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FAIMS based volatile fingerprinting for real-time postharvest storage infections detection in stored potatoes and onions

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ABSTRACT

Field asymmetric ion mobility spectrometry (FAIMS) was evaluated towards rapid and non-destructive detection of storage infections under varied storage conditions. Potato tubers and onion bulbs were inoculated with *P. carotovorum* subsp. *carotovorum* (causing soft rot) and *B. cepacia* (causing sour skin), respectively; and were incubated at room (around 25 °C) and reduced temperature condition (4 °C). Additional tubers and bulbs were inoculated with sterile water, which served as healthy controls. At room temperature, FAIMS could detect potato soft rot and onion sour skin pertinent volatile organic compounds (VOCs) as early as 1 and 3 day(s) after inoculation (DAI) for potato tubers and onion bulbs, respectively. At a reduced temperature (4 °C), the respective detection time frames were 11 and 16 DAI. Principal component analysis (PCA) based contribution analysis on FAIMS dispersion field data revealed a significant range of dispersion field (DF) intensity (52%–72%) and compensation voltage (CV) (−1.30 V to −0.90 V) that can potentially be used to train FAIMS for triggering an alarm during real-time monitoring of soft rot pertinent VOCs. This critical range was 47%–77% DF and −0.24 V to 0.48 V CV for sour skin pertinent VOCs. Naïve Bayes (NB) and linear discriminant analysis (LDA) classifiers tested on PCA datasets reported overall accuracies in the range of 71–100% and 69–100% for soft rot and 63–97% and 58–100% for sour skin, respectively. Higher accuracies were reported as days after inoculation progressed. Baseline sensing of different VOCs using FAIMS revealed that ethanol, acetone, 2-butanone and ethyl acetate were specifically contributing to *P. carotovorum* subsp. *carotovorum* caused soft rot peaks whereas pentane and 1-butanol were associated with healthy as well as inoculated tubers. Dimethyl disulfide, dipropyl disulfide, methyl propyl disulfide, undecane and 2-undecanone were found to be associated with healthy controls as well as with sour skin infected onion bulbs.

1. Introduction

Potatoes (*Solanum tuberosum*) and onions (*Allium cepa*) are two important storage crops in United States (US). A large part of the produce is stored for 9–12 months to meet year-round consumer demand. The recommended storage temperature is 4–7 °C for consumable produce of potatoes (Voss and Timm, 2016), with minimum respiration rate for storage at 5 °C (Potato Council, 2012). The recommended storage temperature for onions is between 1 and 7 °C (USA Onions, 2016). During bulk storage, the crops are susceptible to many bacterial and fungal diseases. Major potato storage diseases include soft rot (*Pectobacterium carotovorum* subsp. *carotovorum*), ring rot (*Clavibacter michiganesis* subsp. *sepedonicus*) and pink rot (*Phytophthora erythroseptica*) (Kushalappa et al., 2002). These storage diseases can cause an average loss of about 7.5% annually in the U.S. potatoes (Olsen et al., 2006).

The major postharvest issue for onions are storage rots, which may be caused by more than 26 pathogens encompassing bacteria, filamentous fungi, and a yeast. Different bacteria like *Burkholderia cepacia, Dickeya chrysanthemi, Enterobacter cloacae* and *Pectobacterium carotovorum* subsp. *carotovorum* are documented to cause storage rots in onions. Sour skin infection in onions usually propagates in the field. However, losses mostly appear under storage causing a yield loss of 5–50% (Schwartz and Mohan, 2008). Bulk of the produce is wasted due to diseases in both the storage crops and losses can be as high as 100% for individual storage facilities (Pelter and Sorensen, 2004). Considering the economic losses to growers due to storage infections; it is imperative to detect such infections as early as possible. Early detection can lead towards better management practices being initiated to minimize the losses in these crops under storage conditions.

Produce under storage (e.g. potato, onion and carrot) naturally

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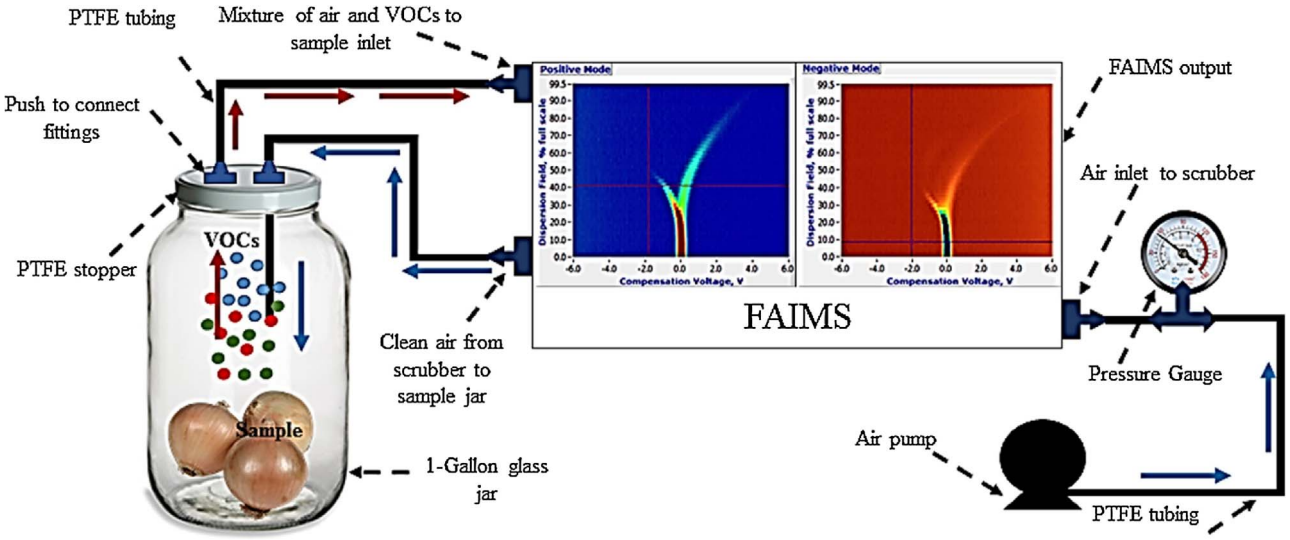

Fig. 1. Schematic for VOCs analysis using a customized module integrated with the portable FAIMS.

produce volatiles, which increase with disease severity, and physical or freeze damage (Toivonen, 1997; Jansen et al., 2011). Efforts towards detecting disease or stress specific volatile organic compounds (VOCs) from produce under storage were completed using gas chromatography mass spectrometry (GC–MS), GC–flame ionization detector (FID) and electronic nose (e–nose) (Varns and Glyn, 1979; Jarvenpaa et al., 1998; Kjeldsen et al., 2003; Prithiviraj et al., 2004; Li et al., 2011; Biondi et al., 2014; Konduru et al., 2015; Rutolo et al., 2016). GC–FID technique was used to discriminate potato tubers (incubated at 20 °C) inoculated with different bacteria (Kushalappa et al., 2002). Rutolo et al. (2016) developed an array of metal–oxide based gas sensors to detect *P. carotovorum* subsp. *carotovorum* related soft rot in potato tubers. The tubers, after inoculation, were kept in sealed plastic boxes and were incubated at 25 °C. de Lacy Costello et al. (1999) used GC–MS technique to identify the VOCs generated by potato tubers inoculated with *E. carotovorum* (now *P. carotovorum* subsp. *carotovorum*), *Bacillus polymyxa and Arthrobacter* sp. incubated at 10 °C. Li et al. (2011) used a gas sensor array to detect Botrytis neck rot (bulbs incubated at 24 ± 2 [mean ± std. dev.] °C) and sour skin (bulbs incubated at 30 °C) in onions, followed by VOCs quantification through GC–MS.

Recently, portable field asymmetric ion mobility spectrometry (FAIMS) was also used to detect huanglongbing disease in citrus (Alexander et al., 2014) and infections in stored potatoes and onions incubated at room temperature (25 ± 1 [mean ± std. dev.] °C) (Rutolo et al., 2014; Sinha et al., 2017a,b). Rutolo et al. (2014), using the potato variety Maris Piper, reported that differences between healthy and inoculated samples were observed in both positive and negative ion matrices of FAIMS. However, Sinha et al. (2017a), using the potato variety Russet Burbank, reported differences to be observed in only the positive ion matrix of FAIMS. FAIMS based biomarkers were studied to detect VOCs pertinent to sour skin infection in onions stored at room temperature (around 25 °C) (Sinha et al., 2017b). It is evident from these studies that most research to detect storage infections have been carried out at room temperature, which is very different from the bulk storage conditions (reduced temperature of 4 to 7 °C). Moreover, very few studies have evaluated FAIMS for disease detection of stored produce, and no study has reported the applicability of FAIMS for detection of postharvest storage disease in onions under reduced temperature condition.

Therefore, overall goal of the study was to study the pattern of release of VOCs from potatoes and onions, inoculated with *P. carotovorum* subsp. *carotovorum* and *B. cepacia* causing soft rot and sour skin respectively, stored under their respective bulk storage temperature conditions using portable FAIMS. The FAIMS based response for the detection of soft rot in potatoes and sour skin in onions were evaluated under room temperature conditions T1 (around 25 °C) and reduced temperature condition T2 (4 °C). Storing the samples was logistically more convenient at T2 and close to the temperature of minimum respiration rate, i.e. 5 °C, of the stored produce. The specific objectives were to evaluate the applicability of FAIMS towards: 1) detection of *P. carotovorum* subsp. *carotovorum* caused soft rot and *B. cepacia* caused sour skin in potatoes and onions respectively under bulk storage conditions, 2) assessment of the detection time frame of storage infections under bulk storage conditions, and 3) characterize FAIMS response for rapid disease onset monitoring and contrast it with infestation related volatile biomarkers.

2. Materials and methods

2.1. Sample preparation and inoculation

P. carotovorum subsp. *carotovorum* strain Ec101 inoculum was prepared as described previously (Sinha et al., 2017a). *Burkholderia cepacia* strain BsWSU1 inoculum was prepared as previously described (Schroeder et al., 2012). Potato tubers (*Solanum tuberosum* cv. Burbank) and onion bulbs (*Allium cepa* cv. Vaquero) were inoculated with *P. carotovorum* subsp. *carotovorum* strain Ec101 and *B. cepacia* strain BsWSU1, respectively. Potato tubers and onion bulbs were also inoculated with sterile water as healthy controls. Potato tubers were obtained from the produce of 2015, which were stored at a commercial storage facility (AgriNorthwest Inc., Prescott, WA). Tuber inoculations with *P. carotovorum* subsp. *carotovorum* strain Ec101 were completed as described previously (Sinha et al., 2017a). Yellow storage onion bulbs from the produce of 2015 (cv. Vaquero) were procured from a local grocery store. The dry skins of onion bulbs with no visible bruises or damage were removed from the bulbs. *B. cepacia* strain BcWSU1 was grown overnight in a 5 ml NBY at 28 °C with agitation, inoculum standardized to 1×10^6 CFU/ml and injected into onion bulbs as previously reported (Schroeder et al., 2012). Tubers and onion bulbs were inoculated with sterile water to serve as controls.

2.2. Experimental module

VOCs released from inoculated samples, and the healthy controls were sampled using a custom sampling module (Fig. 1). The glass jar sealed on top with Polytetrafluoroethylene (PTFE) stopper facilitated the accumulation of VOCs in the headspace. The inlet and outlet ports on the stopper were used to circulate the accumulated VOCs for