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Nitric oxide fumigation for postharvest control of pests and pathogens

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Abstract

Nitric oxide (NO) is a recently discovered fumigant and NO fumigation has been demonstrated to be effective against all insects and mites tested to date, including external and internal pests, fresh and stored product pests. However, as NO reacts with O_2 spontaneously to form nitrogen dioxide (NO_2), NO fumigation must be conducted under ultralow oxygen conditions. As it is impractical to remove all oxygen, NO fumigation always has NO_2 as a result of interaction of NO with residual oxygen in fumigation chamber. Recently, NO_2 was demonstrated to be effective against microbes. Effective control of *Aspergillus flavus* spores was achieved in NO and NO_2 fumigations. Complete control of bacteria and fungi on stored almonds and peanuts were also achieved in NO_2 fumigations. Therefore, NO fumigation has potential to control both pest and pathogens. When conducted properly, NO fumigation is also safe for use on fresh products and helps to maintain storage/shelf-life of fresh fruit. Nitric oxide fumigation does not leave toxic residues on fresh or stored products. These studies indicate that NO fumigation is feasible to control both insects and microbes in a single fumigation treatment. This is important in expanding potential applications of NO fumigation and making NO fumigation more cost effective for potential commercial applications.

Keywords: Nitric oxide, Nitrogen dioxide, Fumigation, Pest control, Microbial control, Almond, Peanuts, Stored products, *Aspergillus flavus*

Introduction

Nitric oxide (NO) is a chemical produced naturally in fossil fuel combustion and lightning and commercially as an intermediate in fertilizer production. Nitric oxide was discovered in 1980s to be a ubiquitous cell messenger molecule and has since been found to play diverse functions in physiological and biochemical processes in organisms (Lamattina et al., 2003). Nitric oxide is 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 (Soegiarto and Wills, 2004; Manjunatha et al., 2012). Recently, NO is found to be a potent fumigant for postharvest pest control and is effective against all insects and mites tested to date (Liu, 2013, 2015; Liu and Yang, 2016, 2018).

Nitric oxide fumigation is also safe to postharvest quality of fresh products when conducted properly (Liu, 2016, 2017; Liu and Yang, 2018; Yang and Liu, 2018a). Nitric oxide fumigation does not leave significant nitrate and nitrite as residues on both fresh and stored products when conducted properly (Yang and Liu, 2017, 2019). In the past few years, NO_2 fumigations were found to be effective against microbes (Liu et al., 2019; Oh and Liu, 2020; Oh et al., 2020). Therefore, it is possible that a single NO fumigation treatment can effectively control pests and microbes on stored products and, thereby, make NO fumigation more useful and cost effective. In this paper, NO fumigation research was reviewed and discussed with an emphasis on the most recent studies on the efficacy of NO fumigation against insects and microbes.

Nitric oxide fumigation

Nitric oxide reacts with oxygen spontaneously to form nitrogen dioxide (NO_2) (Beckman and Koppenol, 1996). Therefore, NO fumigation must be conducted under ultralow oxygen (ULO) conditions to preserve NO. Procedures for NO fumigation have been thoroughly described and demonstrated (Liu and Yang, 2016; Liu et al., 2017). The key points are airtight seal of fumigation chamber and flushing with nitrogen gas at the beginning of the process to establish ULO and at the end to dilute NO in the fumigation chamber to prevent NO_2 formation.

Nitric oxide fumigation has more stringent requirements on fumigation apparatus and procedures due to the requirement of ULO environment and the need to keep the fumigation chamber airtight. For commercial scale NO fumigation, N_2 supply expenses include costs of air compressor and N_2 generator, and operating costs for N_2 generation including energy and maintenance costs. This adds extra expense to NO fumigation and makes NO fumigation more expensive. Costs of fumigation equipment and energy to operate N_2 generation equipment for NO fumigation was previously analyzed (Liu, 2015). Based on the numbers of air exchanges with the N_2 flush, volumes of N_2 and energy cost can be estimated for establishing ULO conditions for NO fumigation. It is estimated that energy cost for initial establishment of ULO conditions for NO fumigation and N_2 flush at the end of NO fumigation is moderate. The added expenses related to N_2 generation may be compensated by the benefits of NO fumigation as compared with fumigations with more toxic fumigants that are less effective and leave toxic residues in fumigated food products (Liu, 2015).

As it is impractical to remove all oxygen in a fumigation chamber especially in commercial scale fumigations, NO fumigation always contains certain levels of NO_2. Nitrogen dioxide; however, has strong antimicrobial property. Nitrogen dioxide fumigation was demonstrated to kill *Aspergillus flavus* spores and control microbes on stored products (Liu et al., 2019; Oh and Liu, 2020; Oh et al., 2020). In fact, NO_2 levels in NO fumigation can be controlled by controlling ULO levels. Therefore, NO fumigation can be established with desired NO and NO_2 levels and may have potential to control both pests and pathogens on stored products.

Efficacy of NO fumigation against postharvest pests

Nitric oxide fumigation has been demonstrated to be effective against over 14 pest species tested to date at different life stages (Liu, 2013, 2015; Liu and Yang, 2016; Liu et al., 2018; Yang et al., 2020) (Table 1). Insect species and their life stages differ in susceptibility to NO fumigation.

Table 1. Fumigation treatments with NO at different concentrations for different durations at different temperatures that resulted in 100% mortality of insects and mites at different life stages.

Species	Life stage	NO (%)	Time (h)	Temp (°C)
Western flower thrips	larva, adult	0.2	8	2
		2.0	2	2
Lettuce aphid	nymph, adult	0.2	12	2
		0.5	9	2
		1.0	3	2
Long-tailed mealybug	nymph, adult	2.0	2	2
Light brown apple moth	egg	3.0	12	2
	larva, pupa	2.0	8	2
Spotted wing drosophila	egg, larva (in cherries)	3.0	8	2
Codling moth	egg, larva, pupa	2.0	48	2
	large larva (in apples)	5.0	24	2
Indianmeal moth	egg	1.0	24	20
Naval orangeworm	egg	2.0	16	25
	larva (in walnuts)	1.0	8	25
Confused flour beetle	egg	2.0	24	10
	larva, pupa	0.5	24	20
	adult	0.5	8	20
Rice weevil	egg	1.0	48	25
	adult	1.0	24	25
Granary weevil	adult	1.0	24	25
False spider mites	larva, adult	0.5	6	2
Bulb mites	larva, adult	2.0	24	20
Ham mites	egg	2.0	8	25
	larva, adult	2.0	4	25

Small external soft body insects on fresh products are more susceptible to NO fumigation than insects on stored product and insects that feed internally on fruits and vegetables. The treatment time is shorter for mobile stages than for pupa and egg stages (Liu, 2013, 2015; Liu and Yang, 2016). It takes a few hours to control external feeding insects including western flower thrips (*Frankliniella occidentalis* (Pergande)), lettuce aphid (*Nasonovia ribisnigri* (Mosley, 1841)), and longtailed mealybug (*Pseudococcus longispinus* (Targioni Tozzetti)) with NO fumigations at ≤1.0% at a low temperature of 2°C (Liu, 2013). Internal feeding larvae of spotted wing drosophila (*Drosophila suzukii* (Matsumura)) in infested cherries takes 8 h to control with 2.5% NO fumigation (Liu and Yang, 2016). Codling moth (*Cydia pomonella* L.) larvae in infested apples take 24 h NO fumigation at 5.0% concentration at 2°C to achieve complete control (Liu et al., 2016). Nitric oxide fumigation at 1-2% concentrations takes 24 to 72 h at 15-25°C to control stored product insects including Indian meal moth (*Plodia interpunctella* (Hübner)), confused flour beetle (*Tribolium confusum* Jacquelin du Val), and rice weevil (*Sitophilus oryzae* (Linnaeus)) (Liu, 2013, 2015; Liu and Yang, 2016). Nitric oxide fumigation under ULO established with CO_2 flush is also effective against stored product insects (Liu, 2020). Nitric dioxide fumigation was also tested against Navel orangeworm (*Amyelois transitella* (Walker)) on an artificial diet and in infested walnuts. Navel orangeworm eggs are more tolerant to NO fumigation than larvae and pupae and complete control of eggs was achieved in 8 and 16 h fumigation with 3.0 and 2.0% NO, respectively (Yang et al., 2020). Nitric dioxide fumigation is also effective against mites. Bulb mites (*Rhizoglyphus spp.*) on infested peanuts were controlled with 2.0% NO in 24 h at 20°C (Liu, 2017). Complete control of false spider mites (*Brevipalpus phoenicis* (Geijskes)) and ham mites (*Tyrophagus putrescentiae* (Schrank)) was also achieved (Table 1). All of these results show that NO fumigation has good efficacy against all pests at any life stages.

Effects of NO and NO_2 fumigation on microorganisms

Both NO and NO_2 can kill microbes (Table 2). However, NO_2 is far more effective in controlling microbes than NO. In 3 h fumigation tests, 0.1% NO_2 had complete control of *A. flavus* spores (Liu et al., 2019). Nitrogen dioxide fumigation is also effective in controlling both bacteria and fungi on almonds and unshelled peanuts (Oh and Liu, 2020; Oh et al., 2020). Unpasteurized almonds were fumigated with NO_2 at 0.1, 0.3, or 1.0% concentrations by inject NO under ambient O_2 for 1 and 3 d at 25°C. A rapid enumeration test was used to determine microbial loads in diluted wash-off samples from NO_2 fumigated almonds and controls. Nitrogen dioxide fumigation treatments showed either greatly reduced microbial loads or complete control of microorganisms, depending on NO_2 concentration and treatment duration. Nitrogen dioxide fumigation was more effective against fungi than against bacteria. Effective control of microbes was also achieved on unshelled peanuts. Bacteria and fungi on outer surfaces and inside unshelled peanuts were effectively controlled with 3 d NO_2 fumigations and complete control of both bacteria and fungi was achieved with 1.0% NO_2 fumigations (Oh et al., 2020) (Table 2). These results suggest that postharvest NO fumigation with proper levels of NO and NO_2 can be used for insect and microorganism control on stored almonds and peanuts.

Safety of NO fumigation

The safety of fumigation treatment for pest and disease management includes preserving product quality and enhancing consumer safety by reducing toxic residues on fumigated products. When conducted properly, NO fumigation is safe to fresh product quality and also does not leave unacceptable residues on fumigated products. In small scale tests, NO fumigation was demonstrated to be 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 no significant differences between the treatment and the control (Liu, 2016; Yang and Liu, 2018a, 2018b). When NO fumigation is terminated by directly opening the fumigation chamber to ambient air without flushing with N_2 gas, 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 when NO fumigation was terminated without N_2 flush (Liu, 2016). The key aspect to ensure safety of NO fumigation to fresh products is to prevent or reduce exposure of products to NO_2 at a high level that causes damage to fresh products.

Table 2. Effects of NO and NO_2 fumigation on relative colony forming unit (CFU) of microorganisms on artificial media and stored products at 25°C.

Source of microorganism	Treatment	Relative CFU (%)
Aspergillus flavus spores on	Control	100
cellulose filter discs	0.1% NO, 3h	79.2
	1.0% NO, 3h	0
	0.1% NO_2, 3h	0
Bacteria and fungi on almonds	Control	100
	0.1% NO_2, 24h	68.5
	0.3% NO_2, 24h	27.5
	1.0% NO_2, 24h	0
Fungi on almonds	Control	100
	0.1% NO_2, 24h	5.5
	0.3% NO_2, 24h	0
	1.0% NO_2, 24h	0
Bacteria and fungi on outer surfaces	Control	100
of intact unshelled peanuts	0.3% NO_2, 72h	8.3
	1.0% NO_2, 72h	0
	3.0% NO_2, 72h	0
Bacteria and fungi on inside and outer	Control	100
surfaces of cracked unshelled peanuts	0.3% NO_2, 72h	6.2
	1.0% NO_2, 72h	0
	3.0% NO_2, 72h	0
Fungi on outer surfaces	Control	100
of intact unshelled peanuts	0.3% NO_2, 72h	2.0
	1.0% NO_2, 72h	0
	3.0% NO_2, 72h	0
Fungi on inside and outer surfaces	Control	100
of cracked unshelled peanuts	0.3% NO_2, 72h	16.9
	1.0% NO_2, 72h	0.2
	3.0% NO_2, 72h	0

Nitric oxide fumigation can extend postharvest storage and shelf life of fresh products due to its antagonistic effects on ethylene biosynthesis (Soegiarto and Wills, 2004; Manjunatha et al., 2010, 2012). Nitric dioxide fumigations for control of western flower thrips and codling moth also result in better postharvest quality of strawberries and apples respectively (Liu, 2016; Liu et al., 2016). These results are consistent with other studies and suggest that NO fumigation for postharvest pest control may also provide additional benefits of extended storage/shelf-life to some fresh products.

Nitrogen dioxide, nitrate, and nitrite are expected residues of NO fumigation and N_2 flush is critical to prevent significant accumulations of these residues. When NO fumigation is terminated with a N_2 flush, most NO will be flushed out to prevent its reaction with O_2 to form NO_2. As NO_2 has a high boiling point of about 21°C and readily reacts with water, NO_2 is expected to be adsorbed onto fumigated products and may be converted to nitrate (NO_3^-) and nitrite (NO_2^-) as residues.

Twenty fresh products and 10 stored products were studied for residues after NO fumigation treatments (Yang and Liu, 2017, 2019). For most fresh products, when NO fumigation is terminated properly with N_2 flush, it does not result in significantly higher nitrate or nitrite levels as compared with controls (Yang and Liu, 2017). For the 10 stored products, NO fumigation also does not significantly increase nitrate or nitrite levels in fumigated stored products as compared with controls (Yang and Liu, 2019). When NO fumigation is terminated without N_2 flush, there are significant increases in nitrate and sometime also nitrite levels in fumigated fresh and stored products (Yang and Liu, 2017, 2019). Nitrate and nitrite naturally exist in food products and have nutritional values as they may contribute to the blood pressure–lowering effects and nitrate is an important part of our bodies' defenses against gastroenteritis (Santamaria, 2006; Hord et al., 2009). Nitric oxide fumigation, therefore, does not leave toxic residues in fumigated products and is safe to food quality and human health.

Prospects of NO fumigation

Efficacy of NO fumigation has been well demonstrated against different life stages of 14 pest species representing diverse pest groups including external and internal feeders, fresh and stored product pests. It is, therefore, reasonably to expect that NO fumigation will be effective against most other pests. Because it is technically feasible to establish NO fumigation with desired levels of NO and NO_2, and NO_2 fumigation was demonstrated to be effective against microbes on stored almonds and peanuts as well as *Aspergillus flavus* spores, NO fumigation has the potential to control pests and pathogens in a single fumigation treatment. This is expected to expand usage of NO fumigation, make NO fumigation more cost effective, and promote its commercial applications. However, approval of NO fumigation for pest and microbial control by regulatory agencies must occur before any prospect for commercial application of NO fumigation can be realized. Active involvement of industry is critical in the regulatory approval process and development of specialized fumigation equipment for NO fumigation. The anticipated expanded use of NO fumigation for control of both pests and microbes is likely to make NO fumigation more attractive to industry to increase efforts to register NO fumigation for commercial applications.

Nitric oxide has advantages in efficacy in comparison with the commercial alternatives: phosphine, sulfuryl fluoride, and ethyl formate. Phosphine, as a major methyl bromide alternative fumigant for postharvest pest control, is not effective against some pests due to tolerance or resistance and phosphine fumigation and, in general, also has long treatment times which may extend over 10 d to achieve effective control of some pests (Hole et al., 1976). Although phosphine fumigation in an oxygen enriched atmosphere (oxygenated phosphine fumigation) has significantly increased the efficacy of phosphine fumigation against phosphine tolerant insects (Liu, 2011), the prospect for commercial application of oxygenated phosphine fumigation remains unclear. Sulfuryl fluoride has the disadvantage of being ineffective against insect eggs (Bell et al., 1998) and having phytotoxicity to fresh products (Aung et al., 2001). The absorbing rate of ethyl formate in fresh products and its phytotoxicity on fresh products are also concerns for wide applications of ethyl formate fumigation (Zoffoli et al., 2013). In contrast, NO fumigation is not only effective against all pests and all life stages but also controls microbes that often occur with pest infestation and need effective control.

For delicate fresh fruits and vegetables, NO fumigation may have additional benefits of extending storage/shelf-life. Some harvested fresh products are treated with chemical agents to maintain proper storage life. Nitric dioxide; however, is an inhibitor of ethylene biosynthesis (Manjunatha et al., 2010) and can also help to maintain postharvest storage life (Soegiarto and Wills, 2004; Manjunatha et al., 2012; Liu et al., 2016; Yang and Liu, 2018a). It is possible that NO fumigation for postharvest pest control can also reduce or replace the usage of chemical agents for postharvest storage of fresh fruit. This suggests NO fumigation may provide additional benefits and enhance food safety.

There have been extensive efforts with limited progresses to find alternative treatments for postharvest control of pests and microbes to replace methyl bromide. Therefore, there is a severe lack of safe and effective alternative fumigants to meet the demand for postharvest pest and disease management. As a recently discovered new fumigant, NO fumigation has high efficacy against a wide variety of pests and associated NO_2 control of microbes. Furthermore, the lack of toxic residues and extended storage life of fresh products treated with NO fumigation should far offset the disadvantages of the complex and strict fumigation procedures and associated costs on acquisition and operation of N_2 generation equipment. Therefore, more efforts are warranted to speed up the commercial applications of NO fumigation including developing effective and safe treatments for specific pests on a variety of products, developing and demonstrating commercial scale treatment protocols, and registration efforts from industries for commercial applications.

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