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Short Communication

Nitric oxide fumigation for control of western flower thrips and its safety to postharvest quality of fresh fruit and vegetables☆

Yong-Biao Liu

Crop Improvement and Protection Unit USDA-ARS, Salinas, CA 93905, USA

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ABSTRACT

Nitric oxide fumigation under ultralow oxygen conditions was studied for controlling western flower thrips and effects on postharvest quality of fresh fruit and vegetables. Four hour fumigation with 1.0% nitric oxide at 2 °C and 3 h fumigation with 2.0% nitric oxide at 5 °C achieved complete control of the thrips. The 4 h treatment was tested on 10 fresh fruit and vegetables including lettuce, broccoli, pepper, squash, tomato, apple, lemon, orange, peach, and pear. When the treatment was terminated by flushing with nitrogen to dilute nitric oxide before exposing the products to ambient air, the treatment had no negative impact on quality of the products. When the treatment was terminated by flushing with air to allow nitric oxide to reacts with oxygen in the air to form nitrogen dioxide, the treatment caused injuries to the majority of the fresh products. Fresh products with thick and robust skins were more tolerant than those with thin and delicate skins to nitric oxide fumigation. The 4 h fumigation with 1.0% nitric oxide of strawberries at 2 °C had positive impact on strawberry quality with enhanced firmness, brighter and richer color than the controls. The study demonstrated efficacy of nitric oxide fumigation in controlling western flower thrips, safety to fresh commodities, and benefits to postharvest quality of strawberries.

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Introduction

Postharvest pest control is critical to international trade and food security. As methyl bromide production is being phased out globally, its future availability for postharvest pest control is bleak and alternative treatments are urgently needed. However, there is a severe lack of new alternative treatments especially for fresh commodities. Phosphine has now became the dominant methyl bromide alternative for postharvest pest control. However, phosphine is not effective against some pests because of tolerance or pesticide resistance and also has long treatment times (Hole et al., 1976; Schlipalius et al., 2002). In comparison, nitric oxide, a newly discovered fumigant, has the potential to become an effective and safe methyl bromide alternative for postharvest pest control.

Nitric oxide (NO) is a natural cell messenger chemical in most biological systems including insects and has been used to treat respiratory and cardiac illnesses (Culotta and Koshland, 1992, Roberts et al., 1993, Rossaint et al., 1993, Müller, 1997, Davies, 2000, Ricciardolo et al., 2004). It is also released as a common pollutant from fossil fuel combustion in power plants and motor vehicles in large quantities and produced commercially as an intermediate in fertilizer production. In agriculture, nitric oxide has been used over 100 years ago for red pigment preservation in preserved meat products (Haldane, 1901). More recently, nitric oxide was found to extend shelf-life and enhance postharvest quality of a wide variety of fresh products including strawberries (Wills et al., 2000; Soegiarto et al., 2003; Soegiarto and Wills, 2004; Wills et al., 2007; Manjunatha et al., 2012; Saadatian et al., 2012).

In a recent study, nitric oxide fumigation under ultralow oxygen atmosphere was found to be effective against a wide variety of insects at different life stages (Liu, 2013). Effective control of western flower thrips (*Frankliniella occidentalis*) and lettuce aphid (*Liriomyza langei*), were achieved in 3 h at a high concentration of 2.0% at 2 °C. All life stages of confused flour beetle (*Tribolium confusum*) and rice weevil (*Sitophilus oryzae*) were controlled in 24 h with 2.0% NO at ≥ 10 °C and 48 h with 1% NO at 25 °C respectively (Liu, 2013). Effective controls of codling moth (*Cydia pomonella*) and spotted wing drosophila (*Drosophila suzukii*) were also achieved (Liu, 2015; Liu et al., 2016). These results indicate the good potential of nitric oxide fumigation for postharvest pest control. Treatment times of nitric oxide fumigation for controlling western flower thrips and lettuce aphid are much shorter than those of low temperature phosphine fumigation. Low temperature phosphine fumigation takes at least 18 h and 72 h to control western flower thrips and lettuce aphid respectively at 2 °C (Liu, 2008, 2012). Therefore, although data are still limited, at least for some insects, nitric oxide fumigation is much more effective than phosphine fumigation. In addition, nitric oxide fumigation does not leave toxic residues on fumigated fresh products (Liu and Yang, 2016).

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E-mail address: yongbiao.liu@ars.usda.gov.

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Nitric oxide reacts with oxygen spontaneously to form nitrogen dioxide (Ashmore et al., 1962). Therefore, nitric oxide fumigation needs to be conducted under ultralow oxygen conditions to minimize oxidation of nitric oxide. Feasibility of practical use of nitric oxide fumigation for postharvest pest control also depends on the safety of the treatment to product quality. Even though nitric oxide fumigation has been demonstrated to be beneficial to shelf-life of many fresh products, the concentrations of nitric oxide used in the treatment for postharvest quality enhancement are much lower as compared with the concentrations used for postharvest pest control (Wills et al., 2000; Soegiarto et al., 2003; Soegiarto and Wills, 2004; Wills et al., 2007; Manjunatha et al., 2012; Saadatian et al., 2012; Liu, 2013). It is therefore important to know whether nitric oxide fumigations at high concentrations can be conducted safely on fresh products and whether the fumigation treatments for pest control can still have beneficial effects on postharvest quality. Strawberries are delicate and have a relatively short postharvest shelf-life. It would be an attractive option if nitric oxide fumigation can be used to control quarantine pests as well as to extend shelf-life of strawberries.

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is a common pest on a variety of crops in the U.S. However, it is a quarantine pest in Taiwan and therefore affects exports of U.S. fresh fruit and vegetables. An alternative treatment is needed to control the pest on fresh commodities. In this study, nitric oxide fumigation for control of western flower thrips was further refined and was tested on fresh fruit and vegetables including strawberries to determine whether nitric oxide fumigation can effectively control western flower thrips and is safe to postharvest quality of fresh fruit and vegetables.

Materials and methods

Chemicals and insect

Nitric oxide (>99.5% purity) in a compressed cylinder was obtained from a commercial source. It was then released and stored in a foil bag (7.5 mils MylarFoil, 20 cm by 40 cm, Impak Corp., Los Angeles, CA) to be used in fumigations tests. Commercial grade nitrogen gas in compressed cylinders from a commercial source (Praxair, Salinas, CA) was used. Western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), from naturally infested lettuce plants in a greenhouse were reared on lettuce plants in a greenhouse at 18–30 °C under natural lighting (Liu, 2008). Both larvae and adults were collected in small plastic vials (3 cm in diameter by 7 cm in height) each containing a piece of lettuce leaf using a vacuum powered aspirator (ca. 20/vial). The vials with thrips were sealed with screened lids and kept at 2 °C overnight in a temperature chamber before being used in fumigation tests.

Nitric oxide fumigation of western flower thrips

Thrips in plastic vials were fumigated in 1.9 L glass jars for 4 h at 2 °C with nitric oxide under ultralow oxygen (ULO) conditions using procedures of Liu (2013). Sealed fumigation jars were first flushed with nitrogen gas to establishing ultralow oxygen levels of ≤30 ppm. Oxygen levels in the jars were monitored using an oxygen analyzer (Series 800, Illinois Instruments, Inc., Johnsburg, IL, USA). Nitric oxide gas was then injected into each treatment jar using an airtight syringe in a fume hood to establish pre-calculated nitric oxide concentrations of 0.3%, 0.5%, 0.7%, and 1.0% based on volume. The syringe and connecting tubing were flushed with nitrogen prior to injection of nitric oxide to avoid oxidation of nitric oxide. Untreated thrips in the normal air and under ULO conditions were used as controls. After nitric oxide injection, the jars were kept at 2 °C in a temperature chamber for the duration of the 4 h to complete the treatment.

Fumigation treatments were terminated by venting the jars under a fume hood. Thrips were kept overnight in an environmental chamber at 22 °C, 90–95% RH, and a 14:10 (L:D) photoperiod before being scored for mortality. Thrips that were motionless, didn't respond to probes with a soft brush, or capable of moving appendages but unable to move were classified as dead. Each treatment was replicated four times. A total of 2433 thrips were used.

Nitric oxide fumigation of fresh fruit and vegetables

A total of 10 fresh fruit and vegetables were subjected to nitric oxide fumigation treatments with 2.0% nitric oxide under ≤50 ppm O_2 for 3 h at 5 °C to determine effects on postharvest quality of fresh products. All products were from supermarkets and they were apple, broccoli, lemon, romaine lettuce, orange, peach, pear, pepper, squash, and tomato. They were fumigated together in 21.8 L chambers modified from pressure cookers. Two fumigation treatments were tested and both were identical except that one treatment (NO-N_2) was terminated by flushing the chamber with nitrogen to dilute NO before opening the chamber to ambient air and the other treatment (NO-Air) was terminated by flush the chamber with air to allow nitric oxide to react with oxygen in the air to produce nitrogen dioxide. As nitric oxide reacts with oxygen spontaneously and exothermally to produce nitrogen dioxide, the opening of the fumigation chambers at the end of fumigation treatments may result in momentary exposure of fumigated products to the reaction between nitric oxide and oxygen as well as its product nitrogen dioxide. The treatment terminated by flushing with nitrogen was to demonstrate whether flushing with nitrogen at the end of fumigation could prevent any possible negative impact of nitric oxide fumigation on product quality. Controls were stored at 5 °C during the fumigations.

In each test, most products had 2–3 individuals per treatment. Apple and pear each had 5 in each treatment. Products were placed in perforated plastic bags in groups and then sealed in the fumigation chambers. Western flower thrips larvae and adults contained in small plastic vials were also included in both treatments to be fumigated. Untreated controls were stored at 5 °C during fumigation. The chambers were flushed with nitrogen through two outlets to establish an ULO condition of ≤50 ppm and were then injected with predetermined volumes of nitric oxide gas to establish a calculated concentration of 2.0% under a fume hood in room temperature. The chambers were then kept in a refrigerator at 5 °C for 3 h to complete the fumigation. At the end of the fumigation, the NO-N_2 treatment chamber was flushed with nitrogen at 5 L/min for 20 min and the NO-Air treatment chamber was flushed with air at 5 L/min for 20 min in a fume hood before they were open to the ambient air. After fumigation, western flower thrips were stored overnight in an environmental chamber before being scored for mortality as described above.

All products from both treatments and the control were placed in plastic boxes and stored at 2 °C for 14 days before being scored for postharvest quality. The test was replicated three times. The products' marketability was visually scored 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, 2012). Injuries from fumigation in the form of dead tissues were also recorded.

Nitric oxide fumigation of strawberries

Harvested fresh strawberries together with western flower thrips were fumigated with 1.0% NO for 4 h at 2 °C to determine efficacy and the effects of the treatment on strawberry quality. Commercial fresh strawberries in retail clamshell plastic boxes were obtained one day after harvest from Driscoll's (Watsonville, CA), chilled to 2 °C in a temperature chamber, and immediately used in fumigation tests. The fumigation test had only one treatment and a control and all fumigations of strawberries were conducted in the 21.8 L chambers. The fumigation treatment was terminated by flushing with nitrogen gas. The fumigation chamber was first flushed with nitrogen gas to establish an ULO level of <50 ppm. Nitric oxide gas from the foil bag was then injected