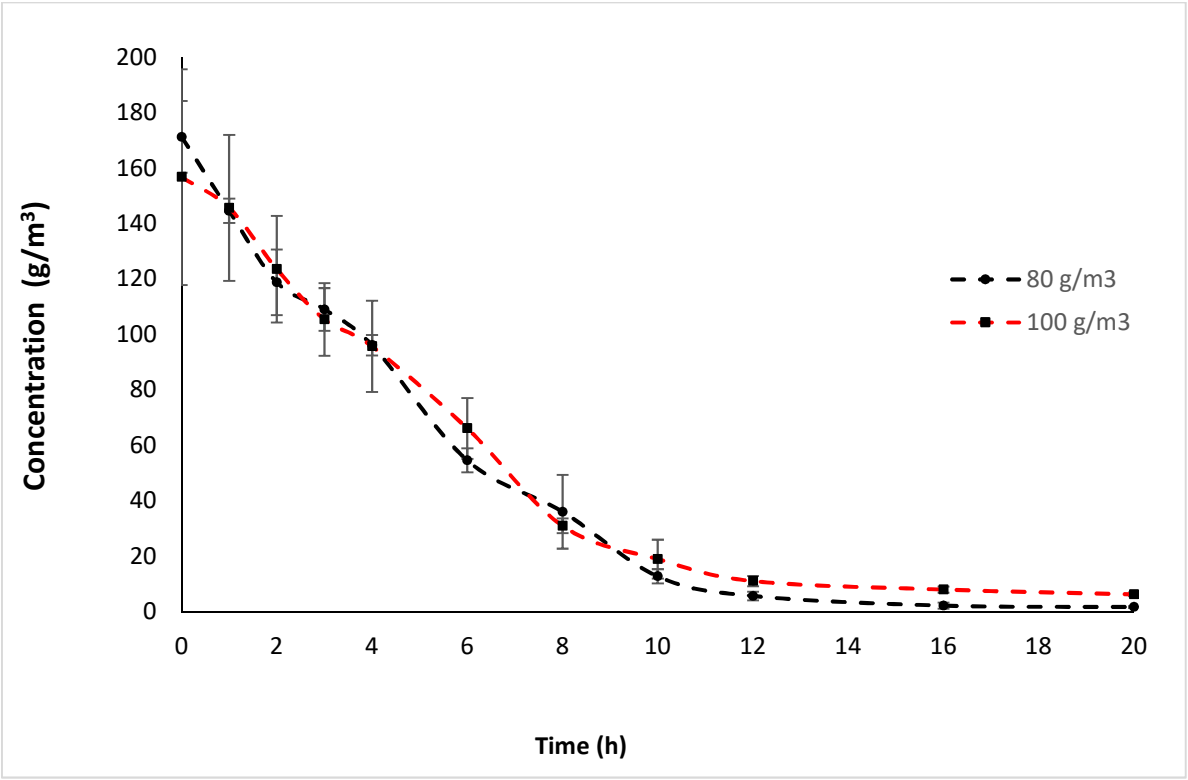

Fig 3. Comparison of EDN concentrations in the treated log stacks for fumigations using either 80 g/m³ or 100 g/m³ EDN for 20 h. Fumigations were conducted on 03-04/04/19.

Table 13. Comparison between the first and last EDN concentrations and the calculated percent EDN remaining under the tarpaulin at the completion of fumigations[a] using either 80 g/m³ or 100 g/m³ EDN for 20 h.

Treatment	Initial concentration		End concentration		Remaining concentration (%)
	ppm	g/m³	ppm	g/m³	
80 g/m³ for 20 h	79,134.4	171.3	751.7	1.6	1.0
100 g/m³ for 20 h	72,416.7	156.8	2,855.4	6.2	3.9

[a] Fumigations were conducted on 03-04/04/19. The fumigation using 80 g/m³ for 20 h was not replicated; the fumigation using 100 g/m³ for 20 h was replicated two more times on 20-21/05/19 to provide three replications total for this EDN concentration and fumigation time.

Table 14. EDN concentrations under the tarpaulin during confirmatory test No. 4 for two fumigation[a] using 100 g/m^3 EDN for 20 h.

Time after EDN application (h)	EDN concentrations under tarpaulin			
	Replication 2		Replication 3	
	ppm	g/m^3	ppm	g/m^3
0	52,854.9	124.1	61,485.2	144.3
1	63,708.6	149.6	65,560.0	153.9
2	57,903.8	135.9	53,215.2	125.0
3	42,181.5	99.0	-	-
4	32,286.4	75.8	31,389.4	74.0
6	19,042.4	44.7	20,097.0	47.2
8	11,481.8	26.9	13,842.4	32.5
10	6,990.9	16.4	4,733.3	11.1
12	2,915.4	6.8	2,287.9	5.3
16	1,210.6	2.8	1,416.7	3.4
20	700.0	1.6	991.7	2.4

[a] Fumigations were conducted on 21-22/05/19

Table 15. Variations across EDN concentrations (in g/m^3) in each of the three culverts used in replication 3, confirmatory test No. 4 using 100 g/m^3 EDN for 20 h.

Culvert No.	Concentration (g/m^3) at sampling times (h) during fumigation										Avg. concentration over 20 h (g/m^3)
	0[a]	1	2	4	6	8	10	12	16	20	
1	180.78	230.25	192.38	103.87	61.98	34.07	11.15	5.36	3.48	2.38	82.57
2	4.16	6.08	6.18	15.15	19.71	30.88	11.04	5.53	3.23	2.27	10.42
3	248.05	225.37	176.20	102.03	59.84	32.53	11.14	5.23	3.27	2.33	86.60

[a] Immediately after EDN was injected into the log stack.

Table 16. Comparison between the first and last EDN concentrations and the calculated percent EDN remaining under the tarpaulin at the completion of fumigations[a] using 100 g/m^3 EDN for 20 h (replications 2 and 3.

Rep.	Initial concentration		End concentration		Remaining concentration (%)
	ppm	g/m^3	ppm	g/m^3	
2	52,854.9	124.1	700.0	1.6	1.3
3	61,485.2	144.3	991.7	2.3	1.1

[a] Fumigations were conducted on 21-22/05/19

Monitoring of log stacks during fumigations for temperature and RH

Table 17 shows a summary of the average temperature and RH data collected from inside the control and treated stacks for all fumigations.

Table 17. Summary of average temperatures and RH inside the control and the treated log stacks for confirmatory tests with EDN conducted at Tokoroa, New Zealand from February through May 2019.

Confirmatory test No. and (replication No.)	Temperature (°C)[e]		RH (%)[e]	
	Control stack	Treated stack	Control stack	Treated stack
1[a] (1)	17.9 ± 0.1 ***21.7 ± 0.8***	17.8 ± 0.1	***80.7 ± 2.4***	NC[f]
1[a] (2)	17.0 ± 0.1 ***18.6 ± 0.1***	16.2 ± 0.1	***92.3 ± 0.4***	95.3 ± 0.2
1[a] (3)	14.2 ± 0.1 ***17.4 ± 0.8***	14.4 ± 0.1	74 ± 1.6	99.72 ± 0.1
2[b] (1, 1)	18.4 ± 0.1 ***21.1 ± 0.4***	18.7 ± 0.1	***84.6 ± 2.0***	97.36 ± 1.0
3[c] (1, 1[c])	***17.0 ± 0.4***	15.5 ± 0.1	***88.0 ± 1.5***	98.52 ± 0.2
4[d] (2, 3)	***11.03 ± 0.2***	***10.9 ± 0.1***	***89.6 ± 0.1***	***85.1 ± 1.3***

[a] Confirmatory test No. 1 consisted of 3 replications using 120 g/m^3 EDN for 24 h

[b] Confirmatory test No. 2 consisted of single fumigations using 120 g/m^3 for either 16 h or 20 h

[c] Confirmatory test No. 3 consisted of single fumigations using either 80 g/m^3 or 100 g/m^3 EDN for 20 h (the 100 g/m^3 EDN fumigation was the first replication of that concentration and fumigation time)

[d] Confirmatory test No. 4 consisted of replications 2 and 3 for fumigations using 100 g/m^3 EDN for 20 h

[e] Values in bold were collected using data loggers (Temprecord International Limited, Auckland, New Zealand). All other values were collected using remote squirrel meter/loggers (Grant 1250 series, CAMBEEP, Cambridge Building Energy & Environmental Portal, UK).

[f] Data not collected

Table 18 shows a summary of the variations in outside the stack temperature, RH, wind average and wind gust for all fumigations.

Table 18. Summary of variations in temperatures and RH outside the stacks for confirmatory tests with EDN conducted at Tokoroa, New Zealand from February through May 2019.

Confirmatory Test	Outside temperature (°C)[e] (minimum-maximum)	Outside RH[e] (%) (minimum-maximum)	Wind average[f] (km/h)	Wind gust[f] (km/h)
No. 1[a]	9.22- 29.00	58.23-93.73	14	20.45
	9.42-33.04	33.93-86.68	6.95	10.45
	8.40-29.25	36.78-90.89	13.35	19.55
No. 2[b]	11.33-33.26	45.59-95.81	6.7	14.25
No. 3[c]	5.43-34.95	31.88-88.26	11.8	18.55
No. 4[d]	8.67-15.50	81.75-89.66	11.75	16.05

[a] Confirmatory test No. 1 consisted of 3 replications using 120 g/m^3 EDN for 24 h

[b] Confirmatory test No. 2 consisted of single fumigations using 120 g/m^3 for either 16 h or 20 h

[c] Confirmatory test No. 3 consisted of single fumigations using either 80 g/m^3 or 100 g/m^3 EDN for 20 h (the 100 g/m^3 EDN fumigation was the first replication of that concentration and fumigation time)

[d] Confirmatory test No. 4 consisted of replications 2 and 3 for fumigations using 100 g/m^3 EDN for 20 h

[e] Values collected using an electronic portable weather station (NIWA EWS).

[f] Values were retrieved from the New Zealand Local Weather Community network online (http://www.localweather.net.nz/smf/).

Confirmatory test No. 1 – replications 1, 2 and 3 of fumigations using 120 g/m³ EDN for 24 h

Table 19. Control and treatment mortality of *H. ater* in three replications[a] of EDN fumigations using 120 g/m³ EDN for 24 h.

Replication and life stage	No. insects		Mortality (%)	
	Control	Treated	Control	Treated
Replication No. 1				
Larvae	1,184	10,834	1.23	100
Pupae[b]	1,994	19,067[c]	2.10	100
Adults	1,172	6,434	2.62	100
Total insects	4,350	36,335	—	—
Replication No. 2				
Larvae	2,546	13,184	1.09	100
Pupae[b]	2,311	13,363[c]	4.14	100
Adults	3,187	7,527	1.55	100
Total insects	8,044	34,083	—	—
Replication No. 3				
Larvae	1,439	6,539	0	100
Pupae[b]	1,798	16,675[c]	0	100
Adults	4,111	16,596	0.02	100
Total insects	7,348	39,810	—	—

[a] Replications 1, 2 and 3 were conducted on 04-05/02/19, 19-20/02/19 and 05-06/03/19, respectively.

[b] The pupal life stage was the target for demonstrating efficacy in the confirmatory tests because laboratory studies showed to be the most EDN-tolerant life stage compared with the eggs, larvae and adults (Najar-Rodriguez et al. 2018).

[c] Total treated *H. ater* pupae with no survivors for all three replications of fumigations using 120 g/m³ for 24 h is 49,105

Confirmatory test No. 2 – fumigations using 120 g/m³ EDN for either 16 h or 20 h

Table 20. Control and treatment mortality of *H. ater* in EDN fumigations[a] using 120 g/m³ EDN for either 16 h or 20 h.

Treatment and life stage	No. insects		Mortality (%)	
	Control[c]	Treated	Control[c]	Treated
120 g/m³ for 16 h				
Larvae	580	2,368	2.50	100
Pupae[b]	1,539	6,994	0	100
Adults	2,580	4,449	0.24	100
Total insects	4,699	13,811	—	—
120 g/m³ for 20 h				
Larvae	580	2,121	2.50	100
Pupae[b]	1,539	6,236	0	100
Adults	2,580	3,116	0.24	100
Total insects	4,699	11,473	—	—

[a] Fumigations were done on 19-20/03/19.

[b] The pupal life stage was the target for demonstrating efficacy in the confirmatory tests because laboratory studies showed to be the most EDN-tolerant life stage compared with the eggs, larvae and adults (Najar-Rodriguez et al. 2018).

[c] One control was used for both treatments.

Confirmatory test No. 3 – fumigations using either 80 g/m³ or 100 g/m³ EDN for 20 h

Table 21. Control and treatment mortality of *H. ater* in EDN fumigations[a] using either 80 g/m³ or 100 g/m³ EDN for 20 h.

Treatment and life stage	No. insects		Mortality (%)	
	Control[c]	Treated	Control[c]	Treated
80 g/m³ for 20 h				
Larvae	703	2,697	0	100
Pupae[b]	2,296	7,852	0.9	100
Adults	1,686	2,154	0	100
Total insects	4,685	13,703	—	—
100 g/m³ for 20 h				
Larvae	703	3,140	0	100
Pupae[b]	2,296	6,921	0.9	100
Adults	1,686	2,017	0	100
Total insects	4,685	12,078	—	—

[a] Fumigations were done on 03-04/04/19.

[b] The pupal life stage was the target for demonstrating efficacy in the confirmatory tests because laboratory studies showed to be the most EDN-tolerant life stage compared with the eggs, larvae and adults (Najar-Rodriguez et al. 2018).

[c] One control was used for both treatments.

Confirmatory test No. 4 – fumigations using 100 g/m³ EDN for 20 h (replications 2 and 3)

Table 22. Control and treatment mortality of *H. ater* in two replications[a] of EDN fumigations using 100 g/m³ EDN for 20 h.

Replication and life stage	No. insects		Mortality (%)	
	Control	Treated	Control	Treated
Replication No. 2				
Larvae	3,639	9,908	0	100
Pupae[b]	2,208	6,087	0	100
Adults	244	1,937	0	100
Total insects	6,091	17,932	—	—
Replication No. 3				
Larvae	3,639	9,335	0	99.9
Pupae[b]	2,208	6,085	0	98.0
Adults	244	1,591	0	100
Total insects	6,091	17,011	—	—

[a] Fumigations were conducted on 21-22/05/19

Discussion

The averaged values show that temperatures inside both the treated and control log stacks did not vary during each confirmatory test conducted at Tokoroa (Table 17). Interestingly, RH values were > 80% for control stacks and > 90% for the treated log stacks. As expected, the variations in minimum and maximum temperatures and RH outside the stack were substantial compared with the variations that were recorded from inside the stack during each confirmatory test (Table 18). The average wind speed and wind gust values were generally low across all the confirmatory tests with lows and highs from 6.7 to 14.0 km/h and 0.45 to 20.5 km/h, respectively (Table 18). Although brief local variations in wind direction occurred, the NIWA station we used showed that the wind direction was generally from the southeast.

EDN concentrations for the nine fumigations (Tables 6, 10, 14) show that variation existed between the initial concentrations measured in the treated log stack relative to the target dose. This is typical of fumigations even when gas dispersion is rapid and is assisted by the movement of the tarpaulin. Initial readings can reflect the position of the sample tubes relative to the gas entry points and sorption can be rapid contributing to the variability observed. In MB fruit treatments, which are a short as 2 h, the first 15 minutes are often excluded when assessing overall treatment concentration because of the inherent variability observed while the gas becomes homogenous (MPI 2018).

The percent sorption across all the completed log fumigations was very consistent with most treatments resulting in less than one percent of the applied concentration remaining at the end of the fumigation.

All insects in the treated logs in each of the nine fumigations were killed (Tables 19 to 21) with the exception of the survival that occurred from six of the eight logs in a single culvert in replication 3 of confirmatory test No. 4 (treated separately below in Confirmatory test No. 4) (Table 22). The use of EDN at the rate of 120 g/m^3 for 24 h could be recommended as a phytosanitary treatment for logs exported from New Zealand. This treatment has been demonstrated to be effective in replicated trials to a probit level >8.7 (i.e. no survivors in a treated population of 48,152 live insects). However, because the 120-g/m^3 EDN concentration for 24 h was selected based on an extrapolated level of efficacy equivalent to the upper 95% confidence intervals of the mean LD_{99} and $LD_{99.99}$ efficacy values from our previous work (Najar-Rodriguez et al. 2019; manuscript in progress), this EDN concentration and/or fumigation time may be excessive. Hence, additional fumigations (Confirmatory tests 2, 3 and 4) were done to examine insect mortality at lower EDN concentrations and/or fumigation times would be equally effective. All these additional fumigations proved effective in achieving complete mortality, with the exception of replication 3 of confirmatory test No. 4.

The two fumigations, one using 120 g/m^3 EDN for 16 h and another using 120 g/m^3 for 20 h, were done to determine whether shortening the fumigation time using the same 120 g/m^3 concentration would adversely affect insect mortality, i.e., result in survival of *H. ater* pupae. The mortality assessments for the two fumigations are shown in Table 19. Neither of the two fumigations using 120 g/m^3 EDN for shorter fumigation times resulted in survival.

The variation in concentrations that were recorded in the treated log stacks over time in the three replications in Confirmatory test No. 1 were also found in the EDN concentrations over time in the two fumigations in Confirmatory test No. 2. Although the two initial concentrations for the calculated amounts (22 kg) of EDN (Table 2) were identical, the initial concentration for the 16-h fumigation (Table 8) was > 75% greater than the initial EDN concentration for the 20-h fumigation (Table 9). However, the final EDN concentration for the 16-h fumigation was about 32% less in the 16-h fumigation compared with the EDN concentration at the same gas sampling interval for the 20-h fumigation (Tables 8 and 9). Interestingly, the end concentrations for both the 16-h and 20-h fumigations were the same (Table 10).

Two further fumigations, one using 80 g/m^3 EDN for 20 h and another using 100 g/m^3 for 20 h were conducted to determine if both the concentration of EDN used to treat the log stacks and the fumigation time could be reduced without resulting in survival of *H. ater* pupae. Neither the 80-g/m^3 fumigation for 20 h nor the 100-g/m^3 EDN for 20 h resulted in *H. ater* survival (Table 21). Although the data indicate that it may be possible to provide quarantine security for export logs with EDN fumigation at concentrations as low as 80 g/m^3 and fumigation times as short as 16 h, both the 80-g/m^3 EDN fumigation for 20 h must be replicated two more times to verify our results.

Confirmatory test No. 4 – replications 2 and 3 for fumigations using 100 g/m^3 EDN for 20 h

All of the insects in replications 1 and 2 for fumigations using 100 g/m3 for 20 h were killed. In the third replicate survivors from six out of eight infested logs in a single culvert were reported (Table 22, Diag. 1).

The mortality data for the middle culvert (Diag. 1) in the replication 3 log stack that included survivors (Table 22) were removed from the confirmatory test results for that log stack and are treated separately here. The middle culvert contained eight infested logs of which six infested logs produced survivors. Of the six infested logs that produced survivors, two logs produced one larval survivor each, and all six logs produced the 103 pupal survivors. The remaining two infested logs in the middle culvert had no survivors. The mortality rates for the *H. ater* larvae and pupae was 99.9% and 98.0%, respectively

(Table 22) for a range of mortality between 98-100%. We believe the survival resulted from the low EDN concentrations in that culvert (average of 15.0 g/m^3) during the first 8 h of fumigation (Table 15) that did not reflect the true EDN concentration under the tarpaulin compared to the other two culverts (average EDN concentrations of 82.9 and 86.6 g/m^3, respectively) (Table 15). Despite the low EDN concentrations in the middle culvert when compared with the two other culverts in the replication 3 treated log stack during the first 8 h of the fumigation (Table 15), still a 98-100% mortality range was achieved (Table 22). The high mortality caused by such low EDN concentrations (Table 22) indicate that EDN concentrations lower than the 120, 100 or 80 g/m^3 that were applied in our confirmatory tests (and achieved 100% mortality) could potentially be used for the phytosanitary treatment of logs stacks before export and maintain quarantine security against the target forest insects.

The log stack used in replication 3 was rebuilt before the fumigation because the dimensions of the original log stack were incorrect. As the log stack was rebuilt by the loader driver, the middle culvert was inadvertently placed into a position where it was surrounded by logs of nearly similar diameter that was consistent along the length of each log, thereby placing the culvert within a "tube" formed by the logs that surrounded it. When the stack was covered with the tarpaulin and the water snakes were laid, concerns arose over the tightness of the tarpaulin and the potential for tears to occur in the tarpaulin caused by the log ends at the top of the stack. Although tears did not occur, it later became apparent that the tarpaulin was fitted so tightly against the sides of the log stack that the tarpaulin was pressed against the ends of the logs surrounding the middle culvert (Diag. 1) to form a barrier that limited the diffusion of EDN into the culvert.

The occurrence of a barrier that limited EDN diffusion into the middle culvert (Diag. 1) in the replication 3 fumigation with 100 g/m^3 EDN was an operational error that occurred during the rebuilding of the stack. This operational error was an anomaly that did not occur in any of the other log stacks used in our confirmatory test fumigations and, because culverts with infested logs are not used in commercial situation, this anomaly would not occur in commercial fumigations of log stacks. Moreover, all other culverts in all of the treated log stacks over the four confirmatory tests exhibited similar increases and declines of EDN regardless of treatment duration (Tables 6, 8, 9, 11, 12 and 14), i.e., normal patterns of EDN dispersion and equilibration throughout the treated log stacks.

For the reasons given here, we removed the middle culvert (both the survival and mortality data for the infested logs in that culvert) from replication 3 of confirmatory test No. 4, and consider replication 3 to be successful based on the two remaining culverts. Therefore, the data from the middle culvert do not affect our conclusions or recommendations below.

Conclusions and Recommendations

The results of the confirmatory tests reported here demonstrate the efficacy of EDN as a potential phytosanitary fumigant for logs exported from New Zealand. In all confirmatory tests, every EDN concentration and fumigation time that was tested controlled *H. ater* larvae, pupae and adults (the pupae being the most EDN-tolerant life stage).

The results of monitoring the EDN concentrations under the tarpaulin in the treated log stacks followed a relatively rapid decrease when the EDN concentrations over time were compared among the fumigations. The percent sorption across all the completed log fumigations was very consistent with most treatments resulting in less than one percent of the applied concentration remaining at the end of the fumigation. AERMOD modelling prepared by Sullivan Environmental for Draslovka was developed

based on the field end-point concentration data (Hall et al. 2016) and supported a 20-m buffer zone. The very low-end point concentration reported first in the field study (Hall et al. 2016) and now confirmed in the tests reported here clearly shows that EDN can be safely vented into the atmosphere without a scrubbing system.

The results of the three replications of fumigations using 100-g/m^3 fumigations for 20 h indicate that this concentration and fumigation time would be an appropriate phytosanitary fumigation schedule for export logs, whereas the 120-g/m^3 EDN concentration for 24 h, although equally efficacious, would be excessive (IPPC 2019). The proposal for EDN approval in New Zealand is 150 g/m^3 for 24 h. However, based on the efficacy results from the our confirmatory tests, 100% mortality of *H. ater* pupae could be achieved using 100 g/m^3 for 20 h, which is 34% lower than the current proposed concentration. A lower initial concentration means a reduced EDN concentration released into the environment during venting.

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