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Review

Using Nitrogen for the Control of Stored Product Insects: One Single Application for Multiple Purposes

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Abstract: Nitrogen treatment can be used as an alternative method to chemical control. Most of the research on nitrogen treatment mainly investigated the optimum concentration of oxygen level as well as duration as a means of insect control. Other parameters such as temperature and different insect species have been extensively studied and recent research focus on the modelling of nitrogen concentration and the efficacy on commodity. In this paper, we briefly review the major parameters (temperature, oxygen level, relative humidity, exposure time) using nitrogen treatment against stored product insects. Exposure to different oxygen levels or different exposure times can remarkably change pest control mortality. Moreover, different insect species and life stages have differing susceptibility to nitrogen treatment. Finally, these studies are reviewed in this paper to illustrate that nitrogen treatment can be used as a part of an IPM strategy.

Keywords: modified atmospheres; nitrogen; low oxygen; stored product insects; alternative to chemicals

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1. From Theory to Practice

Although stored product protection is currently based on a wide range of traditional active ingredients such as contact insecticides and fumigants, the vast majority of these control methods meet with several shortcomings. Hence, most of these chemicals are not compatible with biological products, with very few exceptions, such as the bacterial insecticide spinosad [1–3]. Moreover, the use of these active ingredients has been heavily questioned due to the potential presence of residues in food [4–6]. Finally, there are resistance issues from the continuous use of some of the most widely used contact insecticides and fumigants.

The withdrawal of methyl bromide, and also the removal of many substances from the market for use at the post-harvest stages of durable agricultural commodities, has threaten food security in storage and processing facilities, but also the efficacy of quarantine and pre-shipment treatments in key export scenarios [7,8]. For this purpose, many alternative methods have been evaluated towards this direction by several research groups throughout the world, with good results [9–12]. Among the most promising alternatives is the use of the so-called controlled or modified atmospheres. Although the definition of controlled and modified atmospheres is different, depending on the alteration of the conditions within the area that is to be treated, i.e., change of atmospheric pressure vs change in the ratio of the different gases, both methods are regarded as rather synonymous in terms of efficacy and their overall results, as change of the percentage of certain gases is likely to simultaneously change the atmospheric pressure and vice versa [13,14].

The application of nitrogen has been long regarded as a promising method for the disinfestation of stored products. With the term "nitrogen" we define the addition of nitrogen in a certain volume of air in order to reduce the percentage of oxygen [15–17]. As a result, insects die from a combination of dehydration and low oxygen anoxic atmosphere, thus, in reality, the most accurate expression can be "low oxygen" rather than "high nitrogen" [18,19]. Given that nitrogen and oxygen percentages in the atmospheric air correspond to about 78% and 21%, a further increase of nitrogen will lead to a corresponding decrease of oxygen, causing hypoxia or anoxia [13,14,20]. It is generally considered that insects die in a relatively short period of time when the oxygen level is about 1%, but also levels as high as 3% are, under certain circumstances, very effective against a wide range of target species. In many cases the increase of nitrogen is done with the help of generators that lead the atmospheric nitrogen into the target area, but there are plenty of paradigms where cylinderized nitrogen is used (e.g., silos in order to obtain high purity nitrogen).

In the following, we will refer to the utilization of nitrogen in stored product protection, emphasizing in the control of insects. In this regard, we will focus on both laboratory and field tests, with the latter coming from commercial applications.

2. Application of Nitrogen in Chambers and Silos

Nitrogen chambers are gas tight, often with controlled temperature and air outlets. Athanassiou et al. [17] found that the application of nitrogen to reduce the oxygen level to 1% was lethal for all life stages of the confused flour beetle, *Tribolium confusum* du Val (Cole-optera: Tenebrionidae), eggs and larvae of the cacao moth, *Ephestia elutella* Hubner (Lepidoptera: Pyralidae), and adults of the sawthoothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Sylvanidae). Moreover, Sakka et al. [11] evaluated adults of phosphine-resistant and susceptible populations of the red flout beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *O. surinamensis*, and the rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) in commercial nitrogen chambers exposed to 1% O_2 in different exposure times (e.g., 2.5, 3, and 9 days) and found complete mortality at 40 °C, but some survival when at 28 °C. Agrafioti et al. [19] found 100% mortality of *O. surinamensis* and the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), in different exposure times (2.5–9 days) and temperature ranging from 28 to 40 °C. Nonetheless, in some of these trials, the exposure times that were evaluated were rather increased and often exceeded 14 days. These exposure intervals are longer than the ones that are currently in use for different fumigants, such as phosphine, propylene oxide, and sulfuryl fluoride [21–24]. However, there are ways to sufficiently shorten the application of nitrogen, mostly through the increase of temperature during the application, as it will be analyzed later in this work.

The application of nitrogen in silos is a very complicated procedure, given that leaky structures should be thoroughly improved in their gas-tightness level in order for nitrogen to be successfully applied. In a field trial in large silos in Cyprus, Navarro et al. [25] were able to obtain high mortality levels of *O. surinamensis*, *T. confusum*, and *R. dominica*, which in some of the cases tested, survival was recorded. Moreover, this application exceeded three weeks, which indicates the increased duration that is needed in large structures [25]. However, follow-up trials in silos in the Czech Republic provided complete (100%) mortality of *O. surinamensis*, *T. confusum*, *T. castaneum*, *S. oryzae*, the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), and the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), but again in increased exposure intervals [26].

3. Advanced Sensing Technologies

The advancement of modern nitrogen treatments is that the entire application is based on the occurrence of sensors that measure oxygen and control the operation of the nitrogen generator. There are different sensor devices that are utilized for this purpose, and any measurements can be monitored remotely [22,27]. The sensor technology provides an "interrupted flow" of the gas only when it is necessary. For instance, if the target percentage of oxygen is 1%, then the sensor will trigger the nitrogen flow only if the oxygen percentage is >1% and will terminate the flow if oxygen is again at 1% or lower. As such, the product is under "continuous treatment" without the need to maintain a continuous flow of the gas. A higher number of sensors distributed in various locations within large structures, such as silos, is also beneficial for the entire procedure in order to illustrated possible "oxygen nests". Apart from the controlled flow, there are application systems that can also inform the user when the treatment is successful, which means that mortality of all target species and all of their life stages are 100% [28,29]. Sensing is important also for the quantification of the distribution of the gas in the different locations within the treated area [28–32]. In all cases, nitrogen treatment is carried out in the air that surrounds the product (interstitial space), and penetration into the product mass is gradual [19].

4. Effect of Abiotic Conditions

The most important way to increase the efficacy of nitrogen for the control of stored product insect species is through the increase of temperature. By "temperature" we mean the air temperature that surrounds the product, which means that this has to be operated through an external heat source. The increase of temperature can remarkably shorten the overall application even to intervals that are as short as 2–5 or 3 days [11,17,27]. For instance, Soderstrom et al. [33] tested larvae of *T. castaneum* and found that increasing the temperature to >38 °C increased mortality and reduced exposure time. Similarly, Ofuya and Reichmuth [34] found complete insect control of different life stages of the bean bruchid, *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae), after exposure to 1% nitrogen for 1–9 days. At the same time, the physicochemical and organoleptic properties of the commodity are not affected. In stored currants, Athanassiou et al. [17] found that exposure to high temperatures (38–43 °C) reduced the microbial content (yeast and mold count). Moreover, any alteration in the products' properties might be mostly related with the increased temperature, rather than with the application of nitrogen itself.

It should be noted that the increase of temperature needs several days to be achieved, and this interval should be added to the overall application time. The larger a structure, the longer the exposure time needs to reach the temperature at the target point. Thus, if an application is planned for three days at 38 °C, then a certain amount of days is needed for the temperature to reach the desired level, before the initiation of the actual nitrogen application. The time that is needed for this temperature increase depends on the temperatures prevailing and is longer in the winter time. Consequently, the total duration of the treatment may be prolonged by 2–4 days, or longer, which should be taken into account. Obviously, increased temperature causes elevated stress to insects that eventually die faster [35]. A technique has been evaluated in the so-called "heat treatments" in processing facilities and is now considered as one of the major alternatives to structural methyl bromide fumigations [12,36,37]. Which is in contrast to recent data showing that temperature does not change the distribution of nitrogen and oxygen much, at least in the case of commercial chambers [19]. As it becomes evident, temperature levels can be increased in chambers, but not in silos, which is economically unrealistic and of questionable efficacy.

In contrast with the effect of temperatures, there are extremely few data on the effect of moisture content or relative humidity on the efficacy of modified or controlled atmospheres [13,18,34,38]. Earlier studies have shown a linear relationship between carbon dioxide and r.h. on mortality of the tropical warehouse moth, *Ephestia cautella* (Wlk) (Lepidoptera: Pyralidae) pupae [18]. Moreover, Jay et al. [38] showed that increasing the r.h. in atmospheres with 99% N_2 can increase mortality of *T. confusum*, *T. castaneum,* and *O. surinamensis*. Stress is also a key parameter here that accelerates mortality, but the mechanisms of the actual water stress to insects at modified or controlled atmospheres are poorly understood. Nitrogen enrichment in the atmosphere is likely to cause dehydration, which consequently leads to water loss and eventually weight loss in the treated commodity, e.g., fresh fruits [39–42]. This is an important parameter of the overall application if weight loss reaches undesirable levels. This effect may be alleviated by a controlled relative humidity equipment that can be incorporated in chambers and add water when needed [43,44].

5. Variations among Different Species and Life Stages

As in the case with the other control methods that are used in stored product protection, stored product insect species have a dissimilar susceptibility level to both controlled and modified atmospheres. This is a critical issue as nitrogen-based control protocols should be designed according to the target species [27,45]. For instance, Adler and Reichmuth [45] tested nitrogen treatment with <2% oxygen against *S. granarius*, *T. confusum*, *E. elutella,* and *O. surinamensis,* and complete control mortality was observed after six weeks. Navarro [46] found significant differences in the mortality rates of *T. castaneum* adults between 0.1% and 1% O_2. Navarro [13] reported that adult *S. oryzae* and *R. dominica* are more tolerant to nitrogen than *Tribolium* spp. In general, internal feeders are considered more tolerant than external feeders [12,47]. More recent data show one of the most difficult species to be controlled with nitrogen is the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), as Sakka et al. [27] has shown. This species can survive conditions and exposures that are lethal for many other stored product insect species. In their work, the authors revealed that, among the different life stages of *T. granarium* that were tested, larvae and, to a lesser extent, eggs were the most tolerant life stages [27]. Larvae are the long-lived life stage of this species [48] and are considered tolerant to many substances, such as contact insecticides [49–52]. Furthermore, Vassilakos et al. [52] tested adults of *T. granarium* under laboratory conditions and found complete control after four or six days of exposure to 0.1% O_2. Earlier studies have shown that *T. granarium* is a particularly tolerant species to extreme conditions, such as both heat and cold [52–54]. The relatively reduced susceptibility of *T. granarium* to low oxygen [27,52,55] should be considered when drawing modified/controlled atmosphere-based protocols for pre-shipment and quarantine treatments.

In contrast to other fumigants, eggs are not always the most tolerant life stage. For instance, William et al. [55] found that with the maize weevil, *Sitophilus zeamais* Motchulsky, (Coleoptera: Curculionidae), eggs and adults were more susceptible than pupae. Moreover, Athanassiou et al. [17] found complete control at 38–43 °C of eggs, pupae, and adults of *T. confusum* and some survival was recorded for larvae. Still, the way that the eggs are exposed during these bioassays may cause additional egg mortality through handling [17]. One alternative way to overcome this is to use vials that contain food quantities on which adults are allowed to oviposit for a certain interval, and then these vials can be used in the bioassays, as it will contain a mixture of life stages, including eggs. This scenario is closer to "real world" expectations than using egg samples alone that have been prepared in the laboratory and can simultaneously evaluate the progeny production capacity of the adults in the treated substrate [11,19,27].

One additional parameter that can be considered further is the potential utilization of nitrogen as a "resistance breaker". Recent data have shown that stored product insect populations that are resistant to phosphine could be controlled after exposure to 1% oxygen at the same level with the susceptible populations [11,19]. In this context, modified atmospheres can be incorporated in resistance management protocols, as, apparently the data that have been obtained so far, cross-resistance with phosphine is less likely to occur.

6. Effect on Different Commodities

The effects of increased nitrogen on the commodity itself has not been examined in detail and needs additional investigation, due to the extremely wide variety that can be treated, which ranges from tobacco to dried fruit. In a series of trials in commercial chambers with stored currants, Athanassiou et al. [17] found that nitrogen had no effect on the basic qualitative parameters of the products, while at the same time, the nitrogen-enriched atmosphere caused a considerable reduction of the microbiological load of these commodities, such as yeast and molds. Guarrasi et al. [56] found that after exposure of fresh apples to Modified Atmosphere Packaging (MAP), differences in the aromatic fingerprint were noted. Mattheis and Fellman [57] noted that the use of Controlled Atmospheres and MAP can reduce carbohydrates and titratable acids in fresh fruits and vegetables. In general, it is expected that the effect of nitrogen on the qualitative characteristics of the different amylaceous commodities, such as grains or flour, would be negligible or even zero.

7. Future Trends

One of the most important parameters that determine the wider use of modified atmospheres is the authorization as a plant protection treatment for stored product protection and the cost of the method, as it needs a specialized equipment, which is usually not needed in the case of routine fumigation practices. Generally, the application of nitrogen is much more expensive than the most commonly used chemical control methods [13,25,58]. However, in the long-term, the utilization of nitrogen in storage and processing facilities can be compensated with all the positive effects that have been already mentioned above—especially the "green" characteristics of the method, as most of the cost is related to the establishment of the system (e.g., generator placement). Taking into account the recent increases in energy costs, all parameters of the overall value of the method must be redesigned under this prism, which means that the method might be much more expensive today than two years ago.

Although there are plenty of data for the evaluation of nitrogen for the control of many of the major species that are related with stored products, there is still a number of stored product insects for which there are no data available. The same holds true in the case of the different commodities that are to be treated, as the interactions of decreased oxygen with specific characteristics are poorly understood. For instance, penetration of phosphine on empty containers may be faster than that with currants [28], which may lead to different expected desorption patterns [59].

Considering the serious advantages of the method, and the possible withdrawal of additional chemical compounds that are used in stored product protection, we estimate that nitrogen will continue to play a role as an eco-friendly, non-chemical method that is mostly based on methods that separate atmospheric nitrogen (and to a lesser extent nitrogen cylinders). At the same time, the application of nitrogen can be combined with other methods, such as changes in the air pressure, as well as the simultaneous use of other gases, such as carbon dioxide.

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