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Prospect of nitric oxide as a new fumigant for post harvest pest control

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ABSTRACT

Nitric oxide (NO) is a newly discovered potential fumigant for postharvest pest control. In laboratory tests, complete control was achieved against all insect and mite species tested to date with 0.2% to 5.0% v/v NO fumigations in 2 h to 48 h at 2 to 25°C, depending on species and life stages. Nitric oxide reacts with oxygen spontaneously to produce nitrogen dioxide, which is deleterious to fresh commodities. Nitric oxide fumigation must, therefore, be conducted in ultralow oxygen (ULO) atmospheres. Fumigation may also need to be terminated by flushing with nitrogen to dilute NO at the end of fumigation to avoid damage to delicate fresh products by nitrogen dioxide. In small-scale laboratory tests, when terminated properly with a nitrogen flush, NO fumigation was safe to postharvest quality of fresh products. In addition, NO fumigation enhanced postharvest quality of strawberrie, indicating that nitric oxide fumigation has the potential of not only controlling pests but also enhancing postharvest quality of fresh products. Laboratory studies also indicate that there is no residue concern from NO fumigation based on NO_2 emission and nitrate and nitrite levels in fumigated fresh products. Nitric oxide has the potential to be a safe and effective alternative fumigant to methyl bromide for postharvest pest control on fresh and stored products.

Key words: Fumigation, Nitric oxide, Postharvest quality, Quarantine treatment

Nitric oxide (NO) is a newly discovered potential fumigant for postharvest pest control with high efficacy against insects and mites (Liu, 2013, 2015, 2016; Liu et al. 2016). It was also demonstrated to be safe to fresh commodities and enhance postharvest quality of fresh commodities and is projected to be technical feasible and economical (Liu, 2015, 2016). Nitric oxide fumigation is as effective as methyl bromide against some insects and is much more effective and has much shorter treatment times than phosphine fumigation against all insects tested to date (Liu, 2011; Liu et al., 2013). Therefore, it has the potential to be a safe and effective alternative to methyl bromide fumigation as well as phosphine fumigation for postharvest pest control on fresh and stored products.

However, NO fumigation needs to be conducted under ultralow oxygen (ULO) environments to preserve nitric oxide during fumigation because it reacts with oxygen spontaneously to produce nitrogen dioxide. For fresh products, nitric oxide fumigation may also require to be terminated by flushing with an inert gas such as nitrogen to dilute nitric oxide before exposing fumigated products to ambient air to avoid injuries to the products by nitrogen dioxide (Liu, 2015, 2016).

Nitric oxide is a chemical produced naturally in fossil fuel combustion and lightning, by most organisms including microbes, plants, and animals, and also manufactured for fertilizer production. It functions as a ubiquitous cell signal molecule. It is used in medical fields to treat certain respiratory and cardio vascular conditions (Roberts et al., 1993; Ricciardolo et al., 2004). It also enhances postharvest quality and prolongs shelf-life of fresh fruits (Wills et al., 2000; Soegiarto and Wills, 2004; Manjunatha et al., 2012; Saadatian et al., 2012). Nitric oxide gas is commercially available from various special gas suppliers and, therefore, is readily available for

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fumigation. The cost of nitric oxide fumigation has been discussed previously and is projected to be cost effective for postharvest pest control (Liu, 2015). However, nitric oxide has not been registered in any country as a fumigant for pest control. This paper is intended to provide an update on nitric oxide fumigation research and guidance to conduct nitric oxide fumigation research. New data on efficacy and residues were also presented and discussed.

PROCEDURES OF NITRIC OXIDE FUMIGATION

Nitric oxide fumigation needs to be conducted under ULO conditions to minimize reaction of NO with O_2 to produce NO_2 which can injure fresh products at high concentrations. NO fumigation starts with flushing a fumigation chamber with N_2 gas to reduce O_2 level to a minimum level. For stored product fumigation, CO_2 can also be used to flush the fumigation chamber as CO_2 is unlikely to affect stored products. For most small fumigation tests, we used ULO levels of ≤ 30 ppm O_2. Initially, fumigation chambers were flushed with N_2 at a high flow rate. Once the O_2 level in the chambers close to the desired level, N_2 flow rate was reduced to below 1 L min^{-1}. It is important to use tubing which has low permeability to O_2 such as nylon. O_2 analyzers with zirconia sensors are recommended due to their high sensitivity and longevity.

The length of time to achieve a desired ULO level may vary greatly depending on the type of products to be fumigated. It is relatively easy to establish ULO for small fruits such as cherries and strawberries and leafy vegetables. For fresh products with large solid mass such as apples and pears, large inner cavities such as bell peppers, it may take many hours to establish ULO conditions because of the slow process of air exchange to remove O_2 inside the product. Use of vacuum may accelerate the process of establishing ULO conditions for large fruit products. For NO injection, it is easier to fill a nitrogen-washed foil bag with NO and then take NO samples from the bag using an airtight syringe to inject into fumigation chambers. It is also important to flush injection tubing with N_2 prior to nitric oxide injection.

For fumigation of packaged vegetables such as lettuce wrapped with perforated plastic film sleeves, the packaging material has only small holes for ventilation and poses as a barrier for air exchange in the process of establishing ULO conditions at the start of NO fumigation and also in the termination process of diluting NO with N_2 flush. An effective NO fumigation treatment for a specific pest can be a short treatment with high NO concentration, or a long treatment with a low NO concentration. It is recommended that packaged vegetables be fumigated with NO at low concentrations for longer durations in order to make it easier to dilute NO at the end of fumigation, thereby reducing the risk of injuries caused by NO_2.

There is a lack of suitable instruments currently to monitor NO levels for NO fumigation. High concentration NO sensors in flue gas monitors typically have a maximum concentration limit of 5,000 ppm. Most monitors draw air samples continuously to measure NO levels, and it is a challenge to use them to measure NO levels in small chambers such as 1.9 L jars used in most of our studies. 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 ratios of NO gas and the chamber volumes. For large fumigations, we used a flue gas monitor equipped with a 5,000 ppm NO sensor (Kane 900Plus, Kane International Ltd, Hertfordshire, UK) in combination with a home-made dilution accessory. The dilution device consists 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 2.0% NO can be monitored using the monitor with a 5,000 ppm NO sensor.

Additional details of NO fumigation procedures can be found from Liu (2013, 2015). Liu (2015) also presented detailed descriptions and analyses of commercial scale NO fumigation and concluded that NO is technically feasible and cost-effective. However, as NO is a new fumigant with complex procedures, industrial and scientific efforts are needed to develop suitable equipment as well as protocols for commercial applications of NO fumigation.

EFFICACY OF NITRIC OXIDE FUMIGATION

Nitric oxide is an effective fumigant against all insect and mite species tested to date at different life stages (Liu, 2013, 2015). Complete control was achieved for all pest species tested at different life stages (Table 1). Different insects and life stages varied considerably in susceptibility to NO and had different treatment times and concentration for effective control. Small soft-body external feeding insects including western flower thrips (*Frankliniella occidentalis* (Pergande)), lettuce aphid (*Nasonovia ribisnigri* (Mosley)), and longtailed mealybug (*Pseudococcus longispinus* (Targioni-tozzetti)) were controlled with 1.0% NO fumigation in 4 h and with 2.0% NO

Table 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
		2.0	2	2	
Lettuce aphid	Nymph, adult	0.2	12	2	On lettuce leaves
		0.5	9	2	
		1.0	3	2	
Long-tailed mealybug	Nymph, adult	2.0	2	2	On grape leaves
Confused flour beetle	Larva, pupa	0.5	24	20	On flour diet
	adult	0.5	8	20	
	egg	2.0	24	10	
Rice weevil	Adult	1.0	24	25	On pearled barley
	egg	1.0	48	25	
Indian meal moth	Egg	1.0	24	20	
Light brown apple moth	Larva, pupa	2.0	8	2	On artificial diet
	egg	3.0	12	2	
		5.0	6	2	
Codling moth	Egg, larva, pupa	2.0	48	2	On artificial diet
	larva	5.0	24	2	In apples
Spotted wing drosophila	Egg, larva	3.0	8	2	In sweet cherries
Bulb mites	larva, adult	2.0	24	20	On peanuts

*Data for western flower thrips, lettuce aphid, confused flour beetle, and rice weevil were from Liu (2013) and data for long-tailed mealybug were Liu (2015). Data for codling moth were from Liu et al (2016). Data for all other pest species were unpublished.

fumigation in 2 h, respectively at 2°C. Western flower thrips and lettuce aphid were also controlled with 0.2% NO in 8 and 12 h, respectively, at 2°C (Liu, 2013). Recent tests showed that light brown apple moth (*Epiphyas postvittana Walker*) larvae and pupae were successfully controlled in 8 h fumigation with 2.0% NO, and eggs were effectively controlled with 3.0% and 5.0% NO in 12 and 6 h, respectively, at 2°C (Liu, *unpublished*) (Table 1). NO fumigation was also tested on internal feeding insects in infested fruits. Spotted wing drosophila (*Drosophila suzuki* 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 5.0% concentration at 2°C resulted in 100% larval mortality (Liu et al, 2016). Bulb mites (*Rhizoglyphus* spp.) on infested peanuts (*Arachis hypogalea,* L.) were also controlled with 2% NO in 24 h at 20°C (Liu, *unpublished*) (Table 1). NO fumigation was also effective against granary weevil (*Sitophilus granarius* L.) adults under CO_2 atmosphere with 0.1% O_2. In 12 h, small-scale fumigation tests at 25°C, fumigation with 0.5% NO in CO_2 had a significantly higher weevil mortality of 76.8% than the 42.9% mortality from 0.5% NO fumigation in N_2, indicating that CO_2 can also be used to establish ULO conditions for NO fumigation (Liu, *unpublished*).

Complete control of all pests to date at different life stages indicates that NO fumigation has the potential to be an effective alternative to methyl bromide fumigation for postharvest pest control. Efficacy of NO fumigation increases with increasing concentration, treatment time, and temperature (Liu, 2013). These factors can be modified to suit for different insect species, commodities, and fumigation facilities to find an effective treatment for specific insect species. NO fumigation is more effective against pests than phosphine fumigation based on the long fumigation times and the lack of effectiveness against some pests, especially eggs, for phosphine fumigation (Liu, 2011; Liu et al., 2013).

SAFETY OF NITRIC OXIDE FUMIGATION TO PERISHABLE PRODUCTS

Nitric oxide fumigation when terminated with N_2 flush was safe to all fresh vegetables and fruits including lettuce, broccoli, cucumber, pepper, tomato, strawberries, apple, pear, orange, and lemon in small-scale tests. In a 4 h fumigation of strawberries with 1.0% nitric oxide for controlling western flower thrips at 2°C, the treatment resulted in significantly higher postharvest quality with significantly firmer and brighter, and richer colour as compared with the control one week after treatment (Liu, 2016). For delicate fresh fruits and vegetables, the additional benefits of nitric oxide 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 and tubers for controlling bulb mites with or without flush with nitrogen to dilute nitric oxide also did not have any effects on their germination or growth, indicating nitric oxide fumigation was safe to propagating plant materials (Liu, *unpublished*). Future studies need to determine safety of nitric oxide fumigation in large-scale tests. However, safe commercial application seems to be feasible given the positive outcomes of laboratory tests and the fact that nitric oxide fumigation has been reported in many studies to improve postharvest quality of many fresh products.

RESIDUES OF NITRIC OXIDE FUMIGATION IN FRESH PRODUCTS

Asparagus, broccoli, romaine lettuce, and strawberries from 16 h fumigations with 2.0% NO and apples from 16 h fumigation with 5.0% NO at 2°C were analyzed 24 h after fumigation for NO_2 emission rate, and nitrate (NO_3^-) and nitrite (NO_2^-) contents as residues using a NO analyzer (NOA 280i, GE Analytical Instruments, Boulder, CO, USA). Three treatments were used: fumigation terminated with N_2 flush (NO-N_2), fumigation terminated with air flush (NO-Air), and control. When NO fumigation is terminated any NO in the fumigation chamber is expected to react with O_2 to produce NO_2. Because products were fumigated at low temperature and stored at low temperature after fumigation and NO_2 has a high boiling point of 21°C, some NO_2 is expected to remain on products in liquid form and will evaporate overtime. To measure NO_2 emission rate, products from all three treatments for each species were sealed separately in jars and flushed with N_2 to establish ULO with ≤30 ppm O_2 at 2°C. NO_2 concentrations in the headspace were then measured twice, one hour apart. The measurements were converted to $mgkg^{-1}$ and the difference between the two measurements was NO_2 emission rate. Nitrate and nitrite were measured in filtered liquid from 15 g ground samples in 100 ml water.

NO_2 emissions of apples for both treatments with and without N_2 flush at the end of fumigation were significantly higher than the control. However, there were no significant differences in nitrate and nitrite levels between the NO-N_2 treatment and the control. Lettuce from the treatment without nitrogen flush (NO-Air) had significant increases in NO_2, NO_3^-, and NO_2^-. The NO_2 emission rate increased over 100-fold over that for the treatment flushed with nitrogen. For other products, there were no significant differences between NO-N_2 and the control in NO_2 emission and nitrate and nitrite levels (Table 2). Increases in nitrate

Table 2 NO_2 emission rate and NO_3^- and NO_2^- contents in fresh products at 24 h after nitric oxide fumigation

Product	Treatment	NO_2 (mg kg^{-1}h^{-1})	NO_3^- (mg per 100 g)	NO_2^- (mg per 100 g)
Apple	NO-Air	58.721±8.114a	1.596±0.120a	0.495±0.157a
	NO-N_2	45.613±7.442a	1.364±0.133ab	0.030±0.014b
	Control	0.019±0.005b	0.761±0.280b	0b
Asparagus	NO-Air	3.050±0.704a	2.185±0.132a	0.075±0.042a
	NO-N_2	0.387±0.052b	0.700±0.025b	0a
	Control	0.184±0.073b	0.836±0.074b	0a
Broccoli	NO-Air	0.499±0.165a	18.686±3.754a	0.170±0.063a
	NO-N_2	0.183±0.018ab	18.512±3.416a	0b
	Control	0.081±0.031b	12.258±2.307a	0b
Lettuce	NO-Air	1,643.704±395.573a	112.849±20.170a	7.987±2.015a
	NO-N_2	13.452±5.189b	38.966±5.869b	0.098±0.079b
	Control	14.677±13.652b	40.641±10.806b	0b
Strawberry	NO-Air	3.322±1.147a	6.014±0.620a	0
	NO-N_2	0.334±0.055b	5.299±0.765a	0
	Control	0.079±0.018b	6.162±1.061a	0

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 $P \leq 0.05$ using JMP statistical discovery software (SAS Institute, 2012) content in the NO-Air treatment were likely due to deposition and acidification of NO_2 from the NO and O_2 reaction as the treatment was terminated by flushing with air. The increases in nitrate also coincided with a significant increase of nitrite in apples and broccoli. Though the increases in nitrate and nitrite levels were not very high as compared with their normal ranges (Hord et al., 2009), flushing with N_2 at the end of fumigation is necessary not only to prevent injuries, but also to avoid increases in nitrate and nitrite levels. The higher level of NO_2 emission in the NO-N_2 for apple was likely due to the large solid mass of apples and therefore a slower process of desorption. Longer post-treatment time will likely be required for NO_2 emission to return to a normal level as in the control. The NO_2 levels in the controls were likely due to baseline variations of the instrument and might also include a minor contribution from NO naturally produced by the products. The significant higher nitrate level in lettuce from the treatment without nitrogen flush was likely due to NO_2 deposited on lettuce and NO_2 was converted into nitrate. These experiments indicated that it is necessary to terminate nitric oxide fumigations with N_2 flush to prevent injuries to products as well as any accumulations of nitrate and sometimes nitrite in some fumigated fresh products.

CONCLUSION

There is an urgent need for alternatives to methyl bromide fumigation for postharvest pest control (Fields and White, 2002; Jamieson et al., 2009). Nitric oxide fumigation has been demonstrated to be effective against all insects and mites tested to date with treatment times ranging from a few hours to two days, and is effective against all life stages tested. Its efficacy varies depending on pest species and their life stages and combinations of concentration, time and temperature. Nitric oxide fumigation can also be conducted safely on fresh products when it is terminated properly with N_2 flush to dilute NO. NO fumigation may also enhance postharvest quality of fresh products such as strawberries. NO fumigation did not have a significant accumulation of residues as measured by increases of NO_2 emission and levels of nitrate and nitrite in fumigated fresh products.

Nitric oxide fumigation has not been studied for postharvest pest control on stored products. However, given its efficacy against stored product insects and expected high tolerance of stored products to NO_2 than fresh products, NO fumigation is expected to be suitable for postharvest pest control on stored products. Stored products are typically dry and fumigation will most likely be conducted at ambient temperature instead of low temperature for fresh products. Therefore, NO_2 will more likely to dissipate quickly and less likely reacts with water to form HNO_3 acid and increase nitrate and nitrite levels of the stored products.

The drawbacks of NO fumigation are the complex fumigation procedures, the added cost for an inert gas supply such as N_2, and release of NO_2 pollutant into the atmosphere, though the quantity will be very small as compared with the large quantities being produced from other sources such as motor vehicles and fossil fuel power plants. However, the advantages of nitric oxide fumigation are expected far offset its disadvantages.

Nitric oxide is commercially available and the cost of NO fumigation is moderate. The main cost components are the initial acquisition costs of N_2 generation equipment and fumigation chambers, and subsequent costs of energy and equipment maintenance. Existing fumigation chambers and controlled atmosphere rooms, if sealed properly, can be used for NO fumigation with minimum modifications,e.g., adding or upgrading O_2 analyzers.

Nitric oxide is a promising new alternative fumigant for postharvest pest control. More research and development efforts are warranted to accelerate its commercial applications, including development of safe and effective treatments against a wide variety of pests on different fresh and stored products, commercial treatment protocols and facilities, scrubbing technology to remove nitric oxide at the end of fumigation, and registration of nitric oxide in respective countries as a fumigant for pest control usage.

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