

PROSPECT OF NO FUMIGATION FOR POSTHARVEST PEST CONTROL

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Nitric oxide (NO) is a common signal molecule in biological systems. It regulates diverse physiological processes in insects including reproduction, locomotion, olfaction, learning and memory, and host defense mechanism, and was not known to be insecticidal. However, under ultralow oxygen conditions, nitric oxide was found to be a potent fumigant against a wide variety of insects. This discovery has promise to offer an effective and environmental friendly alternative to methyl bromide and other toxic fumigants for postharvest pest control.

Several insects including western flower thrips (*Frankliniella occidentalis*), lettuce aphid (*Nasonovia ribisnigri*), confused flour beetle (*Tribolium confusum*), and rice weevil (*Sitophilus oryzae*) at various life stages were fumigated with nitric oxide under ≤ 50 ppm oxygen in 1.8 L glass jars. Jars were flushed with nitrogen to establish ≤ 50 ppm ultralow oxygen levels. Nitric oxide gas was injected into the jars to establish a range of calculated concentrations of 0.1 to 2.0% based on volume. The jars were then incubated in temperature chambers at certain temperatures for various durations specific for each pest species and life stage. Fumigation chambers were vented in a fume hood at the end of each treatment. Western flower thrips and lettuce aphid were fumigated with 0.1 to 2.0% nitric oxide for 1 to 12 h at the low temperature of 2°C to be compatible with cold storage condition for fresh products. Different life stages of confused flour beetles and rice weevils were subjected to fumigations with 0.5 to 2.0% nitric oxide for 8 to 48 h at 10 to 25°C.

Nitric oxide fumigation was effective against all insects. At 2°C, western flower thrips were controlled with 8 h fumigation at 0.2% NO and 2 h fumigation at 2.0% NO. Close to 100% mortalities were also achieved in other treatments. Complete control of lettuce aphid was achieved in fumigations of 12 h at 0.2% NO, 9 h at 0.5% NO, and 3 h at $\geq 1.0\%$ NO. All life stages of confused flour beetle were susceptible to nitric oxide fumigations. Complete control of larvae, pupae, and adults was achieved in 24 h with 0.5% NO fumigation at 20°C and complete

control of eggs was achieved in 24 h with 2.0% NO fumigation at 10°C. Rice weevil adults had mortalities of 77.6 and 93.0% in 24 h fumigations with 0.5% NO at 20 and 25°C respectively and 100% mortality in the 24 h fumigation with 1.0% NO at 25°C. The 48 h fumigations with 0.5 and 1.0% NO of infested barley resulted in 99.6 and 100% reductions in adult emergence as compared with the control. These results demonstrated that nitric oxide fumigation is effective against all life stages of different types of insects, and complete control can be achieved in 2 to 48 h depending on insect species and life stages as well as nitric oxide concentrations and temperature. Efficacy of nitric oxide fumigation increased with temperature, time, and concentration. In most tests, controls under ultralow oxygen also had higher mortality rates as compared with the controls under the normal level of oxygen.

To assess suitability of nitric oxide fumigation for fresh commodities, a variety of fresh vegetables and fruits including lettuce, broccoli, bell peppers, cucumber, squash, apple, kiwi, lemon, orange, and pear, were tested. They were held in perforated plastic bags and loaded in three 21.8 L chambers in each test. Two chambers were subjected to 2.0% NO fumigation treatment under ≤ 50 ppm O₂ for 3 h at 5°C, and the third chamber was not sealed and was used as a control. The first treatment was terminated by flushing the chamber with nitrogen at 5 L/min for 20 min to reduce nitric oxide level before opening the cover to expose the contents to ambient air. The second treatment was terminated by flushing with air at 5 L/min for 20 min before opening the cover. Each treatment was replicated 3 times. After fumigation, fruits and vegetables were stored in plastic boxes at 2°C in a walk-in cooler. Postharvest quality was inspected visually after two weeks. Any injuries in the forms of discoloration, dark spots, or patches of dead tissues were recorded.

For the fumigation treatment that was terminated by flushing with nitrogen first, no obvious injury or quality reduction was found. The fumigation treatment that was terminated by direct venting with ambient air resulted in injuries to several products and reduced postharvest quality. Fresh fruits and vegetables varied greatly in sensitivity to nitric oxide fumigation. Lettuce, broccoli, bell pepper, squash, cucumber, and pear sustained injuries in forms of tissue death, localized discoloration, or dark spots, and the extent of injuries varied among different species. Other products including lemon, orange, apple, and kiwi did not show any obvious injury. The difference between the two treatments in their impact on product quality indicates that injuries were due to nitrogen dioxide as nitric oxide reacts with oxygen during the

termination process. These results suggest that nitric oxide fumigations can be used safely without negative impact on sensitive fresh products by either using reduced nitric oxide concentrations or flushing with nitrogen to reduce nitric oxide level in the termination process of fumigation treatments. As nitric oxide reacts with oxygen to produce nitrogen dioxide ($2\text{NO} + \text{O}_2 = 2\text{NO}_2$), the ultralow oxygen levels in the practical nitric oxide fumigation applications need to be controlled based on nitric oxide levels to be applied and product tolerance to nitrogen dioxide to avoid excessive consumption of nitric oxide by oxygen and accumulation of nitrogen dioxide which may impact quality of sensitive fresh products.

Nitric oxide fumigation requires establishment of an ultralow oxygen condition first, and is therefore more complex and will have an additional cost for nitrogen as compared with most other fumigation treatments. However, its advantages of shorter treatment times as compared with phosphine fumigation and better safety to human health will likely outweigh its disadvantages. The cost of nitrogen gas depends on its sources such as nitrogen generator, compressed cylinder, or liquid nitrogen and therefore can be controlled to a certain extent. Nitric oxide is readily available as it is massively produced commercially as an intermediate for fertilizer production. Therefore, nitric oxide fumigation is practical to be adopted commercially.

Nitric oxide has been the subject of intense research interest since its discovery, and a multitude of biological functions have been found. The discovery of nitric oxide as a potent fumigant for pest control marks a significant departure from all other applications. Unlike other fumigants, nitric oxide functions as a signal molecule in all biological systems and is a part of insect life system. Insects are, therefore, unlikely to be able to develop resistance to nitric oxide fumigation. Being a small molecule with a very low boiling point and low solubility in water, nitric oxide can be used to treat perishable products at low storage temperatures and likely has good penetration and dispersion property for treating dense and densely packed products and controlling internal feeding pests. Nitric oxide fumigation offers high efficacy and unsurpassed safety to product quality and human health, and has the potential to become a preferred alternative for postharvest insect control treatment on perishable and stored agricultural products.