Pathogenic *Fusarium oxysporum* f. sp. *cepae* growing inside onion bulbs emits volatile organic compounds that correlate with the extent of infection

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Abstract

Diseases develop during the storage of onions. To minimize losses, new methods are needed to identify diseased bulbs early in storage. Volatile organic compounds (VOCs), the respiration rate, weight loss, and the dry matter content were investigated for 1 to 7 weeks post inoculation of bulbs with water (control) and two strains (Fox006 or Fox260) of *Fusarium oxysporum* f. sp. *cepae*. Photos, multispectral image analysis, and real-time polymerase chain reaction (PCR) showed no infection in the control onions, weak pathogenic infection in Fox006-onions, and strong pathogenic infection in Fox260-onions at week 7 post inoculation. Infected bulbs exhibited increased respiration rate, increased VOC emission rate, and increased weight loss. The control and Fox006-onions did not respond to inoculation and had similar reaction pattern. Forty-three different VOCs were measured, of which 17 compounds had sulfur in their chemical structure. 1-Propanethiol, methyl propyl sulfide, and styrene were emitted in high concentrations and were positively correlated with the extent of infection ($r = 0.82 – 0.89$). Therefore they were the most promising volatile markers of *Fusarium* basal rot infection. For the first time, we show that the extent of fungal infection in onions determined by real-time PCR is related with VOC emission.

Keywords:

Allium cepa L.; food spoilage; SPME; image analysis; real-time PCR; respiration rate.

1. Introduction

Minimizing food loss and waste is crucial for the fresh fruit and vegetable sector (Lipinski et al., 2013). Onions are a major vegetable crop with a global total production of 88 million tons in 2014, making onions the 6th most-produced vegetables of great economic importance in the world (FAOSTAT, 2014). Although the onion bulb is suitable for long-term storage, losses due to moisture loss, sprouting and especially spoilage by microorganisms is a serious problem during storage (Haapalainen et al., 2016; Petropoulos et al., 2016). It has been reported that losses due to diseases can be higher than 90 % of the total production, with *Fusarium spp*. accounting for up to 30 % of the losses (Brown and Leclaire-Conway, 2013; Haapalainen et al., 2016; Petropoulos et al., 2016; Rasiukeviciute et al., 2016).

Pathogens causing diseases in onion bulbs may appear from soil and or be transmitted from flies and herbivores during growth, but they do not reach their manifestation stages before storage (Snowdon, 1990). Most diseases, such as neck rot (*Botrytis allii/aclada/byssoidea*), *Pectobacterium* soft rot (*Pectobacterium carotovorum* subsp. *carotovora*, earlier *as Erwinia*), slippery skin (*Burkholderia gladioli* pv. *Alliicola*), sour skin (*Burkholderia cepacia*), yeast soft rot (*Kluyveromyces marxianus var. marxianus*), *Enterobacter* bulb decay (*Enterobacter cloacae*), *Fusarium* basal rot (*Fusarium oxysporum* f. sp. *cepae*), and *Fusarium* bulb rot (*Fusarium proliferatum*), initiate their development inside the bulbs without visible symptoms on the outside, making early detection of these diseases in onion storage challenging (Schwartz and Mohan, 2007; Snowdon, 1990).

To minimize food losses during storage, managers need tools to identify diseased batches early in storage. Methods of detecting onion diseases include assessment of any disease symptom that may be present by visual inspection of halved bulbs, or identification of spoilage microbes by fungal specific DNA measurements using real-time polymerase chain reaction (PCR) (Haapalainen et al., 2016; Snowdon, 1990). Both methods are destructive and require

63 sampling of bulbs for analyses. New nondestructive methods are emerging, such as image
64 analysis (Islam, 2018; Islam et al., 2018) and volatile organic compound (VOC) profiling using
65 gas sensors or traditional GC-MS (Dudareva et al., 2006; Nezhad, 2014). However, image
66 analysis is not applicable for monitoring during storage, as bulbs must be sampled and analyzed
67 on a regular basis to identify batches that have low storability. In contrast, VOC profiling of
68 onion bulbs is promising, as the VOC method is nondestructive and requires only an air sample
69 for analysis (Li et al., 2011; Luca, 2015; Luca et al., 2015; 2016; Sinha et al., 2018).

70 Many factors influence the VOC emission rate and the type of compounds present in the
71 VOC sample, including the applied analytical method, storage temperature, storage duration,
72 pathogen species and the living plant tissue (Chen et al., 2006; Gouinguené and Turlings, 2002;
73 Li et al., 2011). Several researchers (Li et al., 2011; Prithiviraj et al., 2004; Vikram et al., 2005;
74 Wang et al., 2018) studied the VOC emission from experimentally inoculated onion bulbs and
75 onion pathogens growing in onion medium. Specific volatiles were found, e.g., ethyl
76 cyclobutane was specific to onion bulbs inoculated with *F. oxysporum*; however, the results
77 were not comparable because different sampling and detection methods were applied.

78 Onions are usually kept for many months in cold storage. During storage, diseases may
79 develop slowly without notice if pathogens are present and the growing conditions and storage
80 duration are appropriate (Snowdon, 2010). It is unknown how the VOC profile of infected
81 onions changes over time in storage and whether emitted VOCs correlate with fungal growth
82 and visible disease symptoms. Two strains of *F. oxysporum* f. sp. *cepae* (Fox006 and Fox260)
83 that cause basal rot in onions were selected for the studies. These strains have comparable
84 pathogenic traits in onion seedlings but represent two phylogenetically different groups within
85 the *F. oxysporum* f. sp. *cepae* species (Haapalainen et al., 2016). We hypothesized that the
86 VOC profile of these two strains would be discernable and that the total amount of emitted
87 VOCs would be positively correlated with the levels of fungal biomass and the extent of

infection. Solid-phase micro-extraction (SPME) was applied for extraction of the VOCs from the headspace of inoculated onions, as this method has been previously shown to be applicable for sampling of low-molecular weight sulfur compounds (Luca et al., 2016), with which onion tissue is rich in (Løkke et al., 2012). To study the relationship between the VOC emission rate and the fungal biomass, the infection levels at different numbers of weeks post inoculation (wpi) were quantified by multispectral image analysis and real-time PCR of the same onion bulbs. The objective of this study was to elucidate if fungal infections in onion bulbs, here exemplified by *Fusarium oxysporum* f. sp. *cepae*, are related with increased metabolic activities and VOC emission rates.

2. Materials and Methods

2.1 Plant materials

Yellow onion (*Allium cepa* L.) of the cultivar 'Summit' was grown conventionally in 2016, harvested, artificially dried and stored for one month by a commercial grower (Alex Månsson A/S, Brande, Denmark). One hundred kg of the cultivar was sampled and brought to the university, where it was stored at 0.5 °C and approximately 85% RH until the start of the inoculation experiment.

2.2 Fusarium inoculum preparation and inoculation of onions

Two strains of *F. oxysporum* f. sp. *cepae*, Fox006 and Fox260, were selected from the collection at the Natural Resources Institute of Finland, Jokioinen, Finland (Haapalainen et al., 2016). These strains were isolated from mature onion bulbs, and they were tested on onion seedlings, in which they showed pathogenic traits. Moreover, the strains represent two phylogenetically different groups within the *F. oxysporum* species (Haapalainen et al., 2016).

111 The fungi were grown on PDA plates at room temperature (22 °C) for three weeks before
112 harvesting the spores for the inoculation experiment. The mycelia with spores were scraped off
113 the plates, suspended in sterile water, and filtered through sterile cheesecloth. The spores in the
114 filtrate were counted using a Fuchs-Rosenthal counting chamber (Struers KEBO LAB A/S,
115 Herlev, Denmark) and diluted to 10^6 spores mL^{-1} before use.

116 Healthy, firm bulbs of 100 – 150 g each without wounds or skin cracks were taken from
117 cold storage the day before inoculation and placed in a walk-in storage room (22 °C, < 50%
118 RH). One hundred and thirty-two healthy bulbs were inoculated at 6 time points (22 bulbs x 6
119 times) during a period of seven weeks, corresponding to 1, 3, 4, 5, 6, and 7 weeks post
120 inoculation (wpi). Thus, the bulbs had different levels of infection when analyzed at 7 weeks
121 after start of the experiment.

122 Each time, nine bulbs were inoculated with a 0.1 mL Fox006-spore suspension, nine with a
123 0.1 mL Fox260-suspension, and four with 0.1 mL sterile water (control). The liquid suspension
124 was injected through a sterile needle attached to a 1-mL syringe (TERUMO, Tokyo, Japan)
125 just above the basal plate of the bulb and approximately 1 cm deep into the tissue. The
126 inoculated onions were weighed, placed on egg cardboard trays, and stored in the walk-in room
127 at 22 °C until analysis. Each bulb was treated as an individual replicate.

128 *2.3 Determination of weight loss and respiration rate*

129 The weight of each bulb was recorded again at 1, 3, 4, 5, 6, and 7 wpi. The bulb was then
130 placed in a 1-L glass jar at 20 °C, and the jar was closed with a lid containing a septum for gas
131 and SPME sampling. The weight loss was calculated as a percentage from the weight difference
132 between the bulb weight at inoculation and the bulb weight at the different wpi divided by the
133 bulb weight at inoculation. The respiration rate was determined as the change in the CO_2
134 content during 24 h of incubation (Mahajan et al., 2016) and given in mL CO_2 kg^{-1} h^{-1}. The

CO_2 content was measured at time 0 and time 24 h through the septum of the lid and analyzed on a CheckMate 9900 (Dansensor, Ringsted, Denmark) equipped with a pump for gas sampling and a dual-beam infrared sensor for determination of the CO_2 content.

2.4 Volatile analysis

VOCs were analyzed at 1, 3, 4, 5, 6, and 7 wpi. As all onions were wounded during inoculation, VOCs were sampled first time after 1 week to avoid any volatile signatures from the mechanical damage and wounding of the bulbs at injection (Christensen et al., 2007). VOCs were sampled by SPME after 24 h of incubation of the bulbs in jars. An 85 µm CAR/PDMS fiber (Supelco, Bellefonte, PA) was used for the 5 min extraction. The fiber was transferred to another jar for 5 min at 20 °C for external standard addition (0.90 nmol L^{-1} of 3-methyl-2-pentanone). The sampled VOCs were analyzed as described by Luca et al. (2015) and Wang et al. (2018), with slight modifications. The GC temperature program was 30 °C for 1 min, increasing to 250 °C at 20 °C min^{-1} and then holding at this temperature for 18 min. The total ion chromatogram was recorded on an MS equipped with an inert XL mass-selective detector (Agilent 5975C, Agilent Technologies) over the mass-to-charge ratio (*m*/*z*) range of 29 - 110 atomic mass units (amu) between 4 and 12 min and over the range of 30 - 150 amu between 12 to 30 min.

VOCs were identified and quantified using authentic reference compounds according to the method of Wang et al. (2018). The VOC emission rate in nmol kg^{-1} during 24 h (the incubation time) was calculated from the VOC concentration in nmol L^{-1} of the free headspace in the jar containing the onion and the fresh weight of the bulb. The total volatiles were the concentration of all individual compounds. All authentic compounds were obtained from Sigma-Aldrich Chemie GmbH (Stenheim, Germany).

158 *2.5 Multispectral image analysis and photos*

159 Following VOC analysis, each bulb was inspected for symptoms of disease by multispectral
160 imaging. Bulbs were cut into halves from the neck to the base with a sterile knife, and the
161 reflectance of the surface was recorded with a VideometerLab instrument (Videometer A/S,
162 Hørsholm, Denmark). The instrument consisted of a computer and a monochrome grayscale
163 CCD camera mounted on top of an integrating sphere (Dissing et al., 2011). The sphere was
164 coated inside with matte white to ensure that light was scattered uniformly to give diffuse light
165 upon illumination. The sphere had 18 light-emitting diodes (LEDs) (405, 435, 450, 470, 505,
166 525, 570, 590, 630, 645, 660, 700, 780, 850, 870, 890, 940, and 970 nm) at the rim, which were
167 strobed successively. The instrument was calibrated before analysis following the
168 manufacturer's instructions. Both halves of the bulb, one at a time, were placed in horizontal
169 position under the sphere on an O-ring, with the curved side down. The reflection was acquired
170 in darkness. The multispectral images were analyzed using the VideometerLab software,
171 version 3.0.30. A normalized canonical discriminant analysis was applied to make a
172 segmentation file to minimize the distance to observations within classes and to maximize the
173 distance to observations between classes (Cruz-Castillo et al., 1994). The analysis resulted in
174 highlighted features, which were then segmented using a simple threshold morphology, giving
175 both the diseased and the total area of the bulb halves. From these data, the percentage of
176 diseased tissue out of the total was calculated, and averages were determined for each bulb.
177 Photos of the cut onions were taken with a digital camera (Canon EOS 5D Mark II, Tokyo,
178 Japan) in a photo studio with standardized light settings. The bulb halves were placed similarly
179 to the method for the image analysis.

2.6 Dry matter content

After removal of the outer dry skin and base plate of the onion, the bulb tissue was homogenized in a blender, and from this mixture, 20-30 g tissue was frozen in liquid nitrogen and freeze-dried for one week in a CRIST 173 dryer (Osterode am Harz, Germany). The blender was cleaned with 70 % ethanol between samples to prevent carry-over of DNA between bulbs. The dry matter (DM) content was determined by weighing the tissue before and after freeze-drying and given as a dry matter percentage on a wet weight basis.

2.7 DNA extraction and DNA quantification by real-time PCR

Two to three grams of freeze-dried tissue was pulverized in a shaking mill (MM200, Part no. 20.746.0001, Serial no., 129101108, Retsch GmhH, Haan, Germany). The grinding chamber was cleaned with 4 M HCl between each bulb sample. Fifteen mg of milled powder was mixed with 400 µL Buffer AP1 from a DNeasy Plant Mini kit (QIAGEN, Hilden, Germany) in a 2 mL Lysing Matrix E tube (MP Biomedicals, Santa Ana, California). Each sample was lysed three times on a Powerlyzer (QIAGEN) at a speed of 4500 rpm for 45 s, with chilling on ice for 5 min between each run. DNA was extracted from the lysate using the DNeasy Plant Mini kit according to the manufacturer's instructions. A total of 40 µL (2 x 20 µL) of nuclease-free water was used for elution, and the resulting DNA samples were kept at -20 °C until quantification.

The relative amount of *F. oxysporum* f. sp. *cepae* (Fox) DNA in each sample was determined by real-time PCR, using species-specific primers and a standard curve-based method. The concentrations of Fox DNA extracted from a pure culture of the fungus and onion DNA extracted from healthy onion bulbs were measured by Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) using a Qubit dsDNA High-Sensitivity (0.2 to 100 ng) Assay Kit (Thermo Fisher Scientific) according to the manufacturer's protocols. Two µL of the DNA

solution was mixed in 198 µL of the Qubit working solution. A series of dilutions were prepared from each of these two DNA standards, and run together with the samples in the real-time PCR. To overcome the effects of possible interference by onion DNA when detecting Fox DNA in the extracts, the Fox DNA standard was diluted in a solution containing 8 ng of onion DNA per µL, in line with the onion DNA concentrations found in the samples. The onion standard was diluted in water. Two sets of nine Fox-inoculated samples (Fox006 and Fox260) and four control samples were analyzed from each time point, giving a total of 132 samples.

For quantification of Fox DNA, the primers OMP1049 (5'-TGCGATTTGGACGAGATATGTG-3') and OMP1050 (5'-ATTTGCCTACCCTGTA CCTACC-3') (Validov et al., 2011) were used to amplify a 110-bp fragment of the 18S-28S ribosomal intergenic spacer region. For quantification of onion DNA, new primers AcCOX1F (5'-CGTGCTTACTTCA CCGCAGCT-3') and AcCOX1R (5'-TTCCTGTGAGCCCGCCTA TGG-3') were designed to amplify a 163-bp fragment of the mitochondrial cytochrome c oxidase subunit 1 (COX1) gene (GenBank GU138027.2). Each reaction (20 µL) contained 10 µL of 2×SYBR Green I Master Mix (Roche, Basel, Switzerland) primers at a 300 nM concentration and 5 µL of template DNA (diluted 1:30 prior to measurement). The real-time PCR temperature program for Fox and onion DNA was as follows: an initial denaturation step at 95 °C for 5 min, 45 cycles of denaturation at 95 °C for 10 s, primer annealing at 63 °C for 10 s, and elongation at 72 °C for 10 s. The reactions were run on a Lightcycler® 480 II (Roche) with fluorescence monitoring at the end of each elongation step. For template quantification, a threshold cycle (Ct) value was automatically determined by the Lightcycler software. Melting curve analysis was performed after amplification by heating to 95 °C, cooling to 65 °C, and then heating to 97 °C at increments of 0.11 °C s^{-1}, with data collection five times per degree Celsius. All samples and standards were run in triplicate and the Ct mean values were used for calculations. Two standard curves (Ct mean values vs concentrations) were obtained from the

229 standard dilution series, one for the Fox DNA and one for the onion DNA. Based on these
230 curves, the amount of Fox DNA and the amount of onion DNA were calculated for each sample
231 (absolute quantification) and given as ng of Fox DNA per mg of onion DNA.
232
233 *2.8 Data analysis*
234 The VOC chromatograms were divided into 37 local intervals, and Parallel Factor Analysis
235 2 (PARAFAC2) (Amigo et al., 2010) was applied for the quantification of VOCs based on peak
236 areas using the PLS-Toolbox (PLS-Toolbox ver. 8.0.2, Eigenvector Research Inc., Wenatchee,
237 Washington) in MATLAB® (ver. 9.3.0, The Mathworks Inc., Natick, Massachusetts). One-
238 way analysis of variance (ANOVA) was applied at each wpi to identify differences in the VOC
239 emissions between treatments, and Tukey's honestly significant difference (HSD) test was used
240 for multiple comparisons at $P = 0.05$. Data presented in the figures are the means of 4 control
241 bulbs and 9 Fox006 or 9 Fox260 bulbs ± standard error. Principle component analysis (PCA)
242 was applied in SIMCA-P+ (ver. 15, Umetrics, Umeå, Sweden) to describe the relationships
243 between the treatments (control, Fox006, Fox260) and the total VOCs, respiration rate, weight
244 loss, dry matter content, diseased area, and Fox DNA ratio. Mean values over bulb replicates
245 were used in the PCA, and all data were mean-centered and auto-scaled before analysis. Linear
246 correlations were calculated for all Fox260-inoculated onions (6 times * 9 bulbs, in total 54
247 samples) after ln transformation of the Fox DNA ratios and the individual VOC emission rates.
248 ANOVA, Tukey's HSD test and linear correlations were determined in the R software (version
249 3.4.4, R Development Core Team, 2018).
250

3. Results and discussion

3.1 Symptoms of basal rot and levels of Fusarium infection

Visual inspection of the bulb interior revealed that the control onions showed no symptoms of basal rot or other diseases and bulbs were thus regarded as healthy (Fig. 1). In contrast, the Fox260-inoculated onions (Fox260-onions) showed distinct symptoms of basal rot (Fig. 1). The Fox260-onions first developed mild symptoms beginning at the basal plate (1 wpi), then symptoms became more distinct at weeks 3-4, and finally, the entire bulb was infected at 5-7 wpi. The symptoms were a watery brown to black appearance with lesions beginning at the basal plate, moving upwards and into the inner fleshy scales and then upwards and outwards in the bulb at increasing wpi (Fig. 1). The only symptoms of basal rot in the Fox006-onions were at week 5 post inoculation, but symptoms were weak, hardly visible, and had little progress over time following inoculation (Fig. 1). 'Hylander' bulbs also developed weak symptoms following artificial inoculation with the Fox006 strain at 3 wpi (unpublished data). For that reason, the Fox006 strain was regarded as weakly pathogenic. In contrast, the Fox260 strain was regarded as strongly pathogenic, as bulbs developed severe symptoms of infection shortly after inoculation. These differences in pathogenicity between bulbs are interesting, as Haapalainen et al. (2016) reported that both the Fox006 and Fox260 strains were strongly pathogenic to onion seedlings. However, the pathogenicity to bulbs was not tested. Taylor et al. (2016) tested the pathogenicity of *F. oxysporum* f. sp. *cepae* isolates and found that isolates with high pathogenicity to seedlings also had high pathogenicity to bulbs. Haapalainen et al. (2016) reported that the two tested Fox-strains belonged to different phylogenetic groups, which may explain the observed differences in pathogenicity to bulbs in our study.

Image analysis showed that the area of diseased tissue within the halved bulb area increased in the Fox260-onions compared to the control and Fox006-onions over time, except between 6

and 7 wpi, at which point the diseased area leveled out (Fig. 2A). The diseased area ranged between 0.02 - 0.4% and 0.7 - 2.4% in the control and Fox006-onions, respectively, while it ranged between 8% - 21% in the Fox260-onions (Fig. 2A). At week 1, however, the results of image analysis did not perfectly reflect the visual appearance of the Fox260-onions (Fig. 2A), as bulbs had mild symptoms of fungal infection (Fig. 1) but 8% diseased tissue (Fig. 2A). Nevertheless, the image analysis gave quantitative indications of the disease levels of bulbs post inoculation. Statistical analysis revealed that the disease area percentages were higher at all weeks post inoculation in the Fox260-onions compared to in the control and Fox006-onions and that control and Fox006-onions had nonsignificant differentiated percentages of diseased area, regardless of wpi.

The DNA analysis gave corresponding results to those of the image analysis and the photos at the different wpi, except at week 7 post inoculation (Figs. 1, 2). Control onions contained no fungal specific DNA as the Fox DNA ratio was zero, while the Fox006-onions contained small amounts of fungal specific DNA ranging from 0.01 to 0.5 ng mg^{-1} onion DNA indicating weak pathogenic infection (Fig. 2B). In contrast, there were strong pathogenic infection in the Fox260-onions shown by the high fungal specific DNA content (84.8 ng mg^{-1} onion DNA) at 7 wpi. At week 1, there were no differences in the Fox DNA ratio between treatments, but later, the Fox260-onions had much higher levels than did the control and the Fox006-onions due to fungal proliferation inside the bulbs (Figs. 1, 2B). For the Fox260-onions, the image and DNA analyses showed that the diseased area and the Fox DNA ratios increased from week 1 to week 6, while the diseased area remained unchanged and the Fox DNA ratio increased from week 6 to 7 post inoculation (Fig. 2). These results show that the spreading of the disease eased, while the amount of fungal specific DNA increased.

Overall, the results showed that symptoms of basal rot developed early after artificial inoculation of onion bulbs with the Fox260 strain at room temperature. Shortly after

inoculation, a slight color change was observed, and later, darker-colored scales developed inside the bulbs (Fig. 1). It is known that *F. oxysporum* spp. have a hemibiotrophic ecology, secreting effectors and enzymes that cause cell disruption (De Silva et al., 2016) and brown or black discoloration due to mixing of enzymes and phenolic compounds that are normally present in separate compartments of the cell (Toivonen and Brummell, 2008).

3.2 Physiological responses of bulbs to infection

The total VOC emission rate of the Fox260-onions increased in response to infection (Fig. 3A) and the development of visual symptoms of basal rot (Fig. 1). The emission rates were higher in the Fox260- than in the control and Fox006-onions at 3 - 7 wpi (Fig. 3A). During proliferation, the Fox260 strain grew into the fleshy scales of the bulbs, causing wounding of the cells and discoloration (Fig. 1), which increased the formation of VOCs from the enzymatic degradation of nonvolatile precursors inside bulbs (Christensen et al., 2007). The VOC emission rate of Fox260-onions peaked at week 6, and then it decreased to the level present at week 5, probably due to reduced nutrient availability and a possible shift toward sporulation. The onion weight loss also leveled out between week 6 and 7 (Fig. 2D), which could indicate that moisture loss through evapotranspiration and loss of carbon through respiration (Maguire et al., 2001) decreased due to reduced fungal growth and less progress in wounding of cells.

Generally, the emission rates from the control onions were low (1.12 nmol kg^{-1} d^{-1}), which corresponded to the low VOC emission rates reported from healthy onions in storage (Wang, 2018). The total VOC emission rates of the Fox006-onions at weeks 3 - 7 post inoculation were similar to the levels of the control onions, even though small amounts of fungal specific DNA were measured and weak symptoms of infection were present (Figs. 1, 2B, 3A). Again, this indicated that the Fox006 strain was weakly pathogenic to onion bulbs.

Increased respiration was the first physiological symptom of infection. The respiration rate was more than double in the Fox260-onions compared to that in the control and Fox006-onions at week 1 post inoculation (Fig. 3B). At weeks 3 - 6, the differences between the Fox260-onions and the two other treatments further increased, indicating that the prevalent growth of fungi stimulated bulb respiration. Wang et al. (2016) also found higher respiration rates in newly infected bulbs than in bulbs in later stages of infection.

The DM content was 11 - 12% at week 1, decreasing to 8 – 11% at week 7 post inoculation, with a constantly higher DM content in the control and Fox006-onions compared to the Fox260-onions (Fig. 3C). From week 1 to week 7, the DM content decreased by 0.5 - 1% in the control and Fox006-onions but more than doubled (2.5%) in the Fox260-onions. This result was in line with the higher respiration rate and volatile emission rate of the Fox260-onions compared to the other onions and the continuous higher exploitation of substrate for respiration and volatile emissions (Block et al., 1992; Kays and Paull, 2004; Løkke et al., 2012; Petropoulos et al., 2016) caused by fungal growth and decay. Because of the higher respiration and VOC emission rates of the Fox260-onions, the weight loss was also higher in the Fox260-onions compared to that in the other onions (Fig. 3B, D). Overall, the control and Fox006-onions lost 3% of their bulb weight during 7 wpi, while the weight loss was almost 5 times higher in the Fox260-onions, at a total of 15% (Fig. 3D). Weight loss of fresh produce during storage occurs due to several processes, including moisture loss through evapotranspiration, dry matter loss through respiration (Thamizharasi and Narasimham, 1991), and carbon and sulfur loss through volatile emissions (Fig. 3A-C). The storage humidity was kept below 50% RH after inoculation of the bulbs to prevent the growth of *Penicillium spp.* and other secondary pathogens, which may have enhanced the evapotranspiration from the diseased basal plates of the Fox260-onions.

3.3. Relationship between the levels of infection and the response of bulbs to infection

The relations between the levels of infection (Fox DNA ratio and diseased area) and the response of bulbs to infection (total VOCs, respiration rate, dry matter content, and weight loss) are shown in Fig. 4. Two principal components, PC1 and PC2, explained a total of 93% of the variation in the data, with 77% along PC1 and 16% along PC2. The samples were distributed in three groups in the score plot: those related to control onions, to Fox006-onions and to Fox260-onions (Fig. 4A). Control onions were placed to the left along PC1, followed by Fox006-onions, which were placed to the left but downwards. In contrast, the Fox260-onions were first located in the middle of PC1 and downwards on PC2, and then they moved upwards to the right as basal rot developed post inoculation (Fig. 4A). Only Fox260-onions showed a logic grouping according to wpi, forming three clusters: 1 wpi, 3 - 4 wpi, and 5 - 7 wpi. The distribution of the samples from left to right along PC1 corresponded well to the levels of infection. From the loading plot, it was clear that the total VOCs, respiration rate, and weight loss were related to the Fox DNA ratio and the diseased area caused by pathogenic *F. oxysporum* f. sp. *cepae* infection (Fig. 4B). Thus, the distribution of the samples along PC1 was driven by the diseased area and total VOCs while the Fox DNA ratio drove the distribution along PC2. To further explore the possibility of using VOCs as biomarkers for pathogenic infection of onions in storage, the VOC release was studied at the different wpi to find differences and similarities between healthy (control onions) and diseased onions (Fox260-onions).

3.4 Emission of volatiles from control and Fusarium-infected onions

All bulbs were wounded during injection, which is known to facilitate emission of volatiles from the action of enzymes on non-volatile precursors in onions (Christensen et al., 2007). For this reason, VOCs were collected 1 to 7 weeks after injection to avoid any volatile signatures

371 from the mechanical damage and wounding of the tissue at week 0. Forty-three VOCs were
372 measure as these compounds were above the detection limit of the analytical method. The
373 emission rates ranged from close to zero to 6234 nmol kg^{-1} d^{-1} depending on treatment and time
374 post inoculation (Table 1). The VOC profile of the Fox006-onions was similar to the profile of
375 the control onions, except that some compounds were not present in the headspace of control
376 onions but present in very low, nonsignificant concentrations in the Fox006-onions. Therefore,
377 the results of the Fox006-onions are not shown in Table 1.

378 All VOCs in Table 1 were within the low to medium boiling range, with boiling points
379 ranging from -54 to 164 °C corresponding to the selected method for sampling of VOCs from
380 fresh produce (Luca et al., 2015). Seventeen compounds had sulfur in their chemical structures,
381 and the remaining compounds were acids (1), alcohols (4), aldehydes (4), alkanes (2), alkenes
382 (4), arenes (1), esters (2), furans (3), and ketones (5) (Table 1). Twenty-seven of the VOCs
383 have previously been reported from healthy and diseased onions, fresh-cut onions or onion
384 extracts cultured with *F. oxysporum* f. sp. *cepae* or *F. proliferatum* strains (Løkke et al., 2012;
385 Prithiviraj et al., 2004; Vikram et al., 2005; Wang et al., 2018). The remaining compounds
386 (isoprene, pentane, (*E*)-1,3-pentadiene, C_5H_8, 2-methylfuran, methyl vinyl ketone, 3-
387 methylfuran, 2-ethylfuran, methyl isopropyl sulfide, (*E*)-2-methyl-2-butenal, heptane, 2,4-
388 dithiapentane, styrene, allyl propyl sulfide, 2-heptanone, and 2,2-bis(methylthio)propane) were
389 newly detected in onions.

390 The results in Table 1 show that most compounds were emitted from both control and
391 Fox260-onions, except for propene, methanethiol, (*E*)-1,3-pentadiene, C_5H_8, 1-propanol,
392 methyl isopropyl sulfide, 3-methyl-1-butanol, styrene, and 2-heptanone, which were only
393 detected in the Fox260-onion headspace (Table 1). In theory, these 9 compounds could be
394 potential markers for pathogenic infection in onion bulbs caused by *Fusarium oxysporum* f. sp.
395 *cepae* if compounds are only emitted from the infected Fox260-onions and emission rates

increase with increasing wpi. However, only 5 out of the 9 compounds showed this trend (propene, (*E*)-1,3-pentadiene, C_5H_8, methyl isopropyl sulfide, and styrene) while the remaining 4 compounds (methanethiol, 1-propanol, 3-methyl-1-butanol, and 2-heptanone) had no clear pattern in increase in the VOC emission rates at the different wpi (Table 1). However, other compounds could also be potential markers, e.g., VOCs that constantly have a high relative emission rate compared to those of the control onions (VOC ratio) at the different wpi. The VOC ratios at the different wpi showed that 38 compounds had a VOC ratio that was 2 times or higher (infinity) in the Fox260-onions than that in the control. This result indicates that the pathogenic infection of onion bulbs increased the VOC emission rate of many different compounds and that higher emission rates were linked to infection of bulbs. Some compounds had higher emission rates at all weeks post inoculation (propene, isoprene, pentane, 3-methylfuran, and styrene), while others first differed at week 3 post inoculation (ethanol, (*E*)-1,3-pentadiene, 2-methylfuran, 2-ethylfuran, and the 17 sulfur-containing compounds) (Table 1). This result shows that VOC analysis can already give clear and early indications of pathogenic infections after 1 week given that the compounds can be sampled, when visual symptoms (Fig. 1) and levels of infection (Fig. 2B) do not yet give clear indications. Based on these results, 26 of the 43 compounds were selected as potential markers of pathogenic infection (Table S2), of which 16 compounds had sulfur in their chemical structure and the remaining belonged to other chemical groups (Table 1).

3.5 Markers of Fusarium infection

The VOC emission rates were positively correlated with the Fox DNA ratios for all selected compounds, with correlation coefficients ranging from 0.42 to 0.89 at weeks 1 – 7 post inoculation (Table S2). The emission rates increased with wpi for 11 of the 26 compounds (propene, isoprene, (*E*)-1,3-pentadiene, C_5H_8, 2-methylfuran, 3-methylfuran, 1-propanethiol,

2-ethylfuran, (*E*)-methyl 1-propenyl sulfide, 3,4-dimethylthiophene, and 2,2-bis(methylthio)propane). These compounds were thus sensitive to changes in the fungal growth between wpi. The remaining 15 of the 26 compounds, however, showed a decrease in the emission rate between weeks 6 and 7 (Table 1). These compounds had higher correlation coefficients to the Fox DNA ratio when coefficients were calculated based on data from weeks 1 – 6 (Table S2). Ethanol, 1-propanethiol, methyl propyl sulfide, dimethyl disulfide, styrene, and methyl propyl disulfide drew the most attention because these compounds had high VOC emission rates and were either unique to Fox260-infected onions (styrene) or had high VOC ratios early in infection (ethanol, 1-propanethiol, methyl propyl sulfide, dimethyl disulfide, and methyl propyl disulfide). This result is in line with the data of Sinha et al. (2018), who found a high abundance of dimethyl disulfide and methyl propyl disulfide in response to fungal infection of onions in storage. The average emission rates were 50 nmol kg^{-1} h^{-1} or higher for the selected compounds in the Fox260-onion headspace, and VOC ratios were more than 40 times higher on average over the different wpi (Table 1). 2,2-Bis(methylthio)propane had a high correlation with the Fox DNA ratio, too (r = 0.87-0.88) (Table S2), but this compound was only tentatively identified (Table 1) and was thus excluded from further analysis.

The linear correlations between the selected 6 compounds and the Fox DNA ratios are shown in Fig. 5. Although these 6 compounds were all high in abundance, the plots showed that some compounds were better correlated with the Fox DNA ratio than others. Ethanol had the highest abundance (Table 1) but the lowest correlation coefficient with fungal specific DNA (r = 0.62), followed by dimethyl disulfide (r = 0.68) and methyl propyl disulfide (r = 0.74) (Fig. 5A, D, F). In contrast, 1-propanethiol, methyl propyl sulfide, and styrene had correlation coefficients between 0.82 - 0.89, which made these compounds good candidate markers for pathogenic infection caused by *F. oxysporum* f. sp. *cepae* in onions. Among these three compounds, 1-propanethiol had a steeper slope (0.97) than did methyl propyl sulfide and

445 styrene (slopes were 0.89 and 0.83, respectively), which indicates a faster increase in the
446 emission of 1-propanethiol along with fungal growth and disease development. Only styrene
447 was unique to Fox-inoculated onions (Table 1). The highest emission rates were observed at
448 week 6 post inoculation, with concentrations of 0 and 127 nmol kg^{-1} h^{-1} in the control and
449 Fox260-onions, respectively. Fox006-onions also emitted styrene, and the concentration was
450 2.3 nmol kg^{-1} h^{-1} at week 6 post inoculation (data not shown), which indicates that styrene is
451 produced in response to microbial infection of onion tissue (Nieminen et al., 2008; Vikram et
452 al., 2005). Both 1-propanethiol and methyl propyl sulfide were characteristic compounds of
453 onions derived from S-alk(en)yl-L-cysteine sulfoxides (Boelens et al., 1971; Christensen et al.,
454 2007; Løkke et al., 2012) and were thus emitted from both healthy and diseased onion bulbs
455 but in higher concentrations from diseased onions (Table 1). Overall, 1-propanethiol, methyl
456 propyl sulfide and styrene could be potential markers for the early detection of diseases in
457 onion bulbs.

458 In the present study, VOCs were sampled at 22 ^{o}C and at low RH to enable emission rates
459 above the detection level of the method and to prevent the growth of *Penicillium* spp. and other
460 secondary pathogens during storage. In cold storage, which is closer to 0 ^{o}C, less VOCs will
461 be emitted from the onions as lower temperature decreases on the VOC emission rate (Wang,
462 2018). Nevertheless, the present results show that VOC emission increase along with
463 pathogenic infection to a certain level and then level off. Moreover, we show that VOC
464 emission correlates with the extent of fungal infection, and thus, increased VOC emission rates
465 are related with increased disease levels. In the ideal world, infected and non-infected tissue
466 should not produce the same VOCs. However, we show that very few fungal-specific VOCs
467 emit from fungal infection caused by basal rot. This is in line with recent results showing that
468 Fox-strains growing in liquid media have similar VOC profiles (Wang et al., 2018) and that

469 microorganisms and fresh plant tissue produce similar VOCs but in different concentrations
470 (Wang, 2018).

471 4. **Conclusions**

472 Onions infected by pathogenic *F. oxysporum* f. sp. *cepae* showed distinct disease symptoms
473 starting at 1 week post inoculation. Forty-three VOCs were measured from control onions,
474 slightly infected Fox006-onions, and heavily infected Fox260-onions. Overall, the total volatile
475 emission rate, respiration rate, weight loss and Fox DNA ratio was higher in the Fox260-onions
476 than in the control and Fox006-onions, which showed similar results. Positive correlation
477 coefficients were obtained between the Fox260 DNA ratios and the VOC emission rates for 26
478 selected compounds, ranging from 0.42 to 0.89 at 1 to 7 weeks post inoculation. Ethanol, 1-
479 propanethiol, methyl propyl sulfide, dimethyl disulfide, styrene, and methyl propyl disulfide
480 drew the most attention because these compounds had high VOC emission rates and were either
481 unique to Fox260-infected onions (styrene) or had high VOC ratios early in infection. 1-
482 Propanethiol, methyl propyl sulfide, and styrene had the highest correlation coefficients (r =
483 0.82 - 0.89) to fungal specific DNA, which made these compounds the best candidates for early
484 measurement of pathogenic infection caused by *F. oxysporum* f. sp. *cepae* in onion bulbs. This
485 study shows that fungal activity in onions is related with increased VOC emission rates for the
486 first time.

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492

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583 **Table 1**

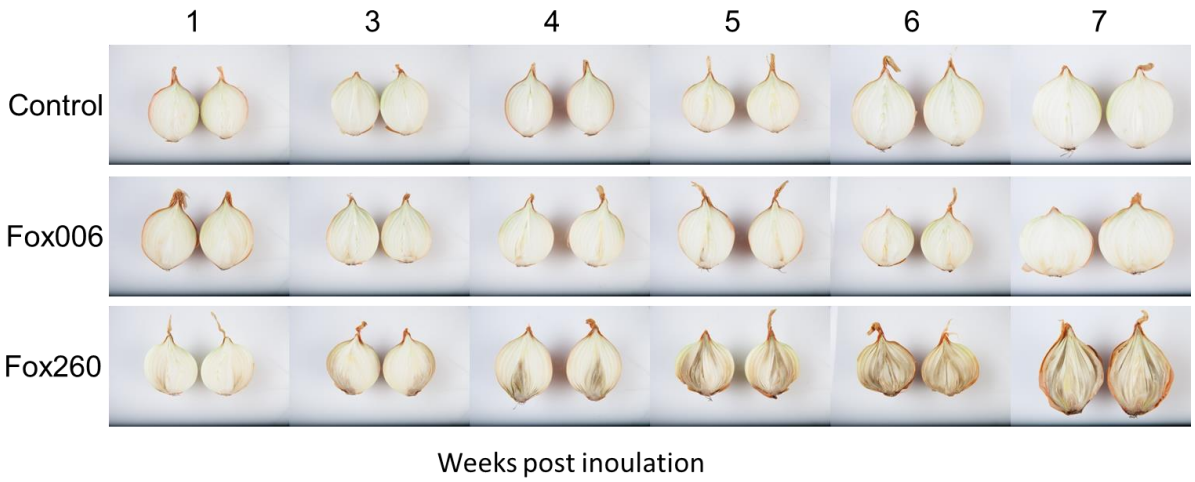
584 The volatile organic compound (VOC) emission rate (nmol kg^{-1} d^{-1}) of control and Fox260-infected onions at weeks 1 - 7 post inoculation.

No	Compound[a]	Retention time min	Boiling point ° C	Chemical group	Treatment	Week post inoculation (wpi)						Average[b]	VOC ratio[c]
						1	3	4	5	6	7		
1	carbonyl sulfide	5.12	-50.2	sulfuric	Control	0.18a[d]	0.25b	0.19b	0.33b	0.22b	0.22b	0.23b	
					Fox260	0.16a	0.5a	0.46a	0.52a	0.59a	0.49a	0.45a	2.0
2	propene	5.85	-48	alkene	Control	nd[e]b	ndb	ndb	ndb	ndb	ndb	0b	
					Fox260	0.1a	0.7a	0.55a	0.83a	1.00a	1.24a	0.74a	∞[f]
3	methanol	7.1	64.7	alcohol	Control	24a	14.4b	26a	13.2b	9.71b	10.3b	16.2b	
					Fox260	33a	100a	78a	83a	225a	62a	97a	6.0
4	acetaldehyde	7.26	21	aldehyde	Control	1.83a	2.15b	4.95a	4.02b	4.38b	3.22b	3.43b	
					Fox260	2.36a	38a	29a	55a	172a	84a	64a	18.5
5	methanethiol	7.52	5.95	sulfuric	Control	nda	ndb	ndb	ndb	ndb	ndb	0b	
					Fox260	nda	7.64a	6.39a	16.7a	43.7a	32.7a	17.8a	∞
6	ethanol	8.6	78.4	alcohol	Control	26a	12.2b	12.3b	5.02b	2.47b	2.22b	10.1b	
					Fox260	24a	1429a	2384a	3511a	6234a	2606a	2698a	268
7	propanal	9.27	48	aldehyde	Control	4.26a	4.46a	6.75a	5.58a	3.86a	3.39a	4.71a	
					Fox260	5.85a	5.79a	3.24a	3.83a	6.58a	5.86a	5.19a	1.1
8	carbon disulfide	9.35	46	sulfuric	Control	1.74a	1.69b	1.45b	2.1b	1.39b	1.23b	1.60b	
					Fox260	1.35a	4.00a	4.86a	5.43a	7.73a	6.53a	4.98a	3.1
9	acetone	9.39	56	ketone	Control	34a	47b	44a	59a	35b	58a	46b	
					Fox260	80a	153a	89a	81a	178a	125a	118a	2.5
10	dimethyl sulfide	9.43	37.3	sulfuric	Control	0.08a	0.05b	0.09b	0.07b	0.13b	0.09b	0.08b	
					Fox260	0.18a	9.35a	7.26a	18.7a	40a	25a	16.7a	198
11	ethyl formate	9.67	54.3	ester	Control	2.46a	2.28a	1.85a	2.13a	1.77b	1.63b	2.02b	
					Fox260	1.97b	2.93a	3.17a	3.85a	5.87a	4.09a	3.65a	1.8
12	methyl acetate	9.71	57.1	ester	Control	0.22a	0.09b	0.69a	1.27a	0.14b	0.03b	0.41a	
					Fox260	0.4a	10.2a	8.75a	10.1a	47a	11.1a	14.6a	36
13	isoprene	9.89	34	alkene	Control	0.17b	0.09b	ndb	0.11b	ndb	ndb	0.06b	
					Fox260	7.06a	36a	37a	27a	24a	45a	33a	530
14	pentane	9.94	36	alkane	Control	0.19b	0.15b	0.13b	0.16b	0.16b	0.15b	0.16b	
					Fox260	2.02a	3.91a	2.99a	3.29a	5.85a	5.2a	3.88a	25
15	(*E*)-1,3-pentadiene	10.15	42	alkene	Control	nda	ndb	ndb	ndb	ndb	ndb	0b	
					Fox260	0.14a	4.67a	2.46a	7.7a	8.75a	12.3a	6.00a	∞
16	C_5H_8[g]	10.2	-[h]	alkene	Control	nda	ndb	ndb	ndb	ndb	ndb	0b	

					Fox260	0.03a	0.83a	0.53a	3.95a	5.24a	6.03a	2.77a	∞
17	1-propanol	10.35	97	alcohol	Control	nda	ndb	nda	ndb	ndb	ndb	0b	
					Fox260	0.04a	35a	78a	108a	318a	86a	104a	∞
18	2-methylfuran	10.74	63-66	furan	Control	nda	ndb	ndb	ndb	0.08b	0.05b	0.02b	
					Fox260	0.19a	0.69a	0.76a	0.96a	1.03a	1.25a	0.81a	40
19	methyl vinyl ketone[i]	10.81	81.4	ketone	Control	0.3a	0.25a	0.18a	0.25a	0.24a	0.19a	0.24a	
					Fox260	0.23a	0.38a	0.22a	0.25a	0.26a	0.44a	0.30a	1.3
20	3-methylfuran	10.88	67	furan	Control	0.24b	0.21b	0.27b	0.49b	0.14b	0.14b	0.25b	
					Fox260	1.13a	11.4a	13.6a	21a	27a	35a	18.1a	73
21	2,3-butanedione	10.97	88	ketone	Control	1.3a	1.23a	1.61a	2.78a	2.46a	0.87a	1.71a	
					Fox260	0.98a	1.52a	2.38a	4.09a	28a	4.08a	6.77a	4.0
22	2-butanone	10.98	80	ketone	Control	0.91a	0.85a	1.15a	2.49a	1.09a	0.74a	1.21a	
					Fox260	0.91a	3.16a	2.61a	4.5a	133a	26a	28a	23
23	1-propanethiol	11.07	68	sulfuric	Control	0.95a	0.79b	1.47b	1.66b	0.56b	0.53b	0.99b	
					Fox260	6.01a	672a	717a	1216a	1929a	2031a	1095a	1102
24	acetic acid	11.55	118.1	acid	Control	23a	17.7b	37b	43a	51b	31a	34b	
					Fox260	28a	72a	99a	113a	378a	229a	153a	4.5
25	2-ethylfuran	12.12	92-93	furan	Control	0.27a	0.26b	0.33b	0.39b	0.25b	0.25b	0.29b	
					Fox260	0.48a	2.08a	3.09a	3.28a	3.83a	4.76a	2.92a	10.1
26	methyl isopropyl sulfide[i]	12.13	84.8	sulfuric	Control	nda	ndb	ndb	ndb	ndb	ndb	0b	
					Fox260	0.03a	0.5a	0.58a	2.59a	5.06a	4.41a	2.19a	∞
27	allyl methyl sulfide[i]	12.26	92	sulfuric	Control	nda	ndb	ndb	0.89b	ndb	ndb	0.15b	
					Fox260	0.09a	6.81a	7.94a	20a	38a	30a	17.3a	116
28	(*E*)-methyl 1-propenyl sulfide[i]	12.33	102	sulfuric	Control	0.28a	0.28b	0.33b	0.37b	0.28b	0.27b	0.30b	
					Fox260	0.4a	1.31a	1.74a	1.86a	2.11a	2.44a	1.64a	5.5
29	methyl propyl sulfide	12.45	96	sulfuric	Control	25a	16.6b	10.7b	5.44b	1.61b	2.08b	10.2b	
					Fox260	12.0a	646a	576a	1372a	1768a	1638a	1002a	98
30	(*Z*)-methyl 1-propenyl sulfide[i]	12.5	102	sulfuric	Control	nda	0.24b	ndb	1.09b	ndb	ndb	0.22b	
					Fox260	0.52a	7.23a	24a	28a	49a	34a	24a	106
31	dimethyl disulfide	12.64	110	sulfuric	Control	3.92a	3.09b	2.25b	1.49b	1.06b	1.07b	2.15b	
					Fox260	3.5a	120a	133a	154a	165a	62a	106a	49
32	(*E*)-2-methyl-2-butenal	12.76	116	aldehyde	Control	nda	nda	3.81a	nda	nda	0.14a	0.66a	
					Fox260	0.05a	0.11a	nda	0.08a	nda	nda	0.04a	0.1
33	heptane	12.78	98	alkane	Control	nda	ndb	ndb	ndb	0.6a	ndb	0.10a	
					Fox260	nda	0.21a	0.31a	2.63a	1.32a	0.65a	0.85a	8.6
34	3-methyl-1-butanol	13	131	alcohol	Control	nda	nda	nda	nda	ndb	nda	0b	
					Fox260	nda	5.69a	9.83a	25a	47a	18.2a	17.6a	∞
35	hexanal	14.08	131	aldehyde	Control	34a	29a	28a	34a	26a	23a	29a	

					Fox260	29a	25a	11.5b	4.04b	3.68b	2.59b	12.5b	0.4
36	2,4-dithiapentane[i]	15.48	147	sulfuric	Control	0.29a	0.25a	0.16a	0.09b	ndb	ndb	0.13b	
					Fox260	0.08b	2.45a	2.00a	14.9a	27a	25a	11.9a	90
37	3,4-dimethylthiophene	15.6	144-146	sulfuric	Control	0.18a	0.14b	0.55b	0.18b	0.03b	0.04b	0.19b	
					Fox260	0.24a	2.64a	3.53a	2.45a	2.29a	3.19a	2.39a	12.8
38	styrene	15.67	145	arene	Control	ndb	ndb	ndb	ndb	ndb	ndb	0b	
					Fox260	0.64a	4.65a	7.8a	111a	127a	99a	58a	∞
39	allyl propyl sulfide[i]	15.86	139	sulfuric	Control	0.13a	0.09b	0.08b	0.19b	0.07b	0.05b	0.10b	
					Fox260	0.09a	1.00a	0.89a	1.68a	2.56a	2.38a	1.43a	14.4
40	2-heptanone	16.41	151	ketone	Control	nda	nda	nda	nda	nda	nda	0a	
					Fox260	nda	0.46a	0.97a	1.14a	8.33a	3.72a	2.44a	∞
41	methyl 1-propenyl disulfide[i]	16.84	140	sulfuric	Control	0.94a	0.53b	0.53b	1.61b	0.02b	0.01b	0.61b	
					Fox260	0.62a	21a	42a	34a	37a	15.4a	25a	41
42	methyl propyl disulfide	16.99	154	sulfuric	Control	23a	8.53b	6.48b	3.47b	ndb	ndb	6.84b	
					Fox260	12.3a	410a	444a	332a	465a	267a	322a	47
43	2,2-bis(methylthio)propane[i]	21.56	164.4	sulfuric	Control	2.59a	1.74b	1.48b	0.74b	0.08b	0.07b	1.12b	
					Fox260	1.02a	27a	23a	116a	128a	184a	80a	71

[a] All compounds were identified (otherwise noted) by comparing the mass spectrum of the compound with those of the NIST v. 2.1.2.1database (NIST, 2008) and authentic reference compounds. [b] The average emission of VOC compounds from each treatment (control or Fox260-onions) 1– 7 wpi. [c] The VOC ratio is the average emissions rate of Fox260-onions relatively to control onions. [d] Different letters within compound and wpi indicate significant differences between treatments at P=0.05. [e] nd, not detected. The values are set to zero in the statistical analysis. [f] ∞, the value is approaching infinity because the compound was not detected in control onions. [g] Tentative identification was not possible from the top abundant ions shown in Table S1. [h] Boiling point not available, as the compound is not identified. [i] Tentatively identified as no reference compounds are available. Further information on these compounds are given in Table S1.

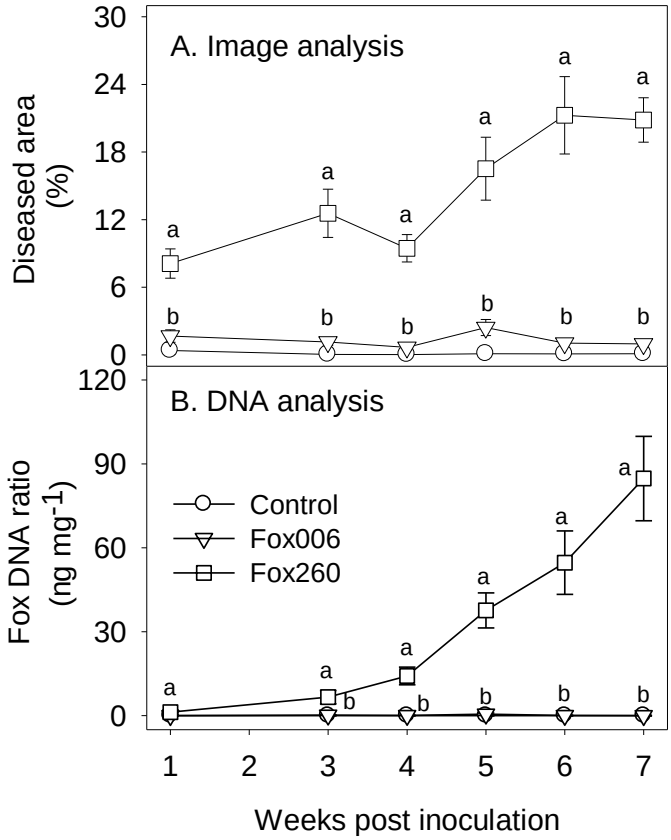


592

593 **Fig. 1.**

594 Photos of the halved onion bulbs following injection with water (control) or two stains of

595 *Fusarium oxysporum* f. sp. *cepae* (Fox006 or Fox260) at weeks 1 to 7 post inoculation.



596

597 **Fig. 2.**

598 Levels of infection determined by image analysis (A) and DNA analysis (B) of onion bulbs
599 following injection with water (control) or two stains of *Fusarium oxysporum* f. sp. *cepae*
600 (Fox006 or Fox260) at weeks 1 to 7 post inoculation. Data are means ± standard error (N = 4
601 for control samples and N = 9 for Fox samples). Means followed by different letters within the
602 figure at each week indicate significant differences between treatments according to Tukey´s
603 HSD test at P = 0.05. The Fox DNA ratio is the amount of fungal specific DNA relative to
604 onion DNA.

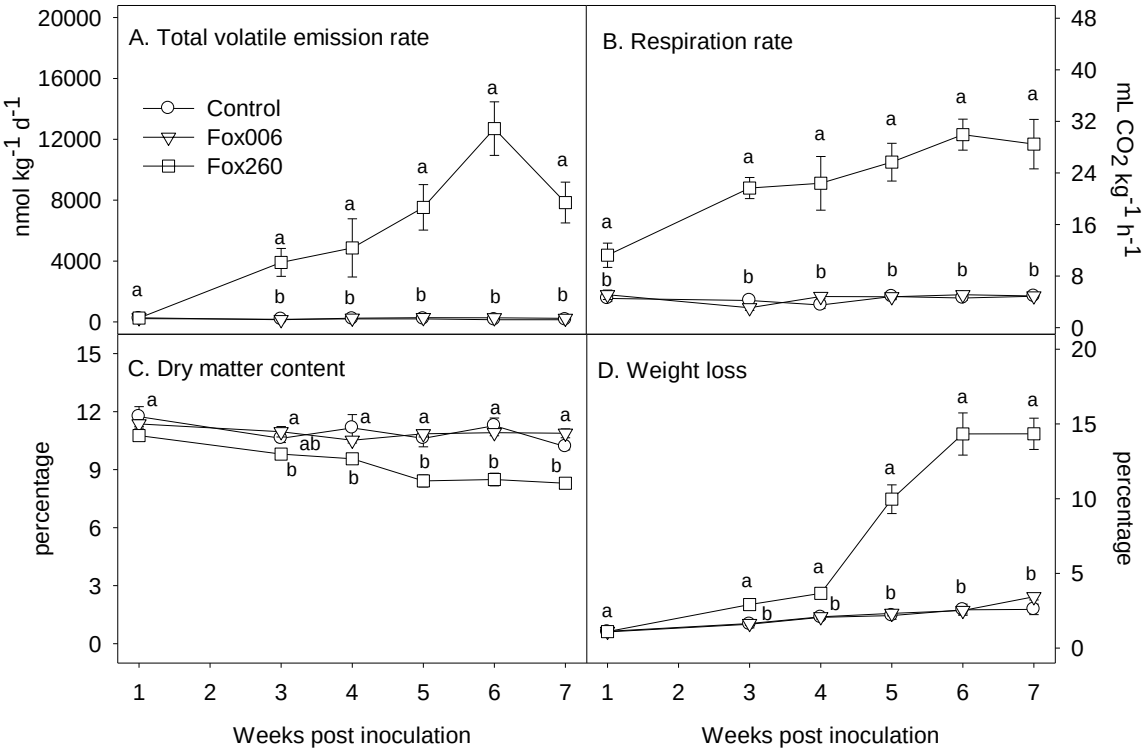
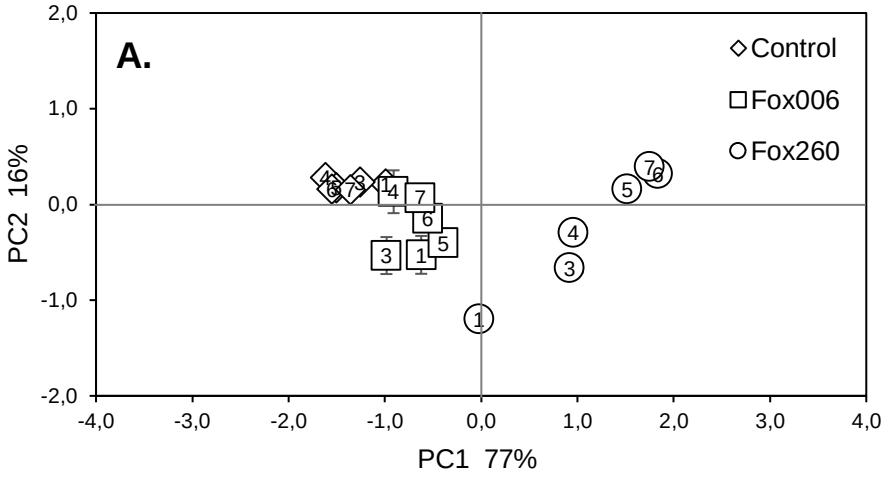
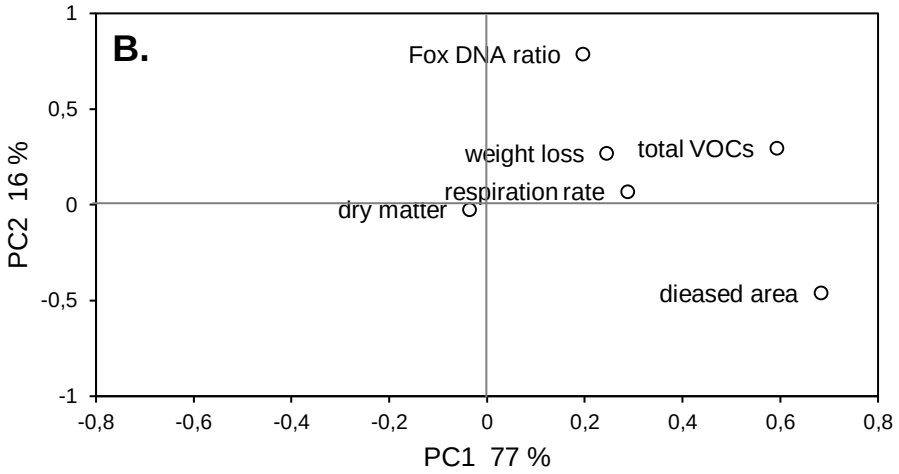


Fig. 3.

Total volatile emission rate (A), respiration rate (B), dry matter content (C) and weight loss (D) of onion bulbs following injection with water (control) or two stains of *Fusarium oxysporum* f. sp. *cepae* (Fox006 or Fox260) at weeks 1 to 7 post inoculation. Data are means ± standard error ($N = 4$ for control samples and $N = 9$ for Fox samples). Means followed by different letters within the figure at each week indicate significant differences between the treatments according to Tukey's HSD test at $P = 0.05$.



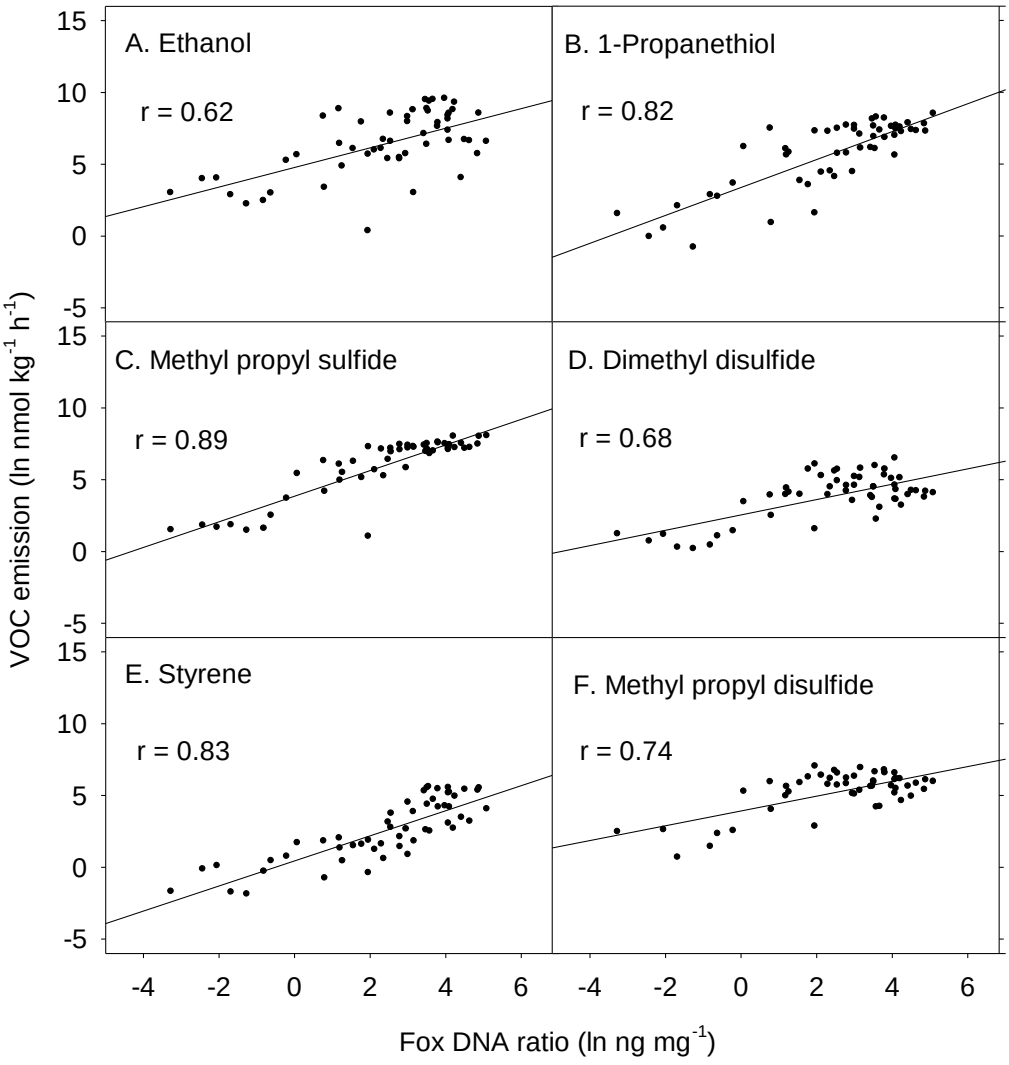
613



614

615 **Fig. 4.**

616 Principal component analysis of the infection level and response of onion bulbs following
617 injection with water (control) or two stains of *Fusarium oxysporum* f. sp. *cepae* (Fox006 or
618 Fox260) at weeks 1 to 7 post inoculation. Data are means ± standard error (N = 4 for control
619 samples and N = 9 for Fox samples). Score plot (A). Loading plot (B).



620

621 **Fig. 5.**

622 Linear correlations between Fox DNA ratios and individual VOC emission rates for all

623 Fox260-inoculated onions at weeks 1 to 7 post inoculation (N = 54). Data are ln-transformed.

Supplementary materials

Table S1

Top abundant ions for the unidentified and tentatively identified compounds in Table 1. The percentages of ions relative to the most abundant ion in the chromatogram are given in parentheses.

No.	Compound	Retention time min	Top abundant ions
16	C_5H_8	10.20	67 (100), 68 (68), 53 (56)
19	methyl vinyl ketone	10.81	55 (100), 43 (80), 70 (39)
26	methyl isopropyl sulfide	12.13	90 (100), 75 (98), 41 (86)
27	allyl methyl sulfide	12.26	88 (100), 73 (81), 45 (58)
28	(*E*)-methyl 1-propenyl sulfide	12.33	73 (100), 88 (99), 45 (79)
30	(*Z*)-methyl 1-propenyl sulfide	12.50	73 (100), 88 (98), 45 (91)
36	2,4-dithiapentane	15.48	61 (100), 108 (87), 45 (38)
39	allyl n-propyl sulfide	15.86	74 (100), 41 (85), 104 (80)
41	methyl 1-propenyl disulfide	16.84	120 (100), 45 (49), 72 (35)
43	2,2-bis(methylthio)propane	21.56	89 (100), 41 (52), 136 (48)

Table S2

The average emission rate (nmol kg^{-1} d^{-1}) of selected VOCs emitted from Fox260-inoculated onion bulbs at weeks 1 – 7. Linear slopes and correlation coefficients (r) between Fox DNA ratios (ng mg^{-1}) and selected VOCs are shown. The correlation analyses were made with ln-transformed data from weeks 1 - 6 (N = 45) or weeks 1 - 7 (N = 54).

No.	Compound	Average emission rate[a]	Weeks 1 - 6 post inoculation		Weeks 1 - 7 post inoculation	
			Slope	Correlation coefficient	Slope	Correlation coefficient
1	carbonyl sulfide	very low	0.19	0.73	0.17	0.69
2	propene	very low	0.38	0.81	0.38	0.83
6	ethanol	high	0.81	0.69	0.68	0.62
8	carbon disulfide	low	0.24	0.63	0.23	0.63
10	dimethyl sulfide	low	0.94	0.86	0.88	0.85
13	isoprene	medium	0.28	0.41	0.28	0.42
14	pentane	low	0.16	0.48	0.16	0.48
15	(E)-1,3-pentadiene	low	0.87	0.85	0.84	0.85
16	C_5H_8[b]	low	0.46	0.59	0.50	0.63
18	2-methylfuran	very low	1.02	0.88	0.27	0.62
20	3-methylfuran	low	0.53	0.80	0.53	0.81
23	1-propanethiol	high	0.98	0.80	0.97	0.82
25	2-ethylfuran	low	0.36	0.69	0.35	0.68
26	methyl isopropyl sulfide[c]	low	0.59	0.78	0.60	0.81
27	allyl methyl sulfide[c]	low	1.02	0.88	0.97	0.88
28	(E)-methyl 1-propenyl sulfide[c]	very low	0.28	0.69	0.27	0.69
29	methyl propyl sulfide	high	0.94	0.88	0.89	0.89
30	(Z)-methyl 1-propenyl sulfide[c]	medium	0.79	0.81	0.74	0.80
31	dimethyl disulfide	high	0.64	0.74	0.53	0.68
36	2,4-dithiapentane[c]	low	1.06	0.86	1.06	0.87
37	3,4-dimethylthiophene	low	0.39	0.69	0.37	0.68
38	styrene	high	0.87	0.82	0.87	0.83
39	allyl n-propyl sulfide[c]	very low	0.53	0.85	0.52	0.86
41	methyl 1-propenyl disulfide	medium	0.66	0.67	0.58	0.64
42	methyl propyl disulfide	high	0.72	0.78	0.63	0.74
43	2,2-bis(methylthio)propane[c]	high	0.95	0.87	0.94	0.88

[a] The average emission rate of compounds by Fox260-onions were taken from Table 1 and ranked into: very low, < 2 nmol kg^{-1} d^{-1}; low, >2 and <20 nmol kg^{-1} d^{-1}; medium, > 20 and < 100 nmol kg^{-1} d^{-1}; high, >300 nmol kg^{-1} d^{-1}. [b] Tentative identification was not possible from the top abundant ions shown in Table S1. [c]Tentatively identified as no reference compounds are available. Further information on these compounds are given in Table S1.