

Flavor Precursor [S-alk(en)yl-L-cysteine sulfoxide] Concentration and Composition in Onion Plant Organs and Predictability of Field White Rot Reaction of Onions

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ADDITIONAL INDEX WORDS. *Allium cepa*, *Sclerotium cepivorum*, disease screening, roots, bulbs, leaves, long-day onions

ABSTRACT. Breeders have found field screening for white rot (*Sclerotium cepivorum* Berk.) resistance in onion (*Allium cepa* L.) to be unreliable since consistently moderate to high disease levels that significantly differentiate cultivars do not occur over field sites and years. The objective was to determine if differences in onion white rot resistance levels were associated with differing S-alk(en)yl-L-cysteine sulfoxide (ACSO) levels. A collection of onion breeding lines and hybrids were evaluated in field trials at six sites in 1999–2001. High performance liquid chromatography was used to analyze ACSOs in onion plant organs. Four main cysteine-sulfoxides exist in *Allium* L. species: methyl (MCSO), 2-propenyl (2-PeCSO), 1-propenyl (1-PeCSO), and propyl (PCSO). 1-PeCSO was predominant in onion leaves, bulbs, and roots. 2-PeCSO was found in trace amounts in onion leaves and roots. There was significantly more 2-PeCSO and total ACSO (roots only) and 1-PeCSO (roots and bulbs) in accessions that were more susceptible to white rot in the field trials. This is the first report of significant differences in ACSO contents among white rot susceptible and resistant onions. A covariance analysis was used to determine if the ACSO levels that significantly distinguished among accessions could predict field onion white rot reaction. 1-PeCSO from both roots and bulbs was the best predictor of field disease incidence in field sites that had low, moderate, and high disease levels. Although the ACSO concentrations were not assessed on an individual plant basis, breeders may be able to screen onions for resistance to *S. cepivorum* by comparing onion root or bulb 1-PeCSO levels based on the results from this research. White rot incidence in the field should be higher in those plants whose roots and bulbs have the highest levels of 1-PeCSO.

Sclerotium cepivorum, the cause of white rot, is a fungus that occurs in almost all *Allium* production areas and is a serious threat to the onion and garlic production worldwide. The specific interaction between the host and pathogen is partially understood. *Sclerotium cepivorum* sclerotia are held in dormancy by fungistasis (Allen and Young, 1968; Coley-Smith and King, 1969) until specific stimulation by volatile organosulfur compounds from *Allium* roots. This was determined using aqueous extracts made from *Allium* species and testing their effects on sclerotial germination (Coley-Smith and Holt, 1966). Volatile organosulfur compounds evolved from intact onion and leek (*A. ampeloprasum* L.) seedlings, condensed vapors evolved from garlic (*A. sativum* L.) juice, and distillates of garlic and onion juice also stimulated sclerotial germination (King and Coley-Smith, 1969). The *Allium* compounds that appear to be stimulatory are the organosulfur compounds that comprise flavor in alliums. These compounds include thiosulfonates, monosulfides, disulfides, trisulfides, and thiophene derivatives (Coley-Smith and King, 1969; King and Coley-Smith, 1969), which are some of the products derived from the reaction between *Allium* flavor precursors, S-alk(en)yl-L-cysteine sulfoxide (ACSO), and the enzyme alliinase (Block, 1992).

Putative resistance to white rot infection has been found in wild (Bansal and Broadhurst, 1992; Brix and Zinkernagel, 1992) and cultivated (Coley-Smith, 1986) *Allium* species. Different ACSOs or ratios of ACSOs in roots may or may not play a role in *Allium* white rot resistance (Coley-Smith, 1986). Most of the nonprotein sulfur in alliums can be found in the form of four principle ACSOs: methyl (MCSO), 2-propenyl (2-PeCSO), 1-propenyl (1-PeCSO), and propenyl (PCSO) (Block, 1992). 1-PeCSO is found in the highest concentration in onions and 2-PeCSO is found in the highest concentrations in garlic with only trace amounts in onions. Among three common *Allium* crops, the total ACSO concentration is typically highest in garlic, intermediate in onion, and lowest in leek (Block, 1992; Coley-Smith, 1986). In field screening trials, garlic tends to have the highest white rot incidence, leek the least, and onions fall in between (Coley-Smith, 1986). Coley-Smith (1986) hypothesized that the plants that have the highest disease incidence among and within *Allium* species emit more total organosulfur and/or different types of organosulfur compounds from their roots than the resistant plants. In specific relation to onions, Rahe (1981) hypothesized that one possible explanation for differential white rot resistance among onion cultivars in field trials may be the differential stimulation of germination of sclerotia of *S. cepivorum*. Therefore, differential stimulation of sclerotial germination may be due to differences in organosulfur root exudates between resistant and susceptible onion cultivars. Studies have shown that the ACSO concentrations in onion cultivars vary (Lancaster et al., 1984; McCallum et al., 2002; Randle et al., 1995).

Received for publication 28 Apr. 2003. Accepted for publication 8 Sept. 2004. The technical assistance of Nick Breitbach and Kathleen Quast and statistical assistance of Brian Yandell is gratefully acknowledged. This research was supported in part by funding from Seminis Vegetable Seeds (Woodland, Calif.).

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Table 1. *Allium* type, source, and putative white rot response used in developing a screen for resistance to *Sclerotium cepivorum*.

Common name	Accession	Type ^z	Source ^y
<i>Onion</i> ^s	1416C	B	S
Onion	1247B	B	S
Onion	Ricochet	H	S
<i>Onion</i>	1415C	B	S
<i>Onion</i>	W461B	B	UW
<i>Onion</i>	XPH15056	H	S
<i>Onion</i>	XPH15055	H	S
<i>Onion</i>	XPH15073	H	S
Leek	Ramona	H	BS
Garlic	RG9902	C	RF

^zB = breeding line, H = hybrid, C = clone

^yS = Seminis Vegetable Seeds, Oxnard, Calif.; UW = breeding line from Univ. of Wisconsin–Madison onion breeding program; BS = Bejo Seeds, Oceano, Calif.; RF = Roger's Food, Inc., Turlock, Calif.

^xAll the onion accessions in italics contain 'Zittauer Gelbe' in their pedigree (a putative white rot resistant plant introduction from Germany).

The main objective of this research was to determine if differences in white rot resistance levels among putatively resistant and susceptible onions were associated with different root and shoot ACSO profiles.

Material and Methods

PLANT MATERIAL. Eight different long-day onion breeding lines and hybrids, and one cultivar each of leek and garlic were included in this study (Table 1). These accessions were evaluated in field trials at six locations, and significant differences were identified between groups of resistant and susceptible accessions (Hovius and Goldman, 2004).

GREENHOUSE PLANT PRODUCTION. All alliums were grown in the greenhouse in 96 cell plug trays (5.08 cm deep) in potting mix (Scotts Metro-Mix 366-P; Scotts, Marysville, Ohio) in a randomized complete-block design with four blocks in 1999 and 2000. Each of the 10 alliums consisted of 120 plants per block. A 100 mg·kg⁻¹ of 20N–3.1P–16.6K soluble fertilizer containing 1.4% sulfur was applied at 5 mL/plant at 7, 11, and 15 weeks after emergence. Plants were fertilized to facilitate normal plant growth, and the addition of an external source of sulfur was to ensure at least moderate levels of ACSO production within the plants (Randle et al., 2002). Thirty plants per *Allium* per block chosen randomly were harvested, washed, and blotted dry, 14 and 18 weeks after emergence for analysis of ACSOs. From seeding to ≈11 weeks after emergence, it was difficult to obtain enough roots tissue for the analysis of ACSOs as well as enzyme generated pyruvate (EPY). The 14-week harvest date was chosen to get a large quantity of roots and because it was not directly after a fertilizer application. The 18 week harvest date was chosen since that is typically when onions in field production begin to senesce (Brewster, 1994). The roots, bulbs, and leaves (green tissue only) from both harvest dates were separated by tissue and stored at –80 °C for analysis of ACSOs.

FIELD PLANT PRODUCTION. Onion accessions were grown in a commercial field in 2001 and were replicated four times in a randomized complete-block design. Each replicate consisted of one 3.7-m row, with ≈45 plants/m per row and 45 cm between rows. Recommended control procedures for fungal and bacterial pathogens, weeds, and insects were followed and administered by the grower. Thirty plants per *Allium* per block chosen ran-

domly were harvested, washed, and blotted dry 18 weeks after emergence. The roots and bulbs from each sample of 30 plants were separated by tissue. The roots were stored at –80 °C and the bulbs were stored at 4 °C for analysis of ACSOs. Leaves were not collected since at 18 weeks after emergence the leaves had senesced and were dry and brown.

IN VITRO PLANT PRODUCTION. Onions and leek were grown in vitro in test tubes in 1999 and 2000 in a completely randomized design with four blocks. Surface-sterilized onion and leek seeds were plated onto solid media in test tubes containing 0.16 mM KNO₃, 2.0 mM NH₄H₂PO₄, 1.94 mM [NH₄]NO₃, sucrose (3%), 0.0039 g·mL⁻¹ of media Gamborgs B-5 and 0.8% agar (Myers and Simon, 1998). The plants were stored in a growth chamber at 21 °C with a 12-h light/dark period. Five weeks after plating onto solid media, plants were transferred to test tubes (1 plant/tube) and grown in liquid media containing the same ingredients as the solid media without the agar. Plants were anchored in the test tube using a 1.5-cm-long foam plug with a slit cut in it in accordance with Tisserat and Manthey (1996). The foam plug surrounded the bulb and ≈0.7 cm of the roots and stem so that the base of the plug was ≈0.2 cm above the liquid media and the roots were submerged in the media. The plants were stored in a growth chamber at 25 °C and a 12-h light/dark period for 5 weeks after which the temperature was increased to 28 °C and the light/dark period was changed to 16/8 h. The onion plants were harvested and the liquid was collected 19 weeks after seed germination. The roots from 30 plants per *Allium* per block were harvested and stored at –80 °C for analysis of ACSOs. The liquid from 30 test tubes (≈2 mL/tube) per *Allium* per block was collected and stored at –80 °C for analysis of ACSOs.

EXTRACTION OF ACSOs. ACSOs were extracted from frozen root, bulb, and leaf samples using a modified method of Randle et al. (1995). Alternative extraction methods were tested in preliminary studies and yielded lower recoveries of ACSOs (data not shown). Bulbs from the greenhouse were small and therefore whole bulbs, without the dry outer scale, were used. The fourth leaf scale in from the outside was the sample used from the field bulbs. The fourth scale was chosen since it was the scale that needed the least amount of cutting (minimizing tissue disruption) to obtain an intact 5-g piece. The scale was frozen at –20 °C overnight prior to extraction. ACSOs were extracted from 5-g and 2.5-g frozen tissue samples by steeping for 24 h at –20 °C in a solution (1:5, w/v) of methanol, chloroform and water (M:C:W, ratio of 12:5:3) containing 1 mM hydroxylamine (Edwards et al., 1994) and ECSO as an internal standard. ECSO is usually found in trace, if even detectable, amounts in alliums (Kubec et al., 2000).

After decanting and collecting the extraction medium, 0.45 and 0.55 volumes of chloroform and water, respectively, were added to phase separate the M:W portion from the chloroform. The M:W portion was siphoned off and then placed in a water bath at 40 °C and the methanol was evaporated by running air over the samples for 7 min/mL of M:W. The remaining water extract was frozen at –80 °C for 8 h and then lyophilized and stored at –20 °C until high-performance liquid chromatography (HPLC) analysis.

A 25-mL sub-sample of the frozen liquid collected from the test tubes in which plants were grown in vitro was lyophilized and stored at –20 °C until HPLC analysis.

SAMPLE DERIVATIZATION. Prior to HPLC analysis, freeze-dried samples were dissolved in 150 μL of 40 mM Li₂CO₃ (pH 9.5) and then 75 μL of dansyl chloride was added (1.5 mg·mL⁻¹ ace-

tonitrile) to achieve amino group derivatization (Yoo and Pike, 1998). This derivatization method was used because Yoo and Pike (1998) showed that a dansylation resulted in a good separation of the derivatives of 1-PeCSO and 2-PeCSO. After reaction in the dark for 35 min, samples were filtered using a 0.2- μm , nylon, nonsterile syringe filter.

HPLC ANALYSIS. ACSOs were analyzed by HPLC using the method of Yoo and Pike (1998), using a 50- μL filtered sample injection volume. The HPLC system included a binary pump, a UV detector set to 254 nm, a data collection system (HP ChemStations; Hewlett-Packard Co., Palo Alto, Calif.), and a Zorbax Eclipse XDB-C8 column (4.6 mm i.d. \times 15 cm long, 5 μm ; Agilent Technologies, Wilmington, Del.), and a C8 guard column. Column temperature was maintained by a modified water jacket at 15 ± 0.25 °C. The HPLC solvent system consisted of two solvents, solvent A (methanol) and B (water), each containing 0.6% acetic acid and 0.008% triethylamine. Solvent mixture was programmed at 30% A for 35 min with a flow rate of 1 mL $\cdot\text{min}^{-1}$ for the separation of ACSOs followed by 100% A for 5 min. Peak identification was based on coincidence with the retention times of ACSO standards and quantification was based on external standard curves after correcting for the internal standard.

ACSO standards were synthesized or isolated in accordance with Shen et al. (2002). When appropriate, corrections were made for impurity of the standards. The threshold for detection of the ACSOs was between 0.01 and 0.001 mg $\cdot\text{mL}^{-1}$. The average correlation coefficients of the standard curves, were $r \geq 0.97$, 0.96, 0.96, 0.97, and 0.96 for MCSO, ECSO, 2-PeCSO, 1-PeCSO, and PCSO, respectively. Based on the recovery of ECSO, the percentage ACSO recovered from the samples was 75% and reported values were corrected for these losses.

STATISTICAL ANALYSIS. All data were analyzed using the SAS system for mixed models (Littell et al., 1996). Data were ranked prior to analysis (Conover, 1999). *Allium* accession, time (harvest date 14 weeks and 18 weeks from each year 1999, 2000, and 2001) and plant organ were analyzed as fixed effects and year and block interactions were analyzed as random effects. To determine which of the ACSOs could be used to predict onion white rot reaction in the field, a covariance analysis was used. Only those ACSOs that significantly distinguished among accessions were used in the covariance analysis. If the P value of the ACSO covariate effect was significant ($P \leq 0.05$) and the P value of the ACSO \times onion accession was less significant, a particular ACSO was considered unconditionally predictive of field disease incidence because the prediction was not based on prior knowledge of onion white rot resistance ranking.

Results

Garlic and leek were included in this research as white rot susceptible and resistant checks, respectively (Brix and Zinkernagel, 1992; Coley-Smith, 1986; Van der Meer et al., 1983). The concentration of total ACSO (mg $\cdot\text{g}^{-1}$ tissue) in garlic cloves, roots and leaves averaged 0.43, 0.64, and 0.63, respectively. In leek pseudostems, roots and leaves these values were 0.31, 0.39, and 0.36, respectively, and in onion bulbs, roots and leaves they were 0.39, 0.39 and 0.40, respectively. 2-PeCSO was the most abundant ACSO in garlic tissues and the most abundant ACSO in leek tissues was 1-PeCSO and MCSO. These results yielded substantially lower values than previous studies (Block, 1992; Coley-Smith, 1986; Edwards et al., 1994; Yoo and Pike, 1998), however they were fairly consistent with levels reported by Thomas

and Parkin (1994). The concentrations of 1-PeCSO (mg $\cdot\text{g}^{-1}$ tissue) in onions, averaged over all eight accessions in bulbs, roots and leaves were 0.17, 0.14, and 0.16, respectively, and in garlic cloves, roots and leaves, 0.01, 0.05, and 0.02, respectively, and in leek pseudostems, roots and leaves, 0.14, 0.10, and 0.14, respectively. Onion bulbs typically have the highest concentrations of 1-PeCSO (Block, 1992; Coley-Smith, 1986; Edwards et al., 1994; Thomas and Parkin, 1994; Yoo and Pike, 1998).

EFFECT OF PLANT ORGAN ON FLAVOR PRECURSOR LEVELS. Significant differences in MCSO and 1-PeCSO were found among onion roots, bulbs and leaves. Leaves from the greenhouse produced plants contained significantly more MCSO than bulbs. Bulbs contained significantly more 1-PeCSO than roots and leaves. The interaction of onion \times plant organ \times harvest date was significant for all four ACSOs and therefore the ACSO content was analyzed separately by plant organ. The analysis of the percent of each ACSO of the total ACSO showed that the interaction of onion \times plant organ \times harvest date was significant for percent MCSO, percent 2-PeCSO and percent PCSO (not shown) and therefore the percent of total ACSO content was analyzed separately by plant organ.

FLAVOR PRECURSOR LEVELS IN LEAVES. ACSO analysis of leaves was performed on greenhouse grown plants only. The interaction of onion \times harvest date was not significant for any of the ACSOs detected. Significant differences among onion breeding lines and hybrids were found for total ACSO and the contrast between accessions measured as resistant versus those measured as susceptible in the field was significant for total ACSO and 2-PeCSO (Table 2). At $P \leq 0.1$, there was significantly more 1-PeCSO in susceptible compared to resistant accessions. The analysis of the percent of each ACSO of the total ACSO showed that the interaction of onion \times harvest date was not significant for any of the ACSOs detected. Significant differences among onion breeding lines and hybrids were found for percent MCSO and percent 2-PeCSO and there was significantly less percent MCSO in susceptible than resistant accessions and significantly more percent 2-PeCSO in susceptible than resistant accessions (not shown).

FLAVOR PRECURSOR LEVELS IN BULBS. Onion bulbs from greenhouse and field grown plants were analyzed for ACSOs. The interaction of onion \times plant production location (greenhouse and field) was tested for all ACSOs by comparing the 18-week harvest date, since field plants were only harvested on this date. The interaction of onion \times location was significant for 1-PeCSO and total ACSO. Significant differences among onion breeding lines and hybrids were found for 1-PeCSO from the bulbs harvested from the field only. There was significantly more 1-PeCSO in susceptible accessions from field-produced ($P \leq 0.05$) and greenhouse-produced ($P \leq 0.1$) bulbs than resistant accessions (Table 2). At $P \leq 0.1$ there was significantly more total ACSO in susceptible field-grown bulbs than from resistant bulbs. The analysis of the percent of each ACSO showed that the interaction of onion \times location was not significant for any percent of total ACSO. Significant differences among onion breeding lines and hybrids were found for percent 1-PeCSO but the contrast between susceptible and resistant was not significant among any of the percent ACSOs.

FLAVOR PRECURSOR LEVELS IN ROOTS. Onion roots from plants grown in the greenhouse, field and in vitro (production location) grown plants were analyzed for ACSOs, as were onion roots from greenhouse and field grown plants. The interaction of onion \times the location that the plants were grown in (greenhouse, field, and in vitro in test tubes) was tested for all ACSOs by comparing

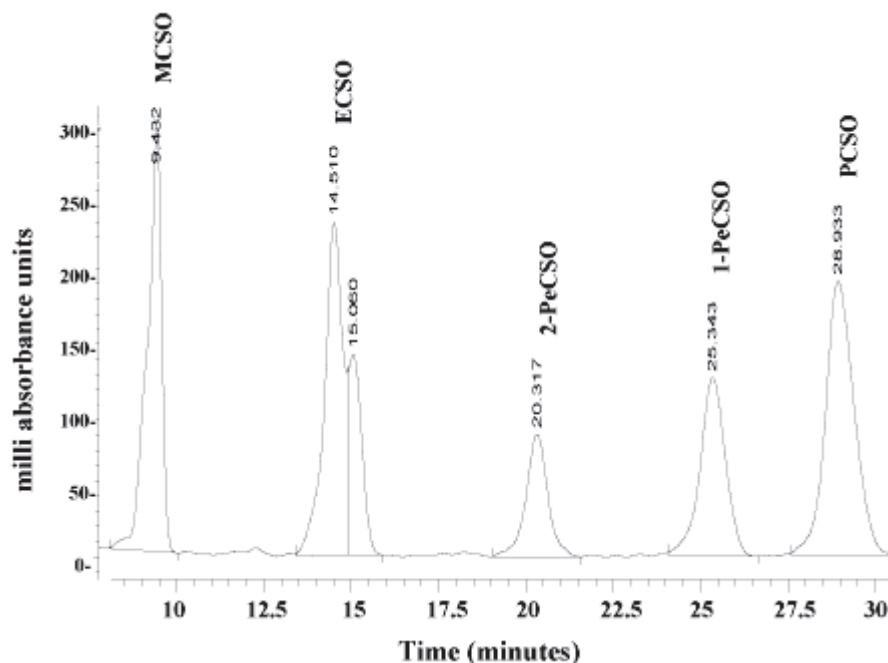


Fig. 1. Retention times of representative high performance liquid chromatography chromatograms of flavor precursor standards: 9.4 min, methyl-L-cysteine sulfoxide (MCSO), 14.5 and 15.1 min, ethyl-L-cysteine sulfoxide (ECSO), 20.3 min, 2-propenyl-L-cysteine sulfoxide (2-PeCSO), 25.3 min, 1-propenyl-L-cysteine sulfoxide (1-PeCSO), 28.9 min, propyl-L-cysteine sulfoxide (PCSO), the respective flavor precursors were measured at 254 nm.

determining effectiveness of the ACSO as a field disease incidence predictor. 2-PeCSO and total ACSO from the roots, root 1-PeCSO from greenhouse, field, and in vitro produced plants, and 1-PeCSO from bulbs from field produced plants were all good predictors of field disease incidence. The best predictor of field disease incidence was 1-PeCSO from roots from greenhouse produced plants since it was predictive of field disease incidence in four out of the six field sites, including field sites with low, moderate, and high disease levels overall. Percent 2-PeCSO from roots and leaves was also predictive of field disease incidence but only for the field site that had high disease levels (results not shown).

Discussion

Significant differences in ACSO content among onion roots, bulbs, and leaves were found among and within production environments and the results agreed with previously reported analyses (Edwards et al., 1994; Lancaster et al., 1984, 2000; McCallion and Lancaster, 1984) even though none of the previous studies looked at all three tissues. The total amounts of ACSOs

the 18-week harvest date, since field plants were only harvested on this date. Only the 2001 seed lot was planted in the field and therefore the comparison was done with the 2001 greenhouse and in vitro results. The interaction of onion \times location was significant for 1-PeCSO only. Pooled over locations, significant differences among onion breeding lines and hybrids were found for 2-PeCSO and total ACSO and 1-PeCSO from field and greenhouse produced roots. Roots from the onions produced in the greenhouse and field contained significantly more 2-PeCSO and total ACSO and 1-PeCSO (greenhouse, field and in vitro locations) in the susceptible than resistant accessions (Table 3). The analysis of the percent of each ACSO showed that the interaction of onion \times location was not significant for any ACSO. Significant differences among onion breeding lines and hybrids were found for percent 2-PeCSO and there was significantly more 2-PeCSO in the susceptible than resistant accessions.

FLAVOR PRECURSOR LEVELS IN LIQUID COLLECTED FROM THE ROOT ZONE OF IN VITRO-GROWN ONION PLANTS. Liquid collected from onion plants that were grown in culture in liquid media was analyzed for ACSO content. ACSO amounts collected from the liquid were lower than that found in onion tissues. One-PeCSO was the most abundant ACSO in the liquid, followed by PCSO and MCSO in all accessions. Two-PeCSO was detected in liquid from all accessions.

PREDICTING FIELD DISEASE INCIDENCE USING FLAVOR PRECURSOR ANALYSIS. The onion accessions used in this study had been previously screened for white rot reaction in the field at six sites. The field disease incidence data were used in the covariance analysis. The ACSOs that predicted field disease incidence (by field site) are shown in Table 2. The best predictor of field disease incidence was considered to be the ACSO that significantly predicted disease incidence for the greatest number of field sites but also had a more significant *P* value than the interaction of the ACSO \times onion. The *P* value differences are important when

detected in this study were in most cases less than those reported previously in onion tissues (Keusgen et al., 2002; Randle et al., 1995; Thomas and Parkin, 1994; Yoo and Pike, 1998). In specific cases, the 1-PeCSO and MCSO levels reported in the present study are similar to previously reported values (Bacon et al., 1999; Lancaster et al., 2000; Thomas and Parkin, 1994; Yoo and Pike, 1998). These differences may have been conferred by our use of immature plants; the accessions evaluated; cultural and climatic conditions, such as temperature, irrigation, sulfur, and nitrogen nutrition; harvest date; and postharvest handling (Block, 1992; Kubec et al., 2000; Lancaster and Boland, 1990; McCallion and Lancaster, 1984; Randle et al., 2002). Another explanation for the reduced levels of ACSOs in the present study might be the rupture of cell membranes during the freezing of onion tissues, which would have caused ACSO lysis prior to extraction and resulted in lower values. In addition, we used a single extraction volume of 5:1 (v:w), which is considerably lower than published reports of extraction volumes of 15:1 (v:w). These factors, taken together, might have been the cause of levels of ACSOs in the present study that are considerably lower than some other published reports. However, the levels reported herein are consistent with a number of studies, including Bacon et al. (1999), Lancaster et al. (2000), Thomas and Parkin (1994), and Yoo and Pike (1998).

Small amounts of 2-PeCSO were found in onion roots and leaves but not bulbs. Trace amounts of 2-PeCSO have been found in onion bulb tissue by Edwards et al. (1994). Calvey et al. (1997) and Brodnitz et al. (1969) using liquid-chromatography mass-spectrometry (MS) on fresh onion bulb juice found low levels of compounds containing the 2-PeCSO group. In our study, 2-PeCSO accounted for <4% of the ACSO pool in roots and leaves and was not detected in bulbs. Since the amounts of 2-PeCSO detected were small and 2-PeCSO was not detected in bulbs, the data for this ACSO should be regarded with caution.

For some onion plant organs, there was significantly more

Table 2. Flavor precursors that significantly predicted field disease incidence in a covariance analysis of onion accessions evaluated in multiple field sites.

Flavor precursor ²	Plant organ	Plant production location	No. of field sites predicted	Disease level of field sites ³	<i>P</i>	
					Covariate	Covariate × onion
2-PeCSO	Roots	Greenhouse, field and in vitro	3	L, M, H	***	**
1-PeCSO	Roots	Greenhouse	4	L, M, H	**	*
		Field	2	M, H	***	**
	Bulbs	In vitro	1	H	***	**
		Field	3	M, H	**	*
Total	Roots	Greenhouse, field and in vitro	2	M, H	***	*

²Flavor precursor used as predictor (covariate): 2-propenyl-L-cysteine sulfoxide (2-PeCSO), 1-propenyl-L-cysteine sulfoxide (1-PeCSO), Total of all flavor precursors.

³L = low, M = moderate, H = high.

*, **, ***Significant at *P* ≤ 0.05, 0.01 or 0.001, respectively, and 18 weeks after emergence.

Table 3. Mean concentration (mg·g⁻¹ of tissue) of flavor precursors in different onion plant organs harvested from different plant production locations and the results of a contrast between the three susceptible onion accessions (SO) and five resistant onion accessions (RO).

Flavor precursor ²	Onion accession	Flavor precursors (mg·g ⁻¹ of tissue) within plant organs and plant production locations							
		Roots				Bulbs			Leaves
		Greenhouse	Field	In vitro	All	Greenhouse	Field	Both	Greenhouse
MCSO	SO	---	---	---	0.14	---	---	0.12	0.14
	RO	---	---	---	0.14	---	---	0.12	0.15
2-PeCSO	SO	---	---	---	0.01***	---	---	0	0.06**
	RO	---	---	---	0.00	---	---	0	0.03
1-PeCSO	SO	0.17***	0.20**	0.14**	---	0.19	0.20***	---	0.17
	RO	0.15	0.18	0.13	---	0.17	0.15	---	0.15
PCSO	SO	---	---	---	0.11	---	---	0.07	0.07
	RO	---	---	---	0.11	---	---	0.07	0.06
TOTAL	SO	---	---	---	0.43**	0.39	0.34	---	0.44
	RO	---	---	---	0.41	0.39	0.33	---	0.37

²Methyl-L-cysteine sulfoxide (MCSO), 2-propenyl-L-cysteine sulfoxide (2-PeCSO), 1-propenyl-L-cysteine sulfoxide (1-PeCSO), propyl-L-cysteine sulfoxide (PCSO).

--- = not applicable, where interaction involving plant production location not significant, all data were pooled and therefore analysis within plant production locations was not necessary.

*, **, ***Contrast between SO and RO significant at *P* ≤ 0.10, 0.05 or 0.01, respectively.

2-PeCSO and total ACSO (roots only) and 1-PeCSO (roots and bulbs) and percent 2-PeCSO (roots and leaves) in susceptible than resistant accessions. Coley-Smith (1986) hypothesized that the plants that obtain the highest disease incidence among and within *Allium* species emit more total organosulfur and/or different types of organosulfur compounds from their roots than the resistant plants. Coley-Smith et al. (1987) tested this hypothesis using European and Egyptian onion cultivars, respectively, considered as resistant and susceptible cultivars. In their field trials, white rot developed faster in the Egyptian cultivars, but field disease incidence ranged from 80% to 100% for both cultivars. No significant differences in ACSO content of roots and stem bases were found between the Egyptian and European cultivars. Also, no evidence was found to suggest that differences in sclerotial germination were due to levels or types of flavor and odor compounds in the onions studied.

Since the specific interaction between host and pathogen begins with organosulfur compounds exuding from roots (Coley-Smith, 1960) analysis of ACSO in root exudates from onion plants grown in vitro in liquid culture were expected to be revealing. The ACSO amounts collected from these root exudates were lower than those found in onion tissues. Interpretation of the relevance of ACSO profiles in root exudates may be compromised by not knowing the mechanism of component exudation. If alliinase has the opportunity to act on ACSO during or after ACSO exudation, then ACSO profiling may not offer a complete view of the relationship

of organosulfur compounds to incidence of white rot. Indeed, the liquid collected from the plants grown in test tubes was analyzed for EPY concentration, which ranged from 0 to 0.09 mmol·mL⁻¹ of collected liquid, averaged 0.02 mmol·mL⁻¹. These low levels of EPY are evidence of some ACSO transformation during exudation, and this could impact the ACSO profiles obtained in this study. Although liquid cultures do not accurately simulate the root environment of plants growing in the field, this growing medium may allow for a rapid screening technique, provided ACSO profiling can be improved and take into account any ACSO transformation as a result of exudation.

Although 2-PeCSO and total ACSO from roots were predictive of onion white rot reaction in field sites that had moderate and high disease levels, they were only predictive of two out of six field sites, the field sites from New Zealand. 1-PeCSO was the best ACSO predictor of field disease incidence. 1-PeCSO (from onion roots) was predictive of onion white rot reaction in field sites that had low, moderate, and high disease levels overall and was predictive of four out of six field sites, including field sites from both Canada and New Zealand. In addition, 1-PeCSO from two different onion tissues, roots (field, greenhouse, and in vitro produced plants) and bulbs (field produced plants), was predictive of field white rot reaction. It is interesting to note that 1-PeCSO from the bulbs produced in the greenhouse was not predictive of field disease incidence. Although the bulbs produced in the greenhouse developed along the same time line as bulbs produced

in the field, the bulbs were not normal morphologically. The bulbs were very small and many had thick necks. In field situations, thick necks do not allow for natural bulb curing, which may account for the differences in 1-PeCSO between the field and greenhouse produced bulbs. The bulbs from the field were stored for 4 months, at ≈ 4 °C prior to extraction. Research has shown that the concentration of 1-PeCSO increases as bulb storage time increases (Kopsell et al., 1999), which could account for the differences between the greenhouse and field produced bulbs. As well, differences in environmental conditions during plant growth could account for the differences in 1-PeCSO detected in this study between the greenhouse and field.

Our results show that variation in 1-PeCSO, more than any of the other ACSOs, explains variation in onion white rot field reaction. This compound is a major contributor to onion flavor compared to the other ACSOs (Block, 1992; Lancaster and Boland, 1990). Onion bulbs typically have higher concentrations of 1-PeCSO than garlic cloves or leek pseudostems, which was corroborated by this study. In our study, the concentration of 1-PeCSO in all onion tissues was higher than any other ACSOs detected. *Sclerotium cepivorum* has evolved to be pathogenic to *Allium* species only (Coley-Smith, 1960; Esler and Coley-Smith, 1984; Walker, 1924) and it is possible that where onions predominate, *S. cepivorum* sclerotial germination is more stimulated by 1-PeCSO than other ACSOs exuding from onion roots.

In studies comparing garlic, onions, and leek, garlic was the most susceptible, followed by onion, and leek was the least susceptible (Brix and Zinkernagel, 1992; Coley-Smith, 1986; Van der Meer et al., 1983). Coley-Smith and King (1969) tested the *S. cepivorum* sclerotia stimulatory capacity of a number of sulfide compounds from *Allium* species and synthetic equivalents in vitro. Sulfides consisting of 2-PeCSO stimulated more sclerotia to germinate than most other sulfides tested but was not different from some of the sulfides containing 1-PeCSO. Methyl, butyl, and ethyl containing sulfides were weakly stimulatory. Therefore, among ACSOs exuding from onion roots under field conditions, the organosulfur products containing 1-PeCSO may stimulate more *S. cepivorum* sclerotia to germinate. The onion plants that exude the most would, therefore, stimulate more sclerotia to germinate and would have an increased risk of infection. Those onions would be more susceptible to white rot than onions exuding less 1-PeCSO.

Since the 1-PeCSO concentrations from the plants grown in the greenhouse were significantly different from those grown in the field, threshold levels for *S. cepivorum* disease resistance could not be determined. Regardless, the results from this study can be used by breeders when breeding for onion white rot resistance. 1-PeCSO was significantly higher in roots and bulbs from susceptible compared to resistant accessions and was predictive of field disease incidence. Although the methods discussed were not assessed on an individual plant basis, the potential for breeders to screen onions for resistance to *S. cepivorum* by comparing onion root or bulb 1-PeCSO levels could be possible. White rot incidence in the field should be higher in those plants whose roots and bulbs have the highest levels of 1-PeCSO.

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