Field Sampling Short-day Onions for Bulb Pungency

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SUMMARY. The marketing of onions (*Allium cepa* L.) based on bulb pungency as a measure of overall flavor intensity is being considered by the onion industry. Pungency is highly variable within and among fields due to genetic and environmental factors. Therefore, a study was undertaken to develop a sampling procedure to estimate onion pungency means and variances from field-grown onions with predetermined degrees of accuracy and confidence. Two short-day onion cultivars, commonly grown in the Vidalia, Ga., area, were each randomly sampled from four different fields. The sampled bulbs were analyzed for enzymatically formed pyruvic acid (EPY) and soluble solids content (SSC) to assess pungency and sugars, respectively. EPY concentration and SSC varied between the two cultivars, among the four fields within cultivars, and among the fifty samples within each field. In a combined analysis of all eight fields, at least 1.3 ten-bulb samples would be needed per acre to come within $\pm 0.5 \mu\text{mol EPY}$ of a field's true EPY mean with 95% confidence. If the accuracy of the estimation was lowered to $\pm 1.0 \mu\text{mol EPY}$ of a field's true mean, then at least 0.4 ten-bulb samples would be needed per acre. Because SSC was less variable than EPY, the number of ten-bulb samples needed per acre to estimate a field's true mean was lower than the number required to estimate EPY. Establishing a sampling method to estimate an onion field's EPY and

SSC will provide the mechanism to standardize onion flavor in the market place and instill greater consumer confidence in purchasing onions.

Onions (*Allium cepa*) are consumed primarily for their unique flavors or their ability to enhance the flavors of other foods. Although sugars, organic acids, and other compounds contribute to the overall flavor of an onion, the organosulfur compounds, collectively known as S-alk(en)yl cysteine sulfoxides (ACSOs), are precursors to the onion's distinctive flavor and aroma (Block, 1992). Three different ACSOs are synthesized and accumulate in onions. Organosulfur compounds are released from the ACSOs following their enzymatic decomposition by alliinase when the onion cells are damaged. Primary decomposition products from the three onion ACSOs are sulfenic acids, ammonia, and pyruvic acid. The sulfenic acids rapidly condense to form various thiosulfinates, which are also unstable and randomly rearrange and disassociate into other S compounds. These ACSO decomposition products are responsible for the different onion flavors, such as heat, pungency, fresh or green onion, etc., and in combination reflect the overall flavor intensity (Randle et al., 1994). Subsequent uses of pungency, flavor, or flavor intensity in this paper will refer to the ACSOs, to their decomposition products, and to the overall contribution these compounds have to the organoleptic experience. However, because the organosulfur compounds are difficult to measure, enzymatically formed pyruvic acid (EPY) has been used to measure indirectly overall flavor intensity (Randle and Bussard, 1993) and has been correlated with overall flavor perception (Schwimmer and Guadagni, 1962; Wall and Corgan, 1992). Even though EPY is an indirect measure of all S-based flavors and overall flavor intensity produced from ACSO decomposition, EPY is routinely associated solely with onion pungency in the scientific literature and within the onion industry. As a result, pungency and flavor intensity are commonly interchangeable in the literature when EPY is assessed. However, EPY is a measure of overall flavor intensity.

Onion flavor is highly variable

(Randle, 1997) and cultivars differ widely in their flavor intensity (Randle, 1992a). In addition, environmental factors such as sulfate and water availability and growing temperature can greatly modify a cultivar's flavor intensity (Randle, 1997). Commercial onion production often occurs in a highly heterogeneous environment. Factors such as soil type, fertility, and irrigation, are commonly nonuniform within a field and affect flavor intensity. For example, S availability affects ACSO concentration and composition (Randle et al., 1995), which in turn can influence flavor intensity and flavor perception (Randle et al., 1994). Thus, the ability to market or purchase onions with a known or desired flavor intensity can be a challenge.

Understanding the specific sources and levels of flavor variation is also important in determining how onions are sampled to accurately assess their flavor intensity. First, significant bulb-to-bulb variation for flavor occurs in an onion cultivar (Randle, 1992b). Consequently, multiple-bulb samples in replicated experiments are often required to determine a cultivar's flavor performance accurately in scientific studies. Factors that contribute to within cultivar variability include 1) an outcrossing breeding behavior that continually introduces genetic variability into individual plants; 2) severe inbreeding depression from self-pollination, which prevents traits from becoming homozygous at all important loci; and 3) little direct selection for flavor during cultivar improvement (Pike, 1986). Second, flavor intensity varies as the plant grows. Flavor compound accumulation is greatest in the leaves up to bulb initiation and then increases in the bulb as the plant approaches maturity (Randle et al., 1993). In the final stages of bulbing, flavor intensity decreases as cells rapidly expand and dilution occurs. Third, flavor intensity is unequally distributed within the plant. In the bulb, flavor compound accumulation is highest in the center scales and at the base and lowest in the outside scales and at the top (Lancaster et al., 1986). Therefore, to make valid comparisons, onions at the same stage of development must be used, the same part of the bulb must be sampled, and a sufficiently large number of bulbs per sample need to be used for each cultivar tested.

Interest exists in the onion indus-

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try in testing field-grown onions for pungency so production lots could be marketed with a known flavor intensity. The objective of this study was to develop sampling procedures to estimate onion pungency means and variance from field-grown onions with a predetermined degree of accuracy and confidence. In addition, sampling procedures to estimate soluble solids contents (SSC) as a measure of total sugar content were also determined.

Materials and methods

Two onion cultivars popular in the Vidalia, Ga., growing area—'Sweet Vidalia' (Rio Colorado Seeds, Yuma, Ariz.) and 'Pegasus' (Asgrow Vegetable Seed, Kalamazoo, Mich.)—were selected for this study. A cultivar can be considered a population within a growing region, and each onion field can be viewed as a subset (stratum) of the cultivar population. When populations are partitioned into different fields (L strata), variability within fields is intended to and should be lower than among fields (Steel and Torrie, 1980). By randomly selecting different onion fields, population parameters can then be estimated and the appropriate sample size can be determined.

Fields were randomly selected from commercial Vidalia onion growers and ranged in size from 4.9 to 14.8 acres (2 to 6 ha). 'Sweet Vidalia' bulbs were randomly sampled from four different fields on 30 Apr. and 1 May 1997, and 'Pegasus' bulbs were sampled from four fields on 14 May. A random sampling procedure was generated for $n = 50$ within field points

according to each field area (SAS, Cary, N.C.). Sampling points represented an x-y field coordinate where a 10-bulb sample was taken. Ten bulbs per x-y coordinate were determined necessary because of the inherent bulb-to-bulb variation for pungency (Randle, 1992b). Before sampling, the onions were undercut, their tops and roots clipped, and the bulbs were placed into burlap field bags by harvest crews. The sample coordinates were found by pacing the x and y axes of the field. Samples were taken from the field bag closest to each x-y coordinate.

Each 10-bulb sample was analyzed for pungency, as measured by EPY concentration and SSC. SSC correlates well with onion sugars (Mann and Hoyle, 1945). A 0.25-cm-thick wedge was cut from each of the 10 bulbs and the wedges were combined and then pressed to extract juice. EPY was then determined according to the method of Randle and Bussard (1993). SSC was determined with a hand-held refractometer (Kernco, Tokyo).

Estimates for EPY and SSC sample and population means and variances, and the sample sizes required to come within ± 0.5 and 1.0 units of each test variable with 95% confidence were calculated (Steel and Torrie, 1980; Thompson, 1992). In spatial sampling, a 10-bulb sample from a given field can be treated as a simple random sample of that field. The field sample mean and variance are used to estimate the field population mean and variance. An estimate of the field population mean can be made using the field sample mean $\mu_h = 1/n_h \sum z_{hi}$, where n_h

= number of samples in field h and z_{hi} = value for the variable of interest for coordinate i in field h. The field sample mean is an unbiased estimator of the field population mean. An estimate of the field population variance (s^2_h) can be made using the field sample variance $s^2_h = 1/n_h - 1 \sum (z_{hi} - \mu_h)^2$. The variance of the field sample mean (μ_h) can be estimated by $\text{var}(\mu_h) = s^2_h/n_h$. The population mean can be estimated by $\mu_{st} = 1/|A| \sum |A_h| \mu_h$, where $|A|$ = total area of all fields; $|A_h|$ = area of field h. The variance of the population mean (μ_{st}) = $\sum (|A_h|/|A|)^2 \mu_h^2/n_h$, which can be estimated by $\text{var}(\mu_{st}) = \sum (|A_h|/|A|)^2 s^2_h/n_h$. The sample size required to estimate μ_{st} within d units of measurement with $(1 - \alpha) \times 100\%$ confidence is calculated by $n = (\sum |A_h| s_h)^2 / (d^2 |A| / z_{\alpha/2})^2 + \sum |A_h| s^2_h$, where $z_{\alpha/2}$ = the standard normal deviate. Estimated samples required per acre or hectare were calculated as $(\sum n/|A_h|)/h$.

Results and discussion

Overall, 'Pegasus' had lower mean EPY and higher mean SSC than 'Sweet Vidalia' (Table 1). The pungency variance of 'Pegasus' was less than that of 'Sweet Vidalia', while the variance in SSC was less for 'Sweet Vidalia' than 'Pegasus'. 'Pegasus' field means ranged from 2.7 to 3.7 $\mu\text{mol}\cdot\text{mL}^{-1}$ EPY onion juice and 7.7% to 9.8% SSC, while 'Sweet Vidalia' field means ranged from 3.8 to 5.9 $\mu\text{mol}\cdot\text{mL}^{-1}$ EPY and 6.6% to 7.8% SSC. Field pungency variances for 'Pegasus' ranged from 0.34 to 1.30, while 'Sweet Vidalia' field pungency variances ranged from 0.53 to 1.30.

Table 1. Field sample means (μ_h), variances (s^2_h), and standard deviations (s) for enzymatically formed pyruvic acid (EPY) and soluble solids content (SSC) for two onion cultivars sampled from four fields each.

Field	n ²	Area		EPY (μmol·mL ⁻¹ juice)			SSC (%)		
		Acres	ha	μ _h	s ² _h	s	μ _h	s ² _h	s
Pegasus									
1	50	9.9	4.0	2.7	1.30	1.14	8.3	0.30	0.54
2	50	7.4	3.0	3.1	0.65	0.80	7.7	0.20	0.45
3	50	9.3	3.8	3.4	0.34	0.58	8.1	0.40	0.63
4	50	14.8	6.0	3.7	0.61	0.78	9.8	0.22	0.46
All	200	41.4	16.8	3.2	0.85	0.92	8.5	0.88	0.94
Sweet Vidalia									
5	50	9.9	4	5.8	1.14	1.07	7.2	0.33	0.58
6	50	4.9	2	3.8	1.3	1.14	7.8	0.13	0.37
7	50	12.4	5	5.9	0.84	0.92	7.6	0.16	0.41
8	50	4.9	2	5.4	0.53	0.74	6.6	0.12	0.34
All	200	32.1	13	5.2	1.65	1.28	7.3	0.39	0.62

²n = Number of 10-bulb samples.

Table 2. Estimated population means (μ_{st}) and variances [$\text{var}(\mu_{st})$] of enzymatically developed pyruvic acid (EPY) and soluble solids content (SSC) for 'Pegasus', 'Sweet Vidalia', and the combined onion cultivars.

	Pegasus	Sweet Vidalia	Combined
EPY ($\mu\text{mol}\cdot\text{mL}^{-1}$ juice)			
μ_{st}	3.3	5.5	4.3
$\text{var}(\mu_{st})$	0.004	0.006	0.002
SSC (%)			
μ_{st}	8.7	7.4	8.1
$\text{var}(\mu_{st})$	0.001	0.001	0.001

Table 3. The number of ten-bulb onion samples needed per acre (hectare) to estimate field means for enzymatically developed pyruvic acid concentration (EPY) and soluble solids content (SSC). Samples per acre (hectare) estimate a field mean within ± 0.5 or ± 1.0 μmol EPY or SSC with 95% confidence. Values are for 'Pegasus', 'Sweet Vidalia', and the combined onion cultivars.

Variable	Pegasus	Sweet Vidalia	Combined
± 0.5 units			
EPY ($\mu\text{mol}\cdot\text{mL}^{-1}$)	0.9 (2.7)	1.5 (5.3)	1.3 (3.8)
SSC (%)	0.4 (1.0)	0.4 (1.1)	0.4 (1.1)
± 1.0 units			
EPY ($\mu\text{mol}\cdot\text{mL}^{-1}$)	0.3 (0.7)	0.5 (1.3)	0.4 (0.9)
SSC (%)	0.1 (0.3)	0.1 (0.3)	0.1 (0.3)

Bulb pungency variation in this experiment was similar to that previously reported for field-grown short-day onions in experimental plots (Randle, 1992b).

ESTIMATING EPY SAMPLE NUMBER.

The number of 10-bulb samples required to come within at least ± 0.5 and 1.0 μmol EPY was selected because a 1 - μmol difference among bulbs is perceptible when tasting onions (Randle, 1997; personal observations). For example, when eaten, an onion with 4 μmol EPY is perceived as more mild than an onion with 5 μmol EPY. Above eight μmol EPY, onions are very pungent and difficult to eat raw by most people in the United States. Because the estimated variance of EPY differed between the cultivars tested (Table 2), the number of samples needed per acre for each cultivar varied. To be 95% confident that a field of 'Pegasus' or 'Sweet Vidalia' was within ± 0.5 μmol EPY of the field's population mean, 0.9 and 1.5 ten-bulb samples would need to be randomly selected per acre, respectively (Table 3). If the precision needed to predict a field population mean was lowered to ± 1.0 μmol EPY, 0.3 and 0.5 ten-bulb samples would be needed per acre for 'Pegasus' and

'Sweet Vidalia', respectively. The sample-to-sample pungency variation expressed by 'Pegasus' and 'Sweet Vidalia' was representative of the range of sample-to-sample variation for other short-day onions (Randle, 1992b). Thus, using all eight fields in a combined cultivar analysis would allow a general estimation of the number of ten-bulb samples needed to predict the mean performance of a selected commercial field of short-day onions in the Vidalia growing area. In the combined analysis, 1.3 and 0.4 ten-bulb samples would be needed per acre to predict a field mean within ± 0.5 and ± 1.0 μmol EPY with 95% confidence, respectively (Table 3).

ESTIMATING SSC SAMPLE NUMBER.

Percent SSC, was less variable than EPY within cultivars among the tested fields (Table 1). As a result, the number of samples needed to predict a field's SSC within ± 0.5 units with 95% confidence was lower than for EPY. Only 0.4 ten-bulb samples would be needed per acre to predict a field's SSC population mean when the values from the combined cultivars are used (Table 3). Commercially, onions intended to be consumed raw are often referred to as sweet onions. While the sugar con-

centration in these onions is important, the sweet taste of an onion is perceived only if the onions are mild. That is to say, the organosulfur compounds dominate the organoleptic experience by producing the pungency and heat sensations (Randle, 1997). The organosulfur compounds need to be sufficiently low in concentration to allow the sugars to be tasted when consumed. Therefore, when evaluating onions for raw consumption, pungency needs to be considered first, and then sugar concentration. The number of samples needed to predict EPY field population means was more conservative (a greater number of samples were needed per acre) than the number of samples for SSC (Table 3).

This is the first study to assess flavor variability in field-grown commercial onions. The recommendations in this paper, however, should be applied only to the Vidalia growing area, or to other areas of similar climate and geography, and to the mild varieties grown there. Statistically sampling onions fields to predict estimated EPY and SSC field means will provide the mechanism to standardize flavor intensity in the market place and instill greater consumer confidence when onions are purchased. However, growers, buyers, and consumers must also be educated as to the inherent bulb-to-bulb variability found in commercially produced onions. For various reasons it is difficult to breed and grow onions with a high degree of plant-to-plant (bulb-to-bulb) flavor uniformity. Every onion will taste somewhat different from the next. As an example, while the mean EPY of a 'Sweet Vidalia' field in our study was 3.8 μmol , the 50 ten-bulb samples ranged from 1.1 to 6.3 μmol . Therefore, understanding the pungency variance, which is a measure of dispersion around the mean, will also be important information for the producer or consumer. A smaller variance will equate to more onions with a pungency closer to the predicted mean.

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Constraints on Developing an Integrated Pest Management Program for Citrus Leafminer (Lepidoptera: Gracillariidae) in Florida Nurseries

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SUMMARY. Florida citrus nursery growers were surveyed to learn about their citrus leafminer (*Phyllocnistis citrella* Stainton) (CLM) management practices as a preliminary step in developing an integrated pest management (IPM) program. All responses were kept anonymous. Survey responses from growers producing ≈4.2 million trees annually were obtained, which represents most of the estimated 5.2 million trees required to annually replant Florida groves. Large nurseries (20%) each produced ≥100,000 trees per year and jointly provided 88% of the trees produced annually. Small nurseries (80%) each produced <100,000 trees per year. The citrus leafminer was ranked the most important pest in nurseries during 1995.

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Pesticides used for CLM control included avermectin, azadirachtin, imidacloprid, fenoxycarb, diflubenzuron, and sulfur, in order of importance. Oil and soap also were used. Growers were concerned about the possibility that the CLM will develop resistance to pesticides. Producers potentially were willing to monitor CLM populations, switch pesticide types to improve survival of parasitoids of the CLM, and leave untreated trees inside the nursery to serve as refuges for CLM parasitoids. In order of importance, pest management advice was provided by private chemical companies, the Florida Citrus Pest Management Guide produced by the University of Florida/Institute of Food and Agricultural Sciences (UF/IFAS), UF/IFAS personnel, grower magazines, private consultants, the Florida Citrus Nurserymen's Association, and other growers.

Efforts have been made since 1993 to protect Florida citrus production from losses caused by the citrus leafminer (CLM) (*Phyllocnistis citrella* Stainton). Most of the work has been directed to protecting groves (Knapp et al., 1995) and little effort has been extended to citrus nurseries, where damage can be even more dramatic than that caused in mature trees (Stansly et al., 1996). A special set of integrated pest management (IPM) tactics may be needed to delay the development of resistance by the citrus leafminer to pesticides used in citrus nurseries. A strong biological control component should be included in any citrus nursery IPM program developed because an effective natural enemy of the citrus leafminer, *Ageniaspis citricola* Logvinovskaya, has been established in a classical biological control program (Hoy et al., 1995). Cultural control tactics and a judicious use of pesticides should be included as well. Unfortunately, plant resistance, physical and mechanical controls, genetic or autocidal control, and behavioral and legal controls are not well developed or can not be implemented to manage CLM populations in Florida nurseries and groves (Villanueva-Jiménez and Hoy, 1996).

When developing an effective IPM program it is important for researchers to understand the problems faced by the end users and to get feedback from potential users regarding tactics that are feasible and potentially implementable. Ideally, this discussion occurs before