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The use of natural plant volatile compounds for the control of the potato postharvest diseases, black dot, silver scurf and soft rot

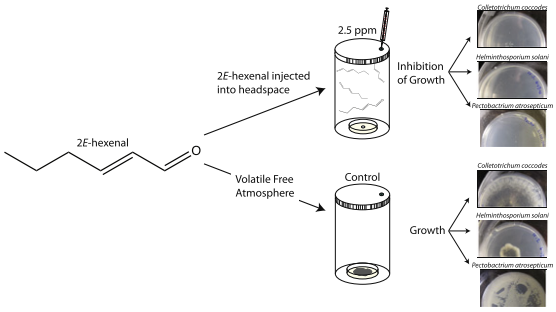
Elisabeth M. Wood, Timothy D. Miles, Phillip S. Wharton *

Department of Plant, Soil, and Entomological Sciences, University of Idaho, Aberdeen, ID 83210, USA

HIGHLIGHTS

- Naturally occurring plant volatiles are known for their anti-fungal properties.
- Acetaldehyde and 2*E*-hexenal tested for anti-fungal properties against potato diseases.
- Acetaldehyde did not prevent growth of potato pathogens *in vitro*.
- 2*E*-hexenal completely inhibited growth of potato pathogens *in vitro* at 10 μL/L.

GRAPHICAL ABSTRACT



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ABSTRACT

Many naturally occurring plant volatile compounds are known for their anti-fungal properties. In this study, acetaldehyde and 2*E*-hexenal were chosen as prototype volatiles in order to investigate the use of volatile compounds for control of blemish pathogens in fresh-pack potato packaging. Pure cultures of the three main potato blemish pathogens, *Pectobacterium atrosepticum* (bacterial soft rot), *Colletotrichum coccodes* (black dot), and *Helminthosporium solani* (silver scurf), were used in the study. Pathogen cultures were exposed to the pure volatiles that were injected into the atmosphere of sealed jars for 4–8 days at 23 °C. Results showed that 2*E*-hexenal was the most effective of the two volatiles with 5 μL/L providing complete inhibition of growth for all three pathogens *in vitro*. Cytological studies showed that a concentration of 2.5 μL/L of 2*E*-hexenal was capable of inhibiting germination in both fungal pathogens. These results suggest that the primary mode of action of 2*E*-hexenal was inhibiting germination for fungi and suppressing bacterial growth. The quantities required to achieve pathogen inhibition are extremely low. This study suggests that these volatiles may be used to effectively manage potato postharvest blemish diseases in storage.

1. Introduction

Pectobacterium atrosepticum (van Hall) Patel and Kulkarni, the causal agent of bacterial soft rot or pit rot of potato, is an important potato pathogen affecting postharvest storage of potatoes and causing significant economic losses. Although it is difficult to estimate exact values for loss of potato crops due specifically to bacterial soft rot, it has been estimated that soft rot bacteria may cause economic losses of $50–100 million in multiple crops world-wide every year (Perombelon and Kelman, 1980). This pathogen infects the lenticels of tubers while in the soil under wet soil conditions and can quickly spread to other potatoes in low oxygen, humid and warm storage conditions (Johnson, 2011). Currently, there are few, if any, postharvest methods of controlling this pathogen. Current control methods rely heavily on keeping harvested tubers well aerated, cool and dry to prevent ideal conditions for pathogen growth and spread (Bourne et al.,1981).

Colletotrichum coccodes (Wallr.) S. Hughes, the causal agent of black dot, and *Helminthosporium solani* (Durieu and Mont.), the

* Corresponding author. Fax: +1 208 397 4311.
E-mail address: pwharton@uidaho.edu (P.S. Wharton).

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causal agent of silver scurf, are other economically important blemish pathogens of potato. These pathogens often initially infect stems and tubers in the field and then spread during storage through distribution of air-borne conidia causing latent infection of tubers (Glais-Varlet et al., 2004, Errampalli et al., 2001). *C. coccodes* is characterized by visible black spots and lesions on the tuber skin that can lead to rejection of the produce by processors and consumers. Similarly, *H. solani* produces a well-defined silvery lesion that can also lead to rejection of the produce. Currently, the most effective methods for control of these pathogens are cultural measures. These include reducing initial field inoculum by not planting infected seed, limiting free moisture in the soil by managing irrigation and reducing the time between vinekill and digging. Studies have also shown that the length of crop duration, from 50 percent emergence to harvest, shows a close relationship to final black dot levels on progeny tubers in storage. The shorter the crop duration the less black dot occurred in storage (Wale et al., 2008). Most current control methods for these pathogens focus on reducing initial field inoculum, but there are very few effective methods of protecting tubers directly in storage.

Many plant volatile organic compounds are known for their anti-fungal properties. 2*E*-hexenal is a well studied volatile organic compound produced by bananas, tomatoes, and other fruits as an aroma volatile associated with a green or grassy smell (Hayata et al., 2002). It is also produced by plants such as common bean (*Phaseolus vulgaris*), gingko (*Gingko biloba*), and cotton (*Gossypium* sp.) in response to pathogenic infection (Croft et al., 1993). 2*E*-hexenal is biosynthesized from the breakdown of linolenic acid by lipoxygenase and hydroperoxide causing the formation of two intermediate compounds *cis*-3-hexenal and *trans*-3-hexenal (Myung, 2005). These intermediates are further altered by two isomerization factors ultimately resulting in the formation of 2*E*-hexenal (Min, 2001). In blueberries and strawberries, it was found that 2*E*-hexenal had antimicrobial properties against *Colletotrichum acutatum*, but the levels present in fruit were not correlated with host resistance (Polashock et al., 2007; Arroyo et al., 2007). Studies by Pérez et al. (1999), found that 2*E*-hexenal was the primary aldehyde in ripening strawberry fruits and its production was due to increases in lipoxygenase activity. An increase in lipoxygenase activity is not necessarily correlated with increased host resistance. In avocados, lipoxygenase breaks down antifungal dienes present in unripe fruits causing them to become susceptible to *Colletotrichum gloeosporioides* infection (Prusky and Keen, 1993). However, in several studies exogenous application of 2*E*-hexenal has been shown to slow the growth of multiple plant pathogens including *Colletotrichum truncatum, Rhizoctonia solani*, and *Sclerotium rolfsii* on soybean, *Penicillium expansum* on conference pears, *Monilinia laxa* on stone fruits, *Pseudomonas syringae* on common bean and *Aspergillus flavus in vitro* (Vaughn and Gardener, 1993; Neri et al., 2006; Neri et al., 2007; Croft et al., 1993).

Acetaldehyde is another volatile compound that is highly effective in slowing the process of fruit ripening in mangos and preventing pathogenic infection of other fruits (Pesis, 2005; Utama et al., 2002). Acetaldehyde is biosynthesized from pyruvic acid by pyruvate decarboxylase, which depends on the cofactors thiamine pyrophosphate and magnesium (Dyda et al., 1993). Exogenous applications of acetaldehyde have also shown that it is fungitoxic to the blueberry pathogens, *C. acutatum*, *Botrytis cinerea*, and *Alternaria alternata* when introduced into the headspace of a jar containing the pathogens (Almenar et al., 2007). Given the effectiveness of these plant volatile compounds in controlling pathogen growth in fruits and other plants tissues, they could also be very useful in providing control of postharvest potato blemish pathogens.

The use of volatile organic compounds is a novel method of controlling postharvest potato blemish pathogens. This method of control has many potential uses such as in storage fumigation, controlled atmosphere storage, and fresh-pack packaging. These volatile organic compounds could provide a new method of protecting tubers directly, and could be an alternative to the few currently available postharvest fungicides. They have less environmental impact as both acetaldehyde and 2*E*-hexenal are naturally occurring plant compounds and registered by the US Food and Drug Administration (FDA) as food grade additives (FDA, 2011). Thus, the objectives of this study were first to determine *in vitro*, the effectiveness of acetaldehyde and 2*E*-hexenal in controlling the growth of the postharvest potato blemish pathogens, *C. coccodes*, *H. solani*, and *P. atrosepticum* and secondly to identify the minimum concentration of the volatiles required to inhibit pathogen growth.

2. Materials and methods

2.1. Fungal isolates and culture preparation

Mono-conidial cultures of the pathogens *C. coccodes, H. solani*, and *P. atrosepticum* were originally isolated from diseased potato tubers grown in Idaho. Cultures of *C. coccodes*, *P. atrosepticum*, and *H. solani* were grown on PDA (potato dextrose agar, VWR International, Randor, PA, USA) in plastic Petri dishes (1 × 10 cm diameter) for 1 week at 23 °C in the light. Conidia and mycelia from the fungal pathogens *C. coccodes* and *H. solani* were collected by flooding the surface of plates with sterile distilled water and gently scraping the surface with a sterile bent glass rod. The suspension (3 mL) was transferred to a sterile 15 mL plastic tube, which was vortexed for three 10 s intervals to dislodge the conidia from the mycelia. Conidial concentrations were determined using a hemocytometer and adjusted to 1×10^5 conidia/mL using sterile distilled water.

Cells from a 24-h-old *P. atrosepticum* culture were collected using a sterile loop (3 mm diameter) and suspended in 1 mL of sterile distilled water in a sterile 15 mL plastic tube. The suspension was vortexed for 10 s to evenly distribute the cells in each step in the dilution series used to obtain a final concentration of cells that was 1:10,000 (v/v) of the original solution.

2.2. In vitro bioassays

For the bioassay, three replicate plates for each pathogen, volatile, and volatile concentration combination were prepared. Ten microliters of each pathogen suspension was pipetted onto the center of separate 5-cm-Petri dishes that each contained exactly 10 mL of PDA. The fungal conidial suspensions were pipetted onto the top of the media in the center of each plate. Bacterial suspensions were pipetted onto the center of the media then spread evenly around the plate using a sterile bent glass stir rod to create a thin layer of the cells on top of the media. The inoculated lidless Petri dishes were carefully transferred in a sterile biosafety cabinet to sterile 1 L glass jars (VWR International). These jars were then sealed using Polytetrafluoroethylene (PTFE) tape (Mil SPEC T-27730A, Merco-Hackensack Inc, Hillburn, NY, USA) around the mouth of the jar and closed with modified screw cap lids fitted with a rubber plug septum (Shimadzu thermo-green from Sigma–Aldrich Corp., St. Louis, MO, USA) and a PTFE liner (Thomas Scientific Swedesboro, NJ, USA) to make sure that they were air-tight.

Liquid volumes of 2.5, 5, 7.5, and 10 μL of either acetaldehyde or 2*E*-hexenal, were injected into the jars using an airtight syringe (25 μL GASTIGHT Hamilton syringe, Reno, NV, USA) through the septum onto the side of the glass container. This allowed the volatile compounds to evaporate into the headspace of the jars. Liquid cyclohexanol was also injected into the jars and used as an internal standard at a constant volume of 2 μL. The jars were stored at 23 °C