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Residue Formations of Phosphorus Hydride Polymers and Phosphorus Oxyacids during Phosphine Gas Fumigations of Stored Products

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With the extent of international usage and the critical role phosphine gas (PH_3) plays in commercial pest control, identification of the residual components deposited during fumigation is mandatory. It has been postulated that these infrequent residues are primarily composed of phosphoric acid or reduced forms of phosphoric acid [hypophosphorous acid (H_3PO_2) and phosphorous acid (H_3PO_3)], due to the oxidative degradation of phosphine. Using environmental scanning electron microscopy, gas phase Fourier transform infrared spectroscopy, and X-ray fluorescence spectroscopy, the structural elucidation and formation mechanism of the yellow amorphous polyhydric phosphorus polymers (P_xH_y) that occur in addition to the lower oxyacids of phosphorus in residues deposited during PH_3 fumigations of select tobacco commodities are explored. This research determined that nitric oxide gas (or nitrogen dioxide) initiates residue formation of phosphorus hydride polymers and phosphorus oxyacids during PH_3 fumigations of stored products.

KEYWORDS: Phosphine; fumigation; yellow residue; tobacco; reconstituted tobacco; *Lasioderma serricorne* Fabricius; cigarette beetle; phosphorus

INTRODUCTION

Various fumigation methods and materials are used to control pests in stored agricultural products such as grains, rice, beans, and tobacco (*1*). The fumigants used are active in the gaseous state, insecticidally toxic at specific concentrations, permeate throughout storage facilities, and are often relatively inexpensive. This disinfestation treatment may occur in a variety of facilities including buildings, containers, silos, bins, warehouses, caverns, railroad cars, barges, or ships. While performing a fumigation, the fumigant may be generated from cylinders or solid material. For optimal efficacy and to prevent human exposure, it is necessary to contain the fumigant during fumigations by sealing the facility in a gastight manner. With appropriate conditions and concentrations, the fumigant penetrates all airspace in and around the stored product, killing targeted insects including all breeding stages (*2*).

Following an appropriate exposure time, the structure is usually ventilated to remove the fumigation gases. Traditionally, structures were exhausted to the atmosphere (e.g., open doors, hatches, vents, etc.). However, as safety and environmental concerns increased, there arose a need to control and/or prevent fumigants from escaping into the atmosphere, and thus, methods of "scrubbing" or removing fumigation gases from exhaust gas streams have been developed (*3*−*7*).

The cigarette beetle, *Lasioderma serricorne* Fabricius, and the tobacco moth, *Ephestia elutella* (Hubner), infest cured tobacco. These pests can be controlled in storage by fumigation. Following trials with phosphine gas (PH_3) fumigations in Europe in the late 1950s and subsequent testing in Japan and the United States, PH_3 became the fumigant of choice in the tobacco industry by 1975. Phosphine may be generated at point of use by exposure of a metal phosphide, such as magnesium or aluminum phosphide, to ambient humidity. Warehouse temperature, humidity, exposure time, and phosphine concentration influence fumigation effectiveness and must be strictly controlled to ensure effective fumigations and prevent the development of resistant populations of cigarette beetles or tobacco moths. Generally, the minimum exposure time and warehouse temperature are 6 days at 16−20 °C or 4 days at greater than 20 °C (*8*, *9*).

With the extent of international usage and the critical role PH_3 plays in commercial pest control, elucidation of the residual components deposited during fumigation is mandatory. It has been postulated that these residues are primarily composed of the phosphoric acid (H_3PO_4) or reduced forms of H_3PO_4 such as hypophosphorous acid (H_3PO_2) and phosphorous acid (H_3PO_3) due to the oxidative degradation proposed by Robinson shown in mechanism 1 (*10*, *11*).

As early as 1954, it was shown that moist food fumigated with PH_3 contained elevated levels of H_3PO_4 (*12*). Later research quantitatively confirmed nonvolatile residue deposition on wheat using isotopic or radioactive-labeled phosphorus, P^{32} (*11*, *13*). However, this research received some scrutiny over the stability of the phosphorus isotope and the validity of the depositions (*10*).

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Mechanism 1:

1) $2\ PH_3 + \frac{1}{2}\ O_2 \rightarrow P_2H_4 + H_2O$
diphosphine

2) $P_2H_4 + H_2O + 1\ \frac{1}{2}\ O_2 \rightarrow 2H_3PO_2$
hypophosphorous acid

3) $2H_3PO_2 + O_2 \rightarrow 2H_3PO_3$
phosphorous acid

4) $2H_3PO_3 + O_2 \rightarrow 2H_3PO_4$
phosphoric acid

Since the early PH_3 studies, speculations of additional residual components have been made including elemental phosphorus (red phosphorus), metallic phosphides, and lower phosphorus hydrides (*10*, *14*). Even trace amounts of these additional components could present off-flavors of food commodities, promote microbial destruction, or even pose latent health effects (*10*).

The research discussed vide infra explores the conditions that initiate the formation of yellow amorphous polyhydric phosphorus polymers (P_xH_y) that occur in addition to the lower oxyacids of phosphorus in residues deposited during PH_3 fumigations of stored tobacco. Because these solid phosphorus hydrides are highly polymeric, typically form at low concentrations, and are insoluble in all common solvents (e.g., inorganic, polar organic, and nonpolar organic solvents), little is known regarding their structure. When conditions for formation are optimal, these relatively stable solid phosphorus hydrides have been observed following exposure to PH_3. These rare depositions are bright yellow and distributed throughout the area of fumigation on all horizontal surfaces. Warehouses that experience this phenomenon appear to be "spray painted yellow". In these cases, the exposed product is typically destroyed. **Figure 1** shows two cases of reconstituted tobacco (sheet material made of tobacco dust, stems, and other byproducts) (*15*) that were topped with pallets and coated with the yellow residue during a PH_3 fumigation.

While a yellow residue deposition is infrequent, significant monetary implications result when a warehouse of stored food or tobacco must be destroyed due to this rare phenomenon. Therefore, a clear understanding of the origin of this residue is essential. The objective of this research was to determine the precursors for the residue formation, which can then be applied to prefumigation screening protocols designed to prevent the formation of this unwanted deposition. Reduction of H_3PO_4 formation during PH_3 fumigation is also of great interest as such formation can be linked to significant corrosion of storage facilities as well as deposition of an unwanted nonvolatile residue.

MATERIALS AND METHODS

X-ray Fluorescence (XRF). XRF analysis was conducted using a Thermo Noran (Madison, WI) QuanX EC. Samples were analyzed under vacuum with no filter (low Za) at 9 kV, autotube current (50% dead time), 0−10 keV, and a medium range for 100 s. The peak height at 2.01 Kα energy was used to estimate the relative phosphorus deposition.

Infrared Spectroscopy. *Residue Analysis.* Fourier transform infrared spectroscopy (FT-IR) analysis was conducted using a Thermo Nicolet (Madison, WI) Nexus 670 ESP equipped with an attenuated total reflectance (ATR) unit and a Smith Detection (Raleigh, NC) single bounce Durascope with a diamond crystal. Samples were collected using 64 scans, 4 cm^{-1} resolution, and Happ−Genzal apodization.

Gas Analysis. Tobacco samples (~50 g) were tightly packed in a Tyvek bag and tied off with wire. Samples were inserted into an incubation chamber with an internal volume of 4.6 L (cylindrical Pyrex tube with aluminum endplates fitted with recessed butyl rubber O-rings and 1/2 in. pipe fittings). The chamber was isolated and heated to 32 °C for 30 min using heating tape and a Variac. Dry CO_2 free air (Balston FT-IR) was then swept through the chamber at approximately 1 L/min into the FT-IR gas cell (Midac, 3.05 m path length and 80 mL volume) for analysis. The Midac FT-IR MN I-2000 (Irvine, CA) used for gas analysis was set to 50 scans and 0.5 cm^{-1} resolution, with triangular apodization.

Residue Formation. The polyhydric phosphorus polymer was synthesized in conjunction with the phosphorus oxyacids at ambient conditions in 0.2 m^3 (8 ft^3) Plexiglas chambers. Approximately 6 kg of a fresh (less than 2 weeks after production) reconstituted tobacco was packed in semipermeable containers (e.g., cardboard boxes, Tyvek bags, or perforated polyethylene) and placed inside the chambers. A 0.8 g amount of Magtoxin (magnesium phosphide) (Degesch, United States) was added to these chambers releasing approximately 1200 ppm PH_3 within about 3 h. Yellow deposition was noted after 24 h of exposure. The residues were also generated by the addition of 2.4% nitric oxide (NO) in nitrogen gas (BOC Gases, Richmond, VA) to empty cardboard boxes or boxes containing flue-cured tobacco in the presence of approximately 1200 ppm PH_3. NO was added in three 1 min increments at a 1 L/min flow rate.

Polymer Isolation. The residue was rinsed from the 0.2 m^3 chamber with approximately 300 mL of methanol. Fifty milliliter aliquots were dispensed into 50 mL centrifuge tubes. Samples were centrifuged for 2 min at 2000 rpm. Methanol was decanted. The yellow polymer was rinsed twice with water with each rinse followed by centrifugation and removal of the liquid. Samples were then rinsed twice with acetone followed by centrifugation and removal of acetone using a disposable pipet. Residual acetone was removed under a stream of nitrogen gas.

Electron Microscopy. The sample of purified yellow residue was mounted directly onto 12 mm diameter carbon adhesive disks that were attached to 12 mm diameter clean aluminum mounts. One sample was sputter coated with 5 nm of Au−Pd using a Cressington 208HR sputter coater operating in Ar, and the other sample was not coated. An FEI XL30 environmental scanning electron microscope (ESEM) operating at 15 kV was used to image the coated and uncoated particles. Elemental analyses were performed using the EDAX energy dispersive spectrometer interfaced with the EDAX Genesis software.

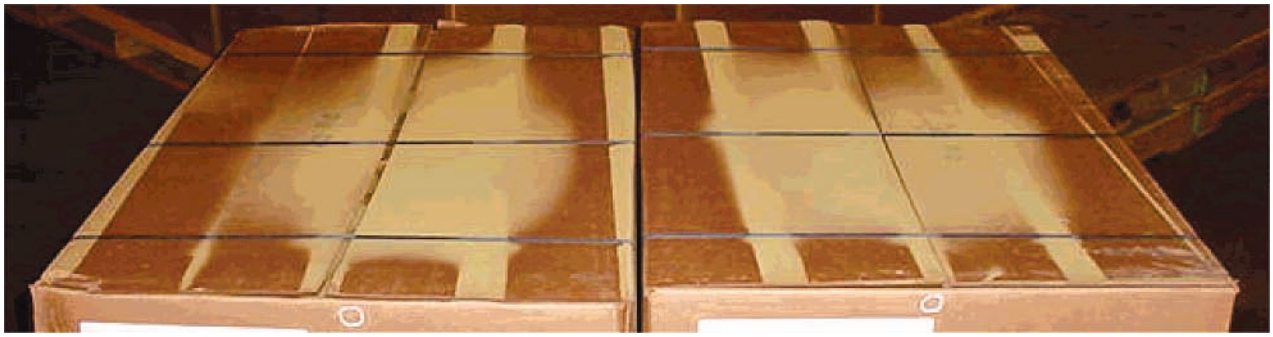

Figure 1. Two cases of export reconstituted leaf with yellow residue deposition following PH_3 fumigations.

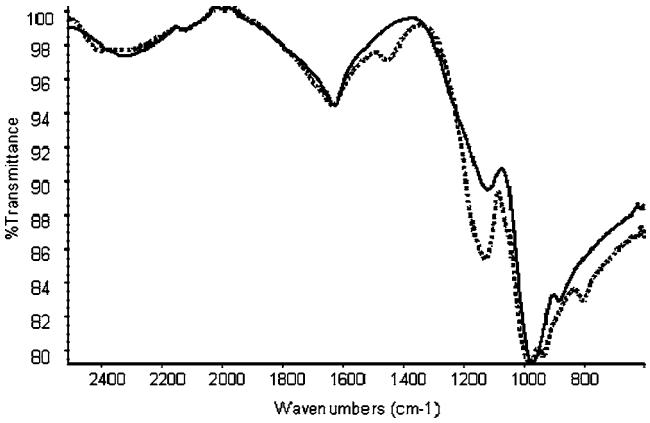

Figure 2. ATR-FT-IR spectra of phosphoric acid (solid line) and the residue deposited following phosphine fumigation (dashed line).

RESULTS AND DISCUSSION

Extensive analytical investigations have been conducted regarding residue formation during phosphine fumigations. It has been previously debated whether the air oxidation of PH_3 (mechanism 1) occurs as a substrate−surface association or as a gas phase mechanism (*10*). Robinson proposed surface sorption of phosphine in a "monomolecular layer" (*10*). However, observations made in this research strongly suggest a gas phase reaction. Preceding all residue depositions observed, the fumigation chambers contained a dense haze as PH_3 was introduced. The haze consists of the oxidation product of the PH_3 (the oxyacids of phosphorus) and in many cases a yellow polymer, which ultimately settled/deposited onto horizontal surfaces.

Relative concentrations of phosphorus depositions during phosphine fumigations of tobacco commodities were measured using XRF. White paper sheets were placed in all fumigation chambers used in this work, and the intensity of the phosphorus deposition was evaluated relative to control paper from the same stock. Within 24 h, the phosphorus deposition was detectable by XRF. It was typically observed that the greater the significance of the haze during fumigation, the greater the XRF phosphorus counts. The phosphorus deposition continues to increase as the fumigation proceeds.

The visible residues deposited in experimental fumigation chambers of select tobacco commodities were collected. The residues were viscous and yellow in color. The viscous component of the residue was presumed to be phosphoric acid or a reduced form of the acid based upon previous publications and confirmed by ATR-FT-IR. **Figure 2** shows the FT-IR analysis of the residue deposition collected after a dense haze was observed. The FT-IR spectrum of pure phosphoric acid is overlaid with the residue spectrum. The residue was spectroscopically analogous to pure phosphoric acid (with the exception of slight silica contamination from the sample extraction) suggesting any of the lower oxyacids of phosphorus.

The yellow component of the residue, isolated from the H_3PO_4 by successive washes, is an amorphous polyhydric phosphorus polymer (P_xH_y). This polymeric phosphorus compound is insoluble in all common solvents and, subsequently, difficult to analyze (*16*). The elemental composition of the isolated phosphorus polymer was confirmed by ESEM. **Figure 3** shows the image at 8000× magnification and elemental analysis (background spectrum not included). Nodules of this polymer are less than 1 μm and composed of only phosphorus and hydrogen (not detected by ESEM), P_xH_y.

Higher phosphorus hydrides have been discussed in the literature, but reports are often conflicting (*16*). As discussed

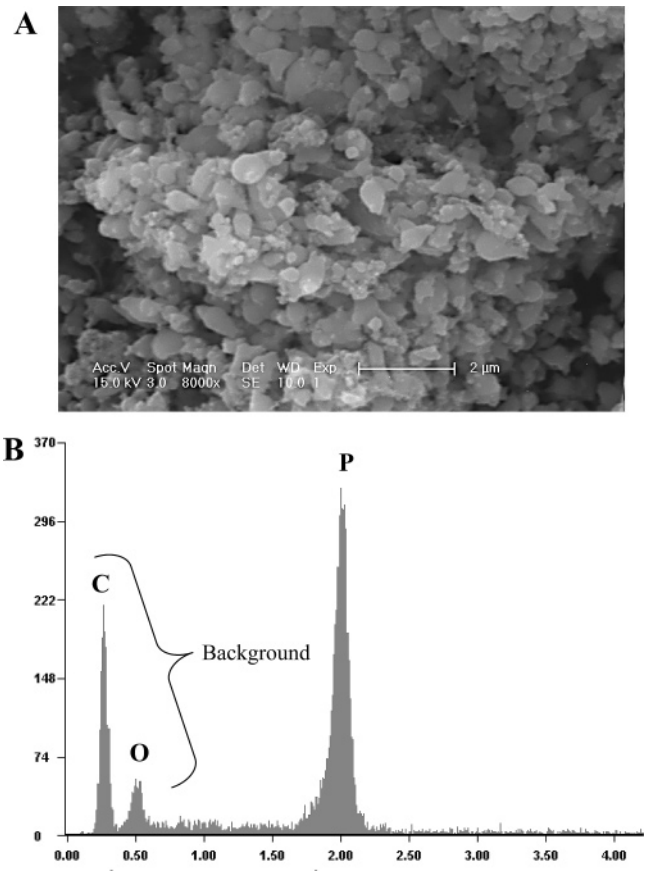

Figure 3. (**A**) Scanning electron microscopy of the yellow residue at 8000× magnification and (**B**) the elemental analysis.

above, PH_3 fumigations of select commodities produce significant hazes and depositions; however, determination of the precursors or initiators for this reaction (excluding PH_3) has been extremely challenging. Preliminary investigations of this phenomenon explored a battery of potential catalysts and reactants for this polymerization including corona discharge, UV light, halides, aldehydes, moisture content, and volatile acids. All potential catalysts appeared to play no role in residue formation. Packaging material was also evaluated and was determined to only play a physical role in the mechanism (discussed below).

The advantage of PH_3 is its excellent penetrating power and fugacity, and it is considered toxic to all life stages of most stored product pests (e.g., *L. serricorne*) (*11*). Semipermeable packaging such as cardboard, perforated polyethylene, and Tyvek are well-suited for PH_3 fumigations. However, this research shows that the physical environment created by these containers ultimately can contribute to the formation of unfavorable phosphorus residues. When packaged in semipermeable containers, fumigation of fresh (less that 2 weeks old) reconstituted tobacco has a high propensity to initiate the formation of residues consisting of the oxyacids of phosphorus in conjunction with the yellow phosphorus polymer. However, when fresh reconstituted leaf is fumigated without packaging, only a white haze (oxyacids of phosphorus) is observed with no notable residue deposition. The semipermeable packaging provides a means of filtering the larger volatile components (e.g., propylene glycol) and allows only very light gases to pass. Subsequently, the likelihood of small reactive gases (e.g., free radicals) interacting with the PH_3 in the airspace of the warehouse (ambient air outside of the packaging) during fumigation is increased.

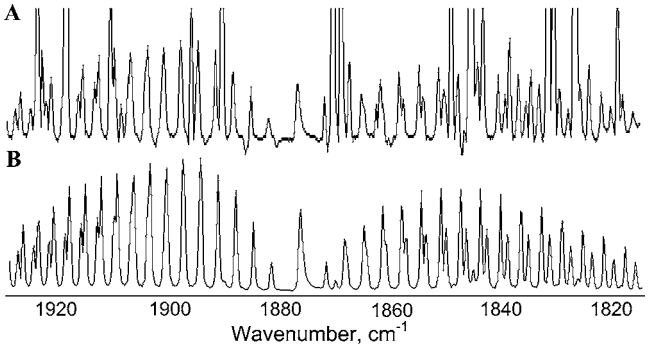

Figure 4. (**A**) Gas phase FT-IR spectrum of reconstituted tobacco subtracted from flue-cured tobacco. (**B**) Reference spectrum of NO gas. NO lines are obscured in spectrum A due to the high water concentration that is difficult to subtract.

To elucidate the volatile precursor emitted from fresh reconstituted tobacco that initiates residue formation, gas analysis was conducted using FT-IR. Gases released from fresh reconstituted tobacco were compared to those released from tobacco products that produced no haze or residue during PH_3 fumigations. The one light gas found unique to the haze and residue-producing tobacco commodities (e.g., reconstituted tobacco) was NO. **Figure 4** shows the gas phase FT-IR spectra of reconstituted tobacco subtracted from that of flue-cured tobacco and compared to a NO reference spectrum.

NO is a free radical that typically originates from internal combustion engines (e.g., fork lifts) by the combination of nitrogen and oxygen under thermal conditions. This colorless gas has been attributed to bacterial metabolism, has significant biological activity, is considered very reactive, and is found in cigarette smoke (*17*−*20*). Its source in the stored tobacco commodities (e.g., reconstituted tobacco) investigated in this research is currently unknown and under investigation.

The research discussed herein suggests that when products that are outgassing NO into the headspace are stored in semipermeable containers, they are prone to promote the formation of both oxyacids of phosphorus as well as the yellow polyhydric phosphorus polymer (*21*). This reaction involves PH_3 interaction with NO and/or nitrogen dioxide (NO_2) (immediately formed from the oxidation of NO on contact with air). The products of this reaction are likely precursors for both the oxyacids of phosphine (mechanism 1, step 2) (*10*, *11*) and the polyhydric phosphorus polymer. Ab initio calculations will be performed to elucidate mechanistic pathways, energetics, and reaction kinetics.

The yellow residue formation has been reproduced by the introduction of NO (~2.4% in nitrogen) into a 0.2 m^3 chamber containing ~1200 ppm of PH_3. However, when the humidity is elevated by the addition of a pan of water, only the oxyacids of phosphorus are formed. Flue-cured tobacco does not outgas significant levels of NO gas; subsequently, PH_3 fumigations of this valuable stored commodity do not result in the formation of a haze or unwanted residues. However, when low levels of NO gas are pumped into a case of flue-cured tobacco during a simulated PH_3 fumigation, the phosphoric acids and the yellow phosphorus hydride polymer are formed.

In conclusion, this research clearly identifies NO gas, or an oxidation product of NO (e.g., NO_2), as a precursor for the formation of both oxyacids of phosphorus as well as a yellow phosphorus hydride polymer in the presence of PH_3 gas. With the availability of portable sensors and the significant monetary implications of stored product contamination, regular screening for these reactive free radicals is recommended prior to all PH_3 fumigations. It is our opinion that when NO_x ($x = 1$ or 2) is detected in stored product intended for PH_3 fumigation, the fumigation should be delayed and the NO_x given time to dissipate.

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