**Project Title:** Prevention of the Contamination of Organic Apples with Diphenylamine During Storage

**PI:** Charles Forney  
**Organization:** Agriculture and Agri-Food Canada, 32 Main Street, Kentville, Nova Scotia  
B4N 1J5 CANADA  
Phone: (902) 679-5714 Fax: (902)679-2311 E-mail: forneyc@em.agr.ca

**Co-PI:** Jun Song  
**Organization:** Agriculture and Agri-Food Canada, Kentville, Nova Scotia

**Cooperator:** Eugene Kupferman  
**Organization:** Washington State University Tree Fruit Research and Extension Center

**Cooperator:** David Parrish  
**Organization:** Scotian Gold Cooperative, Ltd., Kentville, Nova Scotia

**Objectives:**  
The objectives for this two year project were to:

1) Assess the role of potential sources of DPA to contaminate untreated apples by quantifying DPA in the air of commercial controlled atmosphere storage facilities and measuring DPA emissions from untreated apples, storage bins, and plastic liners contaminated with DPA.

2) Determine the amount of DPA retained in CO₂ scrubbers before and after it undergoes its recharge cycle.

3) Evaluate the effectiveness of ozone to remove DPA from storage room air and contaminated surfaces.

**Significant findings:**  
DPA was found in the air of storage rooms containing apples that were not treated with DPA.

CO₂ scrubbers were not significant sources of DPA contamination of the storage room air.

Wood and plastic bin material, bin liners, and foam insulation all had a high affinity for DPA and are potential sources of contamination.

Ozone reacts with DPA and following gaseous ozone treatments off-gassing of DPA from wood and plastic bin material and bin liners was reduced. However, ozone does not appear to be effective in remove all DPA in contaminated materials.

Ozone was ineffective in removing DPA from contaminated apples.

**Methods:**  
*DPA analysis.* Concentrations of DPA in air from storage rooms, CO₂ scrubbers, and that emitted from contaminated surfaces were analyzed using solid phase micro extraction (SPME). DPA in the
air was adsorbed onto 65µm Polyacrylate micro fibers. DPA on the fibers was then desorbed by heating and analyzed by gas chromatography-mass spectroscopy to assure accurate identification and quantification.

**DPA in the air of commercial storages.** Air in four commercial CA storage rooms were monitored monthly for DPA content during the storage season including two rooms that contained apples treated with DPA (Room 1 - 'Cortland' and Room 2 - 'Red Delicious' and 'Golden Delicious') and two rooms that contained untreated apples (Room 3 - 'Idared' and Room 4 - 'Northern Spy'). In addition, DPA in the air streams of the inlets and outlets of two CO2 scrubbers (SCS 600NP and SCS 6006, Storage Control System, MI) treating these rooms were monitored at the same time.

**Affinity of DPA to storage materials.** The adsorption of DPA onto wood and plastic bin material, plastic bin liners, and foam insulation was also assessed. Plastic and wood bin materials were dipped into a 1000 ppm (mg/L) commercial DPA solution for 30 s, dried with paper towel, placed in 4 L glass jars, and held at 23 °C. Jars were periodically sealed to allow the DPA in the headspace air to equilibrate and the DPA in the air was analyzed. In addition, plastic and wood bin materials, foam insulation, plastic bin liner, and apple fruit were sealed in small chambers containing solutions of 1000 ppm DPA at 5 °C for 3 months, or at 23 °C for 24 h. Materials were then placed in 4 L glass jars and headspace DPA was periodically analyzed as described above to measure DPA off gassing.

**Heat treatment.** To measure DPA that remained bound to treated materials, the 4 L jars containing these materials were sealed and held at 45 °C for 72 h. Samples were then taken for headspace DPA at 23 °C. Jars were opened and allowed to air for several days and then the heating was repeated.

**Decontamination of DPA using ozone.** Ozone treatments were conducted in walk-in environmental chambers (Model GR36, Econaire Systems Ltd., Winnipeg, Man.) at 20 °C. Ozone concentrations of 300 ± 10 and 800 ± 20 ppb (nL=L⁻¹) were maintained in chambers using ozone generators (Model SF 300, Aqua-air, Simpson Environmental Corp., Mississauga, Ont.) and small air circulating fans. Ozone concentration in each chamber was continuously monitored with an In-2000 LoCon Ozone Analyzer (InUSA, Inc., Needham, Mass.) and maintained with a data logger (Model 21X, Campbell Scientific Inc., Logan, Utah) connected to an electronic relay that turned the generator on or off in conjunction with programmed ozone set points. A control chamber was equipped with only a circulating fan and had no ozone generator. Samples of wood, plastic bins and apple fruit were dipped in 1,000 ppm DPA for 60 sec and then suspended in the chambers using cloths pins. Samples were removed at various times ranging from 1 to 7 days and DPA off-gassing was measured as described above.

**Results and Discussion:**

**DPA in the air of commercial storages.** Significant quantities of DPA were detected in the air of storage rooms that contained DPA treated apples as well as rooms containing untreated apples, indicating that DPA moved freely within the storage facility, potentially contaminating untreated fruit (Fig. 1). After 2 to 3 months of storage, concentrations of DPA in the storage room air decreased to relatively low levels. However, the presence of DPA in the air of storage rooms containing untreated apples demonstrates the pervasive nature of DPA with in a packinghouse/storage facility. Therefore, to prevent DPA contamination of untreated apples, fruit must be stored and handled in facilities free of DPA and isolated from facilities using DPA.

The concentration of DPA detected in the CO2 scrubbers was consistently lower than that found in the storage rooms, indicating that the scrubbers were not a significant source of DPA contamination.
Affinity of DPA to storage materials. Both wood and plastic bin material adsorbed significant quantities of DPA both from dips and from the air and were found to off-gas significant quantities of DPA for up to 8 d at 23°C (Fig. 2 and 3). Plastic bin material made of both polyethylene and polypropylene had a similar affinity for DPA (Fig. 4). At refrigerated temperatures this off-gassing would occur at a slower rate, but may be more prolonged. In addition to bin materials, plastic bin liners and foam insulation also off-gassed DPA. This high affinity of common materials used in storage room construction and fruit handling indicates that once these materials are exposed to DPA they may play a role in the contamination of untreated fruit. Therefore, placing untreated fruit in containers or rooms that previously held DPA treated fruit may risk contaminating the untreated fruit.

Heat treatment. While off-gassing of DPA appeared to drop to minimal levels in bin materials held at 23 °C, when heated to 45 °C, a significant rise in DPA off-gassing occurred (Fig. 4). This indicates that both plastic and wooden bin materials have a high affinity for DPA and significant quantities of DPA remain adsorbed. Even after aeration, a second heating caused additional off-gassing. Prolonged heating with aeration could be an effective method to reduce DPA contamination, but whether this would be adequate to prevent contamination of untreated fruit in a subsequent season is uncertain.

Decontamination of DPA using ozone. Ozone is a strong oxidizing agent and is effective in oxidizing DPA. To evaluate the effectiveness of ozone to decontaminate apple bins, bin materials were exposed to gaseous ozone for up to 5 days. During the first two days of ozone treatment DPA emissions from wood, polyethylene and polypropylene bin materials, and polyethylene bin liners were reduced significantly (Fig. 5). Treatment with 800 ppb ozone reduced emissions more than the 300 ppb treatment. However, after 3 to 4 days differences in DPA emissions between control and ozone treated materials were minimal. When materials were heated to 45 °C, DPA emissions increased. It appears that ozone is able to react with and destroy DPA on the surface of these materials but DPA that is bound more deeply is unaffected by the applied ozone.

Ozone treatments on DPA-treated apple fruit had no effect on reducing DPA emissions (Fig. 6). DPA appears to bind to apple fruit where ozone can not effectively react with it. Therefore, ozone does not appear to be an effective alternative to decontaminate apple fruit.

Treatment of storage room air with ozone could reduce cross-contamination of untreated apples from air-borne DPA. Ozone can effectively react with DPA vapors reducing the concentration to which fruit may be exposed. However, the practicality of treating air with ozone to reduce cross-contamination is questionable.
Fig. 1. DPA concentration in CA rooms during storage (Room1: DPA treated ‘Cortland’; Room2: DPA treated ‘Red Delicious’/‘Golden Delicious’; Room3: untreated ‘Idared’; Room 4: untreated ‘Northern Spy’).

Fig. 2. Affinity of DPA and its release from storage materials after treatment with DPA at 23 °C. Plastic bin 1, wood and the polyethylene (PE) plastic bin materials were dipped in 1,000 ppm DPA for 30 s, while the plastic bin liner and the foam insulation were sealed in a 23 °C chamber containing 50 mL of 1,000 ppm DPA.
Fig. 3. Affinity of DPA and its release from storage materials after treatment with 3.7 ppm DPA vapor at 3°C for 3 month.
Fig. 4. Effect of heat treatment (45 °C) on removing DPA in storage materials. Plastic bin1, Plastic bin – polyethylene (PE), Plastic bin – polypropylene (PP) materials were dipped for 30 s in 1,000 ppm DPA at 23 °C prior to off gassing monitoring.

Fig. 5. Effects of ozone on DPA emissions from wood, polyethylene bin liner, and samples of polyethylene and polypropylene bin materials. Wood and bin materials were dipped in 1000 ppm DPA for 60 sec and bin liner was sealed in a 4-L jar containing 25 ml of 1,000 ppm DAP. Samples were then placed in chambers containing 0, 300, or 800 ppb ozone. Samples were removed from chambers for measurement of DPA.
Fig. 6. Effects of 800 ppb ozone on DPA emissions from ‘Red Delicious’ apple fruit dipped in 1,000 ppm DPA for 60 sec.

**Budget:**
**Prevention of the Contamination of Organic Apples with Diphenylamine During Storage**

**Charles Forney**

**Project duration:** Two years (2001-2002)

**Project total – 2 years:** $11,000

**Original budget request:**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WTFRC</td>
<td>AAFC&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Salaries&lt;sup&gt;2&lt;/sup&gt;</td>
<td>$5,000</td>
<td>$4,000</td>
</tr>
<tr>
<td>Benefits</td>
<td>$1,000</td>
<td>$800</td>
</tr>
<tr>
<td>Equipment&lt;sup&gt;3&lt;/sup&gt;</td>
<td>$0</td>
<td>$2,200</td>
</tr>
<tr>
<td>Supplies&lt;sup&gt;4&lt;/sup&gt;</td>
<td>$0</td>
<td>$1,500</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>$0</td>
<td>$500</td>
</tr>
<tr>
<td>Total</td>
<td>$6,000</td>
<td>$9,000</td>
</tr>
</tbody>
</table>

<sup>1</sup> Matching funds in Canadian dollars from Agriculture and Agri-Food Canada=s Matching Investment Initiative (Funding approved).

<sup>2</sup>Salary dollars for term scientist.

<sup>3</sup>Air sampling pump, sampling syringes and fibers.

<sup>4</sup>Air sampling supplies, GC/MS operating supplies.