Pungency of Red Radish Storage Roots Is Unaffected by Spent Mushroom Substrate or Yard Waste Compost Treatments Used for Field Production

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Abstract. Field experiments were conducted at Bixby, OK, in 2007. Four compost treatments and an unamended control were compared for field production of eight (spring) or four (fall) red radish (*Raphanus sativus* L.) cultivars. Treatments were either spent mushroom substrate or yard waste compost spread over plots to an average depth of 2.5 or 5 cm and preplant-incorporated ≈ 5 to 7 cm deep. Radishes were direct-seeded into prepared plots and subsequently grown using standard cultural practices. Samples of median-sized marketable storage roots were shredded and juice was analyzed in the laboratory for pungency as measured by isothiocyanate (ITC) concentration (primarily 4-methylthio-3-butenyl isothiocyanate). In the spring, mean ITC concentrations ranged from 28.2 to 36.8 μmol per 100 g juice in storage roots from the four compost treatments, and differences were not significant ($\alpha = 0.05$). There were not enough storage roots to analyze from the unamended control plots as a result of herbicide toxicity. Cultivars differed in mean concentration of ITCs, ranging from a high of 52.9 μmol per 100 g juice for 'Cherry Belle' to a low of 19.2 μmol per 100 g juice for 'Crunchy Royale'. In the fall, mean ITC concentrations ranged from 10.5 to 24.6 μmol per 100 g juice in storage roots from the four compost treatments. Differences were not significant ($\alpha = 0.05$), and there were no differences from the control value of 17.5 μmol per 100 g juice. The mean ITC concentration was 19.9 μmol per 100 g juice for the four cultivars tested in the fall, and the cultivars did not differ. Results indicate that the tested compost treatments did not affect pungency of red radish storage roots as measured by concentrations of ITCs.

Compost use is becoming common in commercial vegetable production, particularly among smaller and more specialized producers (Roe, 2001). Feedstocks for composts evaluated on vegetable crops have included mixed municipal solid waste, biosolids, yard trimmings/waste, and other agricultural wastes (Roe, 2001).

The impact of composted soil amendments on chemical parameters of crop quality, rather than simply on crop productivity,

has received relatively little attention. Amending the soil with composted red clover (*Trifolium pratense* L.) affected the content of S-alk(en)yl-L-cysteine sulfoxides in leek (*Allium porrum* L.) (Lundegårdh et al., 2008). Zhao et al. (2008) reported that organic fertilization (compost + fish emulsion) resulted in higher phenolic concentrations for pak choi (*Brassica rapa* L. Chinensis group) compared with conventional fertilization with mineral fertilizers. The application of sanitized sewage sludges to pepper (*Capsicum annuum* L.) plants improved yield without significantly affecting the concentrations of ascorbic acid, glutathione, and capsaicinoids (Pascual et al., 2010).

Pungency is a major determinant of quality in radish (*Raphanus sativus* L.) storage roots. The pungent principle of radish storage roots was identified by Friis and Kjaer (1966) as a glucoside undergoing rapid enzymatic hydrolysis to 4-methylthio-3-*trans*-butenyl isothiocyanate, abbreviated MTBITC. There is some evidence that levels of glucosinolates in Brassicaceae vegetables can be influenced by growing conditions. Neil and Bible (1973) reported that MTBITC levels in radish storage roots were influenced by photoperiod and by soil type. Foliar fertilization with methionine, a precursor of glucosinolate

synthesis, increased the glucosinolate content in broccoli (*Brassica oleracea* L. Italica group) heads but not in radish hypocotyls (Scheuner et al., 2005). Pant et al. (2012) found that applications of vermicompost tea increased plant growth, nitrogen (N) content, total carotenoids, and total glucosinolates in pak choi tissues.

The work reported here was part of a larger study designed to compare two unblended organic materials—spent mushroom substrate (SMS) and yard waste compost (YWC), both hereafter referred to as composts—applied to the soil surface at two depths (equivalent to 2.5 or 5 cm) and then incorporated ≈ 5 to 7 cm deep for effects on field production of multiple cultivars of red radishes. Details of the larger study, including compost analyses, have been published elsewhere (Kahn et al., 2012). The objective of the work reported here was to determine whether the tested compost treatments would affect pungency levels in the radish storage roots, including analyses of possible compost treatment $\times$ cultivar interactions.

Materials and Methods

Field studies were conducted in Spring and Fall 2007 on a Severn very fine sandy loam [coarse-silty, mixed (calcareous), thermic Typic Udifluvent] with an organic carbon concentration of $\approx 4 \text{ g}\cdot\text{kg}^{-1}$ at a depth of 0 to 15 cm at the Oklahoma Vegetable Research Station in Bixby. Four compost treatments and an unamended control were compared in factorial combination with eight (spring) or four (fall) radish cultivars. Open-pollinated cultivars were Champion; Cherry Belle; Fuego; and Red Silk. Hybrid cultivars were Cherriette; Crunchy Royale; Fireball; and Red Satin. Plots received preplant-incorporated urea to supply $56 \text{ kg}\cdot\text{ha}^{-1}$ N; preplant-incorporated trifluralin at $560 \text{ g}\cdot\text{ha}^{-1}$ for weed control; and sprinkler irrigation. Two harvests, ≈ 1 week apart, were done for each experiment. Harvest dates were treated as samples. For each harvest, all plants in a 1-m section of row were pulled by hand, washed, graded, counted, and weighed.

Median-sized marketable storage roots from the first harvest in each study were used for pungency assessment. Roots were processed through extraction with hexane in accordance with the procedure of Coogan et al. (2001). Roots were divided into two sets and three to five roots were used from each set. Individual weights of each root were determined; the top and bottom quarters were removed and discarded; and the remaining middle portion of root was reweighed. Centered, vertical root cores were then taken with a #11 cork borer ($\approx 1.7 \text{ cm}$ diameter) and weights were obtained. Root cores within each set were then grated into a fine pulp with a vegetable grater and the grated samples were incubated at room temperature for 30 to 45 min to allow time for an endogenous myrosinase to deglycosilate the thiocyanates.

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Duplicate samples of 2 g juice were weighed into 2-dram vials and 2 mL of hexane was added. Samples were vortexed vigorously for 40 s. Vials were then centrifuged in a Speed Vac centrifuge (Savant, Farmingdale, NY) at 3000 g for 20 min to maximize hexane phase separation. The upper hexane phase was removed for ITC analysis, which was conducted either immediately or after storage for no more than 2 d at -20 °C.

Isothiocyanate concentration (primarily MTBITC) of extracts was assayed using the spectrophotometric procedure of Nakamura et al. (2001). Briefly, triplicate samples consisting of 100 µL of hexane layer, 450 µL of high-performance liquid chromatography-grade methanol (Fisher Scientific, Fairlawn, NJ), 450 µL 50 mM borax-HCl buffer (pH 8.5), and 50 µL of 8 mM 1,2-benzenedithiol were placed into plastic tubes and mixed well by vortexing. Duplicate tubes were processed as described previously except that methanol was substituted for 8 mM 1,2-benzenedithiol for use as blanks. Tubes were loosely capped and then incubated at 65 °C in an oven for 1 h. After cooling, absorbance of samples and blanks was determined at 365 nm using a Shimadzu ultraviolet-160 spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). Total ITCs were determined against incubated standards of phenethyl isothiocyanate (10, 25, 50, and 100 nmoles) and converted to µmoles ITCs per 100 g juice.

The experimental design for the Spring 2007 study was a split plot arranged in randomized complete blocks with three replications. The four compost treatments and an unamended control were main plots and the eight radish cultivars were subplots. The experimental design for the Fall 2007 study was a split plot arranged in randomized complete blocks with four replications. This was essentially a repeat of the Spring 2007 study with fewer cultivars and an added replication.

Pungency analyses were not performed on 'Red Silk' in Spring 2007 as a result of low storage root yields resulting from poor adaptation. Also, there were too few marketable storage roots from the unamended control plots in Spring 2007 to allow pungency analyses as a result of apparent phytotoxicity from the trifluralin. Nonetheless, at least 150 samples were available for laboratory and statistical analysis in Spring 2007 and again in Fall 2007. Data were evaluated with analysis of variance procedures using the Statistical Analysis System (SAS) (SAS Institute, Cary, NC). The spring and fall experiments were analyzed for main effects of compost treatments and cultivars and their interactions. Main effects of compost treatments were further partitioned into single df orthogonal contrasts as shown in Table 1. If the main effect of cultivar was significant ($P \leq 0.05$), means were separated by pairwise comparisons using a LSMEANS statement with a PDIFF option and a significance level of 0.05 as shown in Table 2.

Table 1. Summary analyses of variance for effects of compost treatments and cultivars on levels of isothiocyanates in juice from shredded storage roots of red radishes, Spring 2007 and Fall 2007.

Spring 2007		
Source	df	Pr > F
Compost treatments	3	0.452
SMS vs. YWC	1	0.195
2.5 cm vs. 5 cm	1	0.507
Interaction	1	0.576
Cultivars	6	<0.001
Composts × cultivars	18	0.152

Fall 2007		
Source	df	Pr > F
Compost treatments	4	0.408
Control vs. composts	1	0.650
SMS vs. YWC	1	0.354
2.5 cm vs. 5 cm	1	0.246
Interaction	1	0.180
Cultivars	3	0.680
Composts × cultivars	12	0.070

SMS = spent mushroom substrate; YWC = yard waste compost.

Results and Discussion

The yield portion of this work has been reported elsewhere (Kahn et al., 2012). In brief, SMS gave seasonally variable results, including a distinct negative effect when applied in a layer 5 cm deep for fall production. The YWC improved yield compared with unamended soil by mitigating toxicity from the trifluralin, but no benefit was demonstrated in a third fall study in the absence of trifluralin. Compost × cultivar interactions were evident for yield.

Mean concentrations of ITCs did not differ in juice of storage roots from the four compost treatments in spring or fall (Table 2). The comparison with ITC concentrations in juice of storage roots from the unamended control also was not significant in the fall (Table 2), even after further evaluation with Dunnett's test (analysis not presented). We did not find other studies reporting compost effects on ITC concentrations in radish storage roots. However, Eigenbrode and Pimentel (1988) reported that total glucosinolate concentration in leaves of collards (*Brassica oleracea* L. *Acephala* group) was not consistently affected by four fertilizer treatments, including "sheet composted" cow manure, compared with an unfertilized control.

Cultivars differed in pungency as measured by mean concentrations of ITCs in the spring study but not in the fall (Table 2). There were fewer tested cultivars and a higher cv (cv = 25.3) in the fall than in the spring (cv = 12.1). Pungency of 'Crunchy Royale' remained relatively constant across the two seasons, but our study does not provide sufficient evidence to speculate about the genetic stability of the pungency trait in this cultivar. The compost treatment × cultivar interaction was not significant in either season (Table 1). Levels of ITCs in radish storage roots are known to vary among cultivars (Carlson et al., 1985; Okano et al., 1990) and

Table 2. Means for concentrations of isothiocyanates (µmol per 100 g juice) from shredded storage roots of red radishes as affected by compost treatments and cultivars, Spring 2007 and Fall 2007.

Variable	Spring 2007	Fall 2007
Compost treatments		
Control	N/T ^z	17.5
SMS 2.5 cm	32.9	24.6
SMS 5 cm	28.2	10.5
YWC 2.5 cm	32.2	22.2
YWC 5 cm	36.8	23.6
Significance	NS	NS
Cultivars		
Cherry Belle	52.9 a ^y	18.4
Fireball	40.3 b	21.8
Champion	30.9 bc	17.2
Fuego	29.9 bcd	N/T
Cherriette	29.7 cd	N/T
Red Satin	26.6 cd	N/T
Crunchy Royale	19.2 d	21.2
Significance	**	NS

^zN/T = not tested.

^ySeparation in columns using protected pairwise comparisons on least square means, $P \leq 0.05$.

NS, ** Nonsignificant or significant at $P \leq 0.01$, respectively.

SMS = spent mushroom substrate; YWC = yard waste compost.

by season (Coogan et al., 2001; Neil and Bible, 1973).

In conclusion, although our tested compost treatments affected yields, they did not affect pungency of red radish storage roots as measured by concentrations of ITCs.

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