sulfuryl fluoride was developed mainly for use in temperate zone countries, limited efficacy data has been developed for these pests. In 2018, laboratory studies were conducted in California by the Dried Fruit and Tree Nut Association (DFA, Fresno, CA, USA) to determine the dosage required for control of all life stages of these pests. The results of this study coupled with earlier work may allow for inclusion in the Fumiguide® program.

The Fumiguide program is required for use with ProFume to calculate dosage and dose requirements. The program allows users to tailor applications based on job specific parameters to best meet customer needs for cost and time. The new Fumiguide includes improvements in the underlying algorithms, additional functionality for fumigators and the ability to easily add new pests.

Since the purchase of sulfuryl fluoride from The Dow Chemical Company in 2015, Douglas Products has continued to expand product use through new country registrations, expanded efficacy data and development of an updated Fumiguide program. This presentation provides updates on registrations for ProFume, details efficacy work for two insect species of interest in tropical regions, and reviews the updated Fumiguide program, a required tool for dose and dosage determination.

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Nitric oxide as a new fumigant for postharvest pest control

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Abstract

Nitric oxide (NO) is a new fumigant for postharvest pest control. It is effective against all pests tested to date, including external and internal pests of fresh and stored product insects, and mites. Efficacious treatment time ranges from 2 h to 72 h, and NO concentrations range from 0.1% to 5%, depending on species and life stages of the pests.

Nitric oxide fumigation must be conducted under ultralow oxygen conditions because NO reacts with O_2 spontaneously to produce nitrogen dioxide (NO_2), which is toxic to perishable fresh products. Fresh product fumigation must, therefore, also be terminated by flushing with N_2 to dilute NO at the end of fumigation to avoid damage to delicate products by NO_2. Nitric oxide fumigation was safe in small-scale tests to postharvest quality of all fresh commodities when terminated with N_2 flush. In addition, NO fumigation resulted in better postharvest quality of strawberries and apples as compared with controls, indicating its beneficial effects on postharvest quality of fresh products.

Twenty fresh fruit and vegetables and 10 stored products were fumigated with NO to determine residue levels of nitrate and nitrite. When terminated properly with N2 flush, NO fumigation does not increase nitrate or nitrite levels in fumigated products. NO fumigation was demonstrated to be effective against all pests, safe to fresh products, and has no toxic residues and, therefore, has the potential to be a practical alternative to methyl bromide fumigation for postharvest pest control on both fresh and stored products.

Keywords: Nitric oxide, fumigation, quarantine treatment, residue, postharvest quality.

Introduction

There is a severe lack of safe and effective alternative treatments for postharvest pest and disease management after phasing out of methyl bromide. The current main alternatives, including phosphine and sulfuryl fluoride, have difficulties in meeting the need for postharvest pest control on stored products or fresh commodities. Phosphine fumigation typically has long treatment time and is not effective against some pests due to tolerance or resistance (Hole *et al*., 1976; Benhalima *et al*., 2004). Sulfuryl fluoride is not effective against insect eggs (Bell *et al*., 1998) and therefore has limited efficacy in addition to its phytotoxicity to fresh products (Aung *et al*., 2001). Nitric oxide (NO) is a newly discovered fumigant for postharvest pest control and has high efficacy against insects and mites and no toxic residues (Liu, 2013, 2015). It was also demonstrated to be safe to fresh commodities and enhance postharvest quality of fresh commodities (Liu, 2015, 2016, Liu *et al*., 2016). Therefore, NO may have potential to be a practical alternative fumigant for postharvest pest control on both fresh and stored products.

Nitric oxide is a chemical produced naturally in fossil fuel combustion and lightning and commercially as an intermediate in fertilizer production. Since the discovery of NO as a cell messenger molecule in 1980s, NO has been studied extensively and was found to play diverse roles in physiological and biochemical processes in organisms (Lamattina *et al*., 2003). Nitric oxide has also been used in medical fields to treat certain respiratory and cardio vascular conditions (Roberts *et al*., 1993; Ricciardolo *et al*., 2004) and was also found to be an inhibitor of ethylene biosynthesis in plants and can be used to enhance postharvest quality and prolongs shelf-life of fresh fruit and vegetables (Wills *et al*., 2000; Soegiarto and Wills, 2004; Manjunatha *et al*., 2012; Saadatian *et al*., 2012).

As a new fumigant, NO is effective against a wide variety of pests (Liu, 2013, 2015, 2017; Liu and Yang, 2016; Liu *et al*., 2016). Nitric oxide fumigation is also safe to fresh products (Liu, 2016, 2017, Liu et al. 2016). In fact, NO fumigated strawberries and apples have better postharvest quality as compared with untreated controls (Liu, 2016; Liu *et al*., 2016). Nitric oxide fumigation also has no toxic residues on fumigated fresh products (Yang and Liu, 2017). In this paper, NO fumigation past research was reviewed and discussed, and new data on efficacy and residues were also presented and discussed.

Procedures of nitric oxide fumigation

Nitric oxide fumigation must be conducted under ultralow oxygen (ULO) conditions. This is due to the nature of spontaneous reaction between NO and oxygen (O_2) to produce nitrogen dioxide (NO_2). The reaction not only consumes NO, but also produces NO_2 which can cause injuries to sensitive fresh products at high concentrations. Therefore, ULO needs to be established in a fumigation chamber by flushing with nitrogen (N_2) to reduce O_2 concentration to a minimum level. The fumigation chamber also needs to be sealed airtight to prevent O_2 leaking into the chamber. For stored product fumigation, carbon dioxide (CO_2) can also be used to flush the fumigation chamber as CO_2 is unlikely to affect stored products. At the end of fumigation, especially for fresh products, the fumigation chamber also needs to be flushed with N_2 to dilute NO before opening the chamber to prevent the reaction between NO and O_2 and production of NO_2.

Nitric oxide fumigation procedures have been published in a video article (Liu *et al*., 2017) and also described previously (Liu, 2013, 2015; Liu and Yang, 2016; Liu, 2017). Nitric oxide fumigation starts by establishing ULO conditions in an airtight fumigation chamber with a N_2 flush. Greasing with petroleum jelly often is required to achieve an airtight seal of a fumigation chamber. Tubing with low permeability or non-permeable to O_2, such as nylon tubing, should be used. Oxygen analyzers with zirconia sensors are recommended for their high sensitivities and longevity. To have high efficiency in establishing ULO conditions, the fumigation chamber can be flushed with N_2 at a high flow rate at the beginning and then reduce the flow rate when O_2 is close to a desired level. The ULO levels for NO fumigation can vary depending on NO concentrations and products. Higher ULO levels will consume NO and therefore reduce effective NO levels for pest control. Some fresh products are also sensitive to high NO_2 levels. We used $\leq$30 ppm O_2 in all of our small scale NO fumigation tests.

The length of time to achieve a desired ULO level may vary greatly depending on the type of products to be fumigated and the quantities of the products. Large fruit such as apples can take long time to establish ULO conditions as they contain large volume of air inside. Many fresh products are packed in perforated plastic bags or wraps with very limited air exchanges capabilities and therefore limit efficiency of N_2 flush in establishing ULO conditions. Vacuum may also be used to increase the efficiency in establishing ULO conditions.

Nitric oxide concentration, fumigation time, and temperature will depend on pest species and products. For packaged fresh products, it is recommended to use lower NO concentration but longer treatment time, because N_2 flush is needed to dilute NO at the end of fumigation and lower NO concentrations are easier to be diluted than higher concentrations. The level of dilution may also change depending on sensitivity of fresh products to NO_2. For fumigations of leafy vegetables and delicate fruits, it is preferably that NO be diluted to 100 ppm or lower before opening the chamber to ensure safety to products.

Currently there is no suitable analyzer to monitor NO concentrations in NO fumigations. The high concentration NO sensors in commercial flue gas monitors typically have a maximum concentration limit of 5000 ppm. Because of its reactive nature with O_2, NO cannot be quantified using a gas chromatograph. Therefore, NO concentrations in small chamber fumigations were calculated based on NO gas volumes used and the chamber volumes. For large fumigations, a flue gas monitor with a high concentration NO sensor can be used in conjunction with a dilution device to monitor NO concentrations in NO fumigations. The dilution device we made and used consisted of four equal length micro-tubes with one tube for sample gas and the other three for nitrogen. Under the condition of equal air pressure in the fumigation chamber and nitrogen in a foil bag, the air sample can be diluted four times and, thereby, a fumigation with 2% NO can be monitored using the monitor with a 5000 ppm NO sensor. However, a custom built NO analyzer with suitable maximum NO level for NO fumigation is possible from certain vendors.

Efficacy of nitric oxide fumigation

Over 10 species of insects and mites have been tested with NO fumigation. Nitric oxide fumigation is effective against all pest species tested to date at different life stages (Table 1) (Liu, 2013, 2015; Liu and Yang, 2016). However, there are considerable variations among species and life stages in susceptibility to NO fumigation. Nitric oxide fumigation is particularly effective against small external soft-body insects on fresh products. Western flower thrips (*Frankliniella occidentalis* (Pergande)), lettuce aphid (*Nasonovia ribisnigri* (Mosley)), and longtailed mealybug (*Pseudococcus longispinus* (Targioni Tozzetti) can be controlled in a few hours with 1-2% NO at a low temperature of 2°C (Liu, 2013).

Nitric oxide fumigation is also effective against internal feeding insects. Spotted wing drosophila (*Drosophila suzukii* (Matsumura)) larvae in infested cherries were controlled in 8 h with 2.5% NO fumigation. For codling moth (*Cydia pomonella* (L.)) larvae in infested apples, NO fumigation treatments of 24 h at 5% concentration at 2°C resulted in 100% larval mortality (Liu *et al*., 2016). Nitric oxide fumigation at 1-2% concentrations takes 24 h to 72 h at 15-25°C to control stored product insects such as Indianmeal moth (*Plodia interpunctella* (Hubner)), confused flour beetle (*Tribolium confusum* (Jacquelin du Val)), and rice weevil (*Sitophilus oryzae* (Linnaeus)). The treatment time is shorter for mobile stages than for pupa and egg stages (Liu, 2013, 2015; Liu and Yang, 2016). Bulb mites (*Rhizoglyphus spp*.) on infested peanuts were also controlled with 2% NO in 24 h at 20°C (Liu, 2017) (Table 1).

The efficacy of NO fumigation increases with concentration, time, and temperature. Concentration x Time (C×T) products correlate well with mortality and can be used to determine NO fumigation treatments. Effect of temperature on efficacy of NO fumigation is lower as compared with concentration and time (Liu, 2013).

Safety of nitric oxide fumigation to product quality

Safety of NO fumigation to product quality includes possible injuries to fresh products and likely residues in fumigated products. In small scale tests, NO fumigation is safe to all fresh products tested to date including lettuce, broccoli, cucumber, pepper, tomato, strawberries, apple, pear, orange, and lemon when terminated with N_2 flush as there are not significant differences between the treatment and the control (Table 2) (Liu, 2016). When NO fumigation is terminated by directly opening the fumigation chamber to ambient air without flushing with N_2, NO reacts with O_2 to produce NO_2 in the fumigation chamber and results in stains on delicate fresh products including leafy vegetables, broccoli, squash, and peach. Stains also occur to some apples (Liu, 2016).

Tab. 1 Summary of nitric oxide fumigation treatments that had 100% control of different pest species at specified life stages*

Species	Life stage	NO (%)	Time (h)	Temp (°C)	Note
Western flower thrips	larva, adult	0.2	8	2	on lettuce leaves
		2	2	2	
Lettuce aphid	nymph, adult	0.2	12	2	on lettuce leaves
		0.5	9	2	
		1	3	2	
Long-tailed mealybug	nymph, adult	2	2	2	on grape leaves
Confused flour beetle	larva, pupa	0.5	24	20	on flour diet
	adult	0.5	8	20	
	egg	2	24	10	
Rice weevil	adult	1	24	25	on pearled barley
	egg	1	48	25	
Indian meal moth	egg	1	24	20	
Light brown apple moth	larva, pupa	2	8	2	on artificial diet
	egg	3	12	2	
		5	6	2	
Codling moth	egg, larva, pupa	2	48	2	on artificial diet
	larva	5	24	2	in apples
Spotted wing drosophila	egg, larva	3	8	2	in sweet cherries
Bulb mites	larva, adult	2	24	20	on peanuts

* Reprint from Liu and Yang (2016).

For some fresh products, properly conducted NO fumigation not only is safe to product quality but also help to extend storage/shelf-life. Nitric oxide fumigations for control of western flower thrips results in better postharvest quality of strawberries with significantly firmer and brighter, richer color as compared with the control one week after treatment (Liu, 2016). Nitric oxide fumigation for control of codling moth larvae in apples also results in better apple quality as compared control four weeks after fumigation (Liu *et al*., 2016).

Residues of nitric oxide fumigation

Nitric oxide fumigation can result in nitrate (NO^{3-}) and nitrite (NO^{2-}) as residues as NO reacts with O_2 to produce NO_2 which can be further converted to nitrate and nitrite. However, both nitrate and nitrite occur in varying quantities in fresh and stored agricultural products. Twenty fresh products and 10 stored products have tested for residues. For most fresh products, NO fumigation does not lead to significantly higher nitrate or nitrite if the treatment is terminated properly with N_2 flush (Yang and Liu, 2017). For the 10 stored products, there were also no significant increases in nitrate or nitrite in fumigated stored products as compared with the controls (Yang and Liu, unpublished). When NO fumigation is terminated without N2 flush, there are significant increases in nitrate and sometime also nitrite levels in fumigated fresh and stored products (Yang and Liu, 2017, unpublished).

Nitrogen dioxide release rates and nitrate and nitrite levels were evaluated for different treatments from five selected fresh products and five selected stored products at 24 h after NO fumigation. This study showed considerable differences between fresh and stored products and among different fresh products, as well as among different stored products (Tab 3). Apples from both NO-N_2 (NO fumigation terminated with N_2 flush) and NO-Air (NO fumigation terminated without N_2 flush) treatments had similar significantly higher NO_2 release rates as compared with the control. Lettuce from the NO-Air treatment, however, had a NO_2 release rate which was about 1000 times as those of the NO-N_2 treatment and the control. For other fresh products: asparagus, broccoli and strawberries, NO-Air treatments had significantly higher NO_2 release rates as compared with NO-N_2

and controls. Higher retention of NO_2 on the fresh products led to corresponding higher levels of nitrate and nitrite in the products (Yang and Liu, 2017).

Tab. 2 Effects of nitric oxide fumigation treatments on postharvest quality of fresh fruit and vegetables after 14 days post-treatment storage at 2°C*

Product	Treatment	N	Quality score (Mean±SE)	ANOVA	
Lettuce	Control	7	6.4±0.9a	df = 2, 18	F = 15.754
	NO-N_2	7	4.9±0.6a		P = 0.0001
	NO-Air	7	1.4±0.2b		
Broccoli	Control	7	8.0±0.3a	df = 2, 17	F = 9.193
	NO-N_2	7	7.9±0.5a		P = 0.0020
	NO-Air	6	5.2±0.7b		
Pepper	Control	15	8.1±0.2a	df = 2, 42	F = 9.026
	NO-N_2	15	7.3±0.3a		P = 0.0005
	NO-Air	15	6.0±0.5b		
Squash	Control	7	7.1±0.3a	df = 2, 18	F = 9.546
	NO-N_2	7	6.6±0.2a		P = 0.002
	NO-Air	7	4.1±0.8b		
Tomato	Control	9	8.3±0.3a	df = 2, 24	F = 2.886
	NO-N_2	9	7.6±0.4a		P = 0.075
	NO-Air	9	6.9±0.5a		
Apple	Control	15	7.9±0.2a	df = 2, 42	F = 11.667
	NO-N_2	15	8.1±0.2a		P < 0.0001
	NO-Air	15	6.3±0.3b		
Lemon	Control	7	8.4±0.3a	df = 2, 18	F = 0.214
	NO-N_2	7	8.3±0.3a		P = 0.809
	NO-Air	7	8.1±0.3a		
Orange	Control	7	8.4±0.2a	df = 2, 18	F = 0.079
	NO-N_2	7	8.4±0.2a		P = 0.924
	NO-Air	7	8.3±0.4a		
Peach	Control	7	8.4±0.2a	df = 2, 18	F = 6.584
	NO-N_2	7	7.4±0.3ab		P = 0.007
	NO-Air	7	5.1±1.1b		
Pear	Control	9	8.1±0.3a	df = 2, 24	F = 5.375
	NO-N_2	9	8.3±0.2a		P = 0.012
	NO-Air	9	6.8±0.5b		

* Reprint from Liu (2017). All products from the treatments and the control were stored at 2°C for 14 days before being scored for postharvest quality. The visual quality was scored for marketability using the 1 (extremely poor) to 9 (excellent) scale for lettuce (Kader *et al*., 1973) with 3, 5, and 7 representing poor, fair with major defects, and good with minor defects (Liu, 2016).

For stored products, NO_2 release rates were much lower as compared with the fresh products. The variations among the three treatments were also much smaller as compared with the three treatments for the fresh products (Table 3). However, for the most products, NO-Air treatment still had significantly higher NO_2 release rate as compared with NO-N_2 treatment and the control. Nitrate and nitrite levels also varied among the three treatments in consistence with the NO_2 release rate variations. Higher NO_2 release rates corresponded with higher nitrate and nitrite levels for all of the stored products (Table 3).

Discussions

Nitric oxide fumigation is effective against all pests tested to date, is safe to fresh product quality and leaves no toxic residues in fumigated products when terminated properly. Over 10 tested pest species have been effectively controlled and they represent different taxonomical groups, both external and internal feeders, both fresh and stored product pests, and different life stages. The efficacy data suggest that NO fumigation is likely effective against all insect pests and mites. Because of large variation in susceptibility to NO fumigation among different species and life stages, different pests will likely need different combinations of NO concentration and treatment time at certain temperatures to achieve effective control. CxT products can be used to determine appropriate NO fumigation treatments because they correspond well with mortality for individual species.

Nitric oxide has advantages in efficacy in comparison with other methyl bromide alternatives, including phosphine, sulfuryl fluoride, and ethyl formate. Phosphine is the major methyl bromide alternative fumigant for both fresh and stored product pests. However, phosphine fumigation is not effective against some pests due to tolerance or resistance. In general, phosphine fumigation also has long treatment times which may extend over 10 days to achieve effective control of some pests (Hole *et al*., 1976). Although recently developed oxygenated phosphine fumigation has significantly increased efficacy of phosphine fumigation against phosphine tolerant insects (Liu, 2011; Liu *et al*., 2013), the prospect of commercial application is still unclear. Sulfuryl fluoride is not effective against insect eggs (Bell *et al*., 1998) and is also phytotoxic to fresh products (Aung *et al*., 2001). Therefore, it has limited effectiveness against postharvest pests. Ethyl formate has high absorbing rates in fresh products and also has phytotoxicity on fresh products (Stewart and Mon, 1984; Zoffoli *et al*., 2013). In contrast, NO is effective against all pests and all life stages and has high efficacy against small external pests on fresh products with short treatment times and very low absorbance in fresh products.

Tab. 3 Nitrogen dioxide (NO_2) release rate and nitrate (NO^{3-}) and nitrite (NO^{2-}) contents in selected fresh and stored products at 24 h after nitric oxide fumigation*

Product	Treatment	NO_2 (mg kg^{-1} h^{-1})	NO^{3-} (mg/kg)	NO^{2-} (mg/kg)
Fresh products				
Apple	NO-Air	58.721±8.114a	15.96±1.20a	4.95±1.57a
	NO-N_2	45.613±7.442a	13.64±1.33ab	0.30±0.14b
	Control	0.019±0.005b	7.61±2.80b	0b
Asparagus	NO-Air	3.050±0.704a	21.85±1.32a	0.75±0.42a
	NO-N_2	0.387±0.052b	7.00±0.25b	0a
	Control	0.184±0.073b	8.36±0.74b	0a
Broccoli	NO-Air	0.499±0.165a	186.86±37.54a	1.70±0.63a
	NO-N_2	0.183±0.018ab	185.12±34.16a	0b
	Control	0.081±0.031b	122.58±23.07a	0b
Lettuce	NO-Air	1643.704±395.573a	1128.49±201.70a	79.87±20.15a
	NO-N_2	13.452±5.189b	389.66±58.69b	0.98±0.79b
	Control	14.677±13.652b	406.41±108.06b	0b
Strawberry	NO-Air	3.322±1.147a	60.14±6.20a	0
	NO-N_2	0.334±0.055b	52.99±7.65a	0
	Control	0.079±0.018b	61.62±10.61a	0
Stored products				
Almond	NO-Air	0.034±0.008a	16.86±1.10a	4.22±0.37a
	NO-N_2	0.024±0.008ab	12.21±1.83ab	1.91±0.89b
	Control	0.020±0.008b	11.34±0.79b	0b
Barley	NO-Air	0.037±0.010a	26.36±0.50a	6.23±0.35a
	NO-N_2	0.031±0.008b	8.29±1.10b	2.04±0.36b
	Control	0.018±0.005b	8.48±0.56b	0c
Pinto beans	NO-Air	0.017±0.005a	39.58±3.53a	9.54±1.47a
	NO-N_2	0.013±0.001b	33.62±9.0b	1.12±0.16b
	Control	0.001±0.001c	28.37±5.84b	0b
Rice	NO-Air	0.042±0.009a	14.41±2.02a	3.44±0.28a
	NO-N_2	0.033±0.008a	8.53±1.60ab	1.69±0.13b
	Control	0.034±0.009a	7.76±0.71b	0c
Walnut	NO-Air	0.023±0.008a	19.04±3.61a	3.20±0.07a
	NO-N_2	0.015±0.007b	11.73±2.12a	0.82±0.47b
	Control	0.016±0.007b	13.84±0.22a	0b

*Fresh products: apple, asparagus, broccoli, lettuce, and strawberries were fumigated with 5, 3, 3, 2, and 2.5% NO respectively for 16h at 2°C. Stored products were fumigated with 3% NO for 24 h at 20°C. Treatments NO-Air and NO-N_2 refer to nitric oxide fumigation that was terminated by flush with air and N_2 respectively. For each product, the values in each column followed by different letters were significantly different based on Tuckey HSD multiple range tests at $P \leqslant 0.05$ (SAS Institute, 2012). Data on fresh products are from a previous article (Liu and Yang, 2016).

In small scale fumigation tests, NO fumigation is safe to fresh products if it is terminated properly with N_2 flush to dilute NO prior to open the fumigation chamber to ambient air. These results are encouraging and need to be demonstrated in large scale trials. Commercial fresh products often are sealed in plastic packing materials such as perforated wraps and bags with very limited air exchange ability and then packed in cartons and crated on pallets. All of these restrict air exchange and increase difficulty in establishing ULO conditions for NO fumigation and diluting NO at the end of fumigation.

For delicate fresh fruits and vegetables, the additional benefits of NO fumigation for pest control on postharvest quality can have significant economic impact as it increases shelf-life and enable wider distribution of the products. Fumigation of flower bulbs for controlling bulb mites with or without N_2 flush to dilute NO also did not have any effects on their germination or growth, indicating NO fumigation was safe to propagating plant materials (Liu, 2017).

Some harvested fresh products are treated with chemical agents to maintain proper storage life. For example, diphenylamine (DPA), a plant growth regulator, is used to control storage scald of apples in USA. Nitric oxide, however, is an inhibitor of ethylene biosynthesis (Manjunatha *et al*., 2010) and can also help to maintain postharvest storage life (Wills *et al*., 2000; Soegiarto and Wills, 2004; Manjunatha *et al*., 2012; Saadatian *et al*., 2012; Liu *et al.,* 2016). It is possible that NO fumigation for postharvest pest control can also reduce or replace the usage of chemical agents such as DPA for postharvest storage of fresh fruit. This potentially bring additional benefits of NO fumigation and enhance food safety.

Nitric oxide fumigation does not leave toxic residues on fumigated products. When NO fumigation is not terminated properly by directly opening the fumigation chamber to ambient air without prior N_2 flush, the fumigation will likely cause significant increases in nitrate levels and sometimes also nitrite levels in fresh products. However, nitrate and nitrite are nutrients and they exist in both fresh and stored products at various levels (Santamaria, 2006; Hord *et al*., 2009) and the increases after NO fumigation are also well within their normal ranges in prospective products.

Nitrogen dioxide has a boiling point of about 21°C and high solubility in water. This is likely the main reason for higher NO_2 release rate 24 h after fumigation on fresh products than on stored products. Fresh products were fumigated at 2°C and stored products were fumigated at 20°C. At the end of fumigation, NO_2 from oxidation of NO will more likely to stay on fresh products because of the low ambient temperature and high relative humidity than on stored products. Retaining of NO_2 not only cause increases in nitrate and nitrite contents, also affect management of fumigated products due to increased health risks related to workers' exposures to released NO_2 from fumigated products. So, even the increases in nitrate and nitrite from NO fumigation are acceptable, it is preferably to terminate NO fumigation with N_2 flush to avoid prolonged emission of NO_2 from fumigated products, especially for fresh products since NO_2 at high levels can also cause injuries to delicate fresh products (Liu, 2016).

Due to the reactive nature of NO with O_2, NO fumigation, however, must be conducted under ULO conditions in airtight fumigation chambers. Therefore, NO fumigation requires complex and strict procedures and efforts are needed to develop protocols for commercial applications of NO fumigation. The complex fumigation procedures also add costs to NO fumigation. These costs include initial capital expenses on N_2 generation equipment and fumigation chambers and operation related costs including electricity, equipment maintenance, and NO supply. Nitrogen generation equipment is widely available commercially. Electricity cost varies depending on locations. Nitric oxide gas is also available commercially. Previous analysis suggests that NO fumigation is technically feasible and cost effective (Liu, 2015).

Nitric oxide has been studied extensively over past three decades since it was found to be a ubiquitous cell messenger. However, as a newly discovered fumigant, the mode of action of NO for pest control is still unknown. In addition, NO is known to be toxic to humans. Therefore, even NO is produced naturally by almost all organisms, it still needs to be registered as a chemical pesticide in USA in order to be used for postharvest pest control. Nitric oxide will also need regulatory approval in other countries in order to be used commercially for postharvest pest control. Participation of industry will be critical for eventual registration and commercial use of NO for postharvest pest control.

There have been extensive efforts to find alternative treatments for postharvest pest control since the start of global phase out of methyl bromide production. However, progresses are very limited and there is a severe lack of safe and effective alternative fumigants to meet the demand for postharvest pest management. Nitric oxide fumigation has high efficacy against a wide variety of pests, no toxic residues on fumigated products, and can be used on both fresh and stored products. In addition, NO fumigation has potential to extend storage life of fresh products. All these advantages of NO should far offset the disadvantages of complex and strict fumigation procedures and associated costs on acquisition and operation of N_2 generation equipment. More efforts are needed in several fronts in order to speed up the commercial applications of NO fumigation. They include research to develop effective and safe treatments for various pests on a variety of products, developmental efforts for suitable and reliable systems and protocols for commercial scale NO fumigation, including techniques to reduce emission of NO into atmosphere and registration efforts from industries to attain regulatory approval from respective countries for commercial applications.

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Bluefume (HCN) and EDN® as fumigation alternatives to methy bromide for control of primary stored product pests

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Abstract

The presented paper provides preliminary results on the fumigation potential of two preparations: Bluefume (HCN - hydrogen cyanide) and EDN®. (Ethane-dinitrile). Their biological efficacy was tested on Granary weevil (*Sitophilus granarius*; Curculionidae; Coleoptera) as a primary stored product pest in the Czech Republic. In fumigation chamber, we tested temporal survival of various *S. granarius* strains following exposure of a dose of 9 $g.m^{-3}$ HCN (Bluefume). We compared differential sensitivity of one laboratory (i.e. sensitive) CRI-strain and 9 field strains collected from the Czech stores and mills. The HCN Ct products required to kill the tested *S. granarius* strains ranged from CTp= 30.5 $g.m^{-3}.h^{-1}$ to CTp= 51.7 $g.m3.h^{-1}$. The efficacy of EDN (30 $g.m^{-3}$) on various developmental stages *S. granarius* was tested in a fumigation chamber. No live individual of *S. granarius* belonging to any life stage was recorded following 18 hours of EDN exposure.

Keywords: gas, ethane dinitrile, hydrogen cyanide, Granary weevil, Sitophilus granarius,

Introduction

Fumigation of stored product pests has become a real challenge for both farmers and pest control professionals (PCOs) in the last two decades. The reason is that broad-spectrum pesticide methyl bromide is no longer available and pest resistance to the remaining major fumigant phosphine is on the rapid increase (Nayak, et al., 2017). Therefore, the alternatives to methyl bromide or "resistance phosphine breakers" (e.g., Nayak et al., 2016) are urgently needed. However, there are only few candidate active ingredients available even at the worldwide scale (Ducom, 2006). Currently two of them (EDN and HCN) are produced in the Czech Republic (Lucebni zavody Draslovka Kolin a.s.).

HCN (Bluefume)

Various formulations of hydrogen cyanide (HCN) has previously been used for pesticide/biocide fumigation in several countries, including USA, South Korea, France, Germany, Czech Republic, and Switzerland (Rambeau et al., 2001). HCN as an active ingredient shows quick and high efficacy on structural pests infesting mills (Bond 1984, Rambeau et al. 2001, Aulicky et al., 2015a) and ships (Monro, et al., 1952). Aulicky et al., (2015a) demonstrated a higher activity of HCN on *Tribolium confusum* eggs than the one documented for phoshine during the commercial mill fumigations in Czechia (Aulicky et al., 2015b). HCN has been historically used for the fumigation of many dry