Variations in Concentrations and Interrelationships of Phytate, Phosphorus, Magnesium, Calcium, Zinc, and Iron in Wheat Varieties during Two Years

Aratoonnaz Nahapetian* and Abdollah Bassiri

Variations in concentrations and interrelationships of phytate, P, Mg, Ca, Zn, and Fe were studied in grains of seven Iranian (Deihim, Derakhshan, Jolgeh, Koohrang, Navid, Ommid, and Roshan) and five foreign (Akowa, Inia, Penjamo, Ciano, and Tobari) varieties of wheat (*Triticum aestivum* L.) in two successive years. Protein concentrations and yields of the grains were also determined. There were significant annual variations in yield and concentrations of phytate, P, Mg, Ca, and Zn. The effect of year was not significant for concentrations of protein and Fe. Significant varietal differences were also found for most of the variables. There was a highly significant relationship between total P and phytate P ($r = 0.93$). Estimation of phytate P from total P data and possibility of changing concentrations of the variables with emphasis on phytic acid in the wheat grain by agricultural practices are discussed.

Previous studies have shown that in wheat phytate P varied from 49 to 80% of total P of the grain (Knowles and Watkins, 1932; Booth et al., 1941; Asada et al., 1968; Nelson et al., 1968; O'Dell et al., 1972; Abernethy et al., 1973; Nahapetian and Bassiri, 1975). It has been suggested that phytic acid in the cereal grains is present as a mixed insoluble salt of Mg, Ca, and K (Averill and King, 1926). In contrast, other studies have indicated that it is present in a soluble form in barley (Sandegren, 1948) and in beans (Lolas and Markakis, 1975). The primary role of phytic acid may be as P store which is gradually utilized during germination by the grain (Hall and Hodges, 1966; Asada et al., 1968; Williams, 1970).

It has been shown that phytic acid might cause decreased physiological availability of dietary Ca (Harison and Mellanby, 1939; McCance and Widdowson, 1942; Krebs and Mellanby, 1943; Hoff-Jorgensen et al., 1946; Cullumbine et al., 1950; Nelson et al., 1968; Berlyne et al., 1973; Reinhold et al., 1973b), Fe (Sharpe et al., 1950; Reinhold, 1975a), Zn (O'Dell and Savage, 1960; Prasad et al., 1963; O'Dell, 1969; Reinhold, 1971, 1975a,b; Halsted et al., 1972; Reinhold et al., 1973a), and Mg (McCance and Widdowson, 1942; Roberts and Yudkin, 1960; Likuski and Forbes, 1965).

Nutritional studies in Fars villages of Iran have indicated the incidence of Ca, Fe, and Zn deficiencies (Prasad et al., 1961; Halsted, 1968; Ronaghy et al., 1968; Ronaghy, 1970; Reinhold, 1971, 1975a,b; Haghshenass et al., 1972; Halsted et al., 1972; Reinhold et al., 1973a) although the intake of these minerals was adequate. Low availability of Ca, Fe, and Zn from predominantly cereal diets of the villagers has been suggested to be the major cause of the mineral deficiencies (Reinhold, 1971, 1975a,b; Reinhold et al., 1973a).

Reinhold (1975b) has reported that Lavash and Tanok (Iranian village breads made from whole meals of 95–100% extraction) constituted 50 to 80% of total dietary calories of the villagers. The breads were found to contain 0.5 to 1.3% phytate (Reinhold, 1972) and 2.5% of fiber (Reinhold et al., 1976). Thus, excessive intakes of phytate (Reinhold, 1971, 1972; Reinhold et al., 1973a) and fiber (Reinhold, 1975a,b; Reinhold et al., 1976) were suggested to be responsible for the mineral deficiencies.

Departments of Food Technology and Nutrition and Agronomy, College of Agriculture, Pahlavi University, Shiraz, Iran.

Providing villagers with wheat or flour with a low phytic acid content for bread making might be a practical means of increasing availability of their dietary minerals. To reach this goal, wheat varieties should be found that not only have the above merit but also have high yields and reasonable amounts of the minerals and protein in different growing seasons. The present study was initiated to investigate the possible year-to-year fluctuations in concentrations and interrelationships of phytic acid, minerals (P, Mg, Ca, Fe, and Zn), and protein together with an important agronomic characteristic (yield) in seven Iranian and five introduced wheat varieties.

MATERIALS AND METHODS

Seven Iranian (Deihim, Derakhshan, Jolgeh, Koohrang, Navid, Ommid, and Roshan) and five foreign (Akowa, Inia, Penjamo, Ciano, and Tobari) varieties of wheat (*Triticum aestivum* L.) were used in this study. These varieties were planted in two successive years (Dec 10, 1972 and Nov 11, 1973) at the College of Agriculture Experimental Station, Pahlavi University, Shiraz, Iran. In both years the experiment was laid out in a randomized complete block design with four replications. The field used in each year was kept fallow the preceding year and was not fertilized either previously or during the course of the experiment. The plots were irrigated when necessary. Each plot consisted of eight 5-m rows at 50-cm distance. The four middle rows of each plot were harvested at maturity and the grains were dried at 100 °C for 24 h, ground in a coffee mill, and stored in plastic bags until analysis.

Concentrations of the phytate and the minerals were determined by the methods reported earlier (Nahapetian and Bassiri, 1975). Protein concentration was determined by the AOAC (1970) Kjeldahl method.

Data for the characters in individual years were subjected to the analyses of variance, coefficients of variability calculated for each year, and the combined data of both years were analyzed as a split-plot over time as described by Steel and Torrie (1960). Means were compared using the Duncan's new multiple range test (Duncan, 1955).

RESULTS

Comparison of values for the variables in the two successive years (Tables I, II, and III) showed that, in general, values obtained in 1973 were significantly lower than those obtained in 1972, except for concentrations of Mg, Fe, and protein. The effect of year was not significant for the concentrations of Fe (Table II) and protein (Table III). Concentrations of Mg, in the wheat grains harvested

Table I. Concentrations of Total P, Phytate P, Nonphytate P, and Phytate P as Percent of Total P in Grains of Wheat (*Triticum aestivum* L.) Varieties during Two Successive Years

Varieties	Total P,[a] mg/100 g		Phytate P,[a] mg/100 g		Nonphytate P,[a] mg/100 g		Phytate P as % of total P[a]	
	1972	1973	1972	1973	1972	1973	1972	1973
Akowa	428ab	285bc	288a	156def	140a	129a	67b	55d
Ciano	449a	339a	318a	244ab	132ab	95ab	71ab	72abc
Deihim	368c	249bc	272a	167cde	84bc	82ab	77ab	67abcd
Derakhshan	375bc	231bc	308a	157def	67c	74b	83a	68abcd
Inia	348c	322a	261a	254a	88bc	68b	76ab	78a
Jolgeh	435ab	281ab	317a	197bcd	118abc	84ab	74ab	69abc
Koohrang	367c	200c	286a	109f	81bc	91ab	78ab	55d
Navid	369c	201c	261a	124ef	105abc	78ab	71ab	62cd
Ommid	397abc	190c	285a	120ef	112abc	70b	72ab	63bcd
Penjamo	357c	279ab	266a	212abc	91abc	67b	75ab	76ab
Roshan	397abc	195c	279a	137ef	118abc	58b	71ab	70abc
Tobari	377bc	332a	268a	251a	109abc	81ab	71ab	76ab
Av	389	259	284	177	104	81	74	68
C.V.,[b] %	6.8	14.7	6.7	19.9	35.9	25.8	8.4	10.5

[a] Means in each column followed by the same letter are not significantly different at the 1% probability level (Duncan's test). [b] Coefficient of variability.

Table II. Concentrations of Mg, Ca, Zn, and Fe in Grains of Wheat (*Triticum aestivum* L.) Varieties during Two Successive Years

Varieties	Mg,[a] %		Ca,[a] ppm		Zn,[a] ppm		Fe,[a] ppm	
	1972	1973	1972	1973	1972	1973	1972	1973
Akowa	0.14c	0.14e	735ab	610a	53abc	34bcd	62abc	65a
Ciano	0.16bc	0.29b	815ab	515a	52abc	73a	87a	57a
Deihim	0.13c	0.40a	685b	705a	39c	35bcd	58abc	62a
Derakhshan	0.27a	0.15de	775ab	515a	38c	26cd	83a	40a
Inia	0.14c	0.26bc	630b	315a	47bc	53b	40bc	44a
Jolgeh	0.13c	0.14e	620b	535a	53abc	42bc	44bc	39a
Koohrang	0.21b	0.18de	835ab	560a	70a	33bcd	48bc	48a
Navid	0.27a	0.13e	815ab	515a	46bc	31cd	67ab	56a
Ommid	0.12c	0.13e	1065a	410a	50abc	22cd	63abc	34a
Penjamo	0.14c	0.14e	690ab	530a	64ab	23cd	60abc	48a
Roshan	0.11c	0.13e	485b	455a	38c	17d	29c	46a
Tobari	0.14c	0.21cd	500b	545a	63ab	29cd	42bc	38a
Av	0.16	0.19	721	518	51	34	57	48
C.V.,[b] %	14.2	19.8	30.1	25.6	18.8	31.5	36.1	27.8

[a] Means in each column followed by the same letter are not significantly different at the 1% probability level (Duncan's test). [b] Coefficient of variability.

in 1973, were significantly higher than those harvested in 1972.

Data on P are shown in Table I. The ranges of average values for wheat varieties harvested in 1972 and 1973 were, respectively, as follows: for total P, 348–449 and 190–339 mg/100 g; for phytate P, 261–318 and 109–254 mg/100 g; for nonphytate P, 67–140 and 58–129 mg/100 g; and for phytate P as percent of total P, 67–83 and 55–78%.

Data on other minerals are presented in Table II. The ranges of average values for wheat varieties harvested in 1972 and 1973 were, respectively, as follows: for Mg, 0.11–0.27 and 0.13–0.40%; for Ca, 480–1060 and 320–700 ppm; for Zn, 38–70 and 17–73 ppm; and for Fe, 29–87 and 34–65 ppm.

Data on protein and yield are shown in Table III. The ranges of average values for wheat varieties harvested in 1972 and 1973 were, respectively, as follows: for protein, 8.53–13.60 and 8.55–11.67%; and for yield, 1863–3077 and 1261–2287 kg/ha.

There were significant varietal differences for all the variables except for phytate P in 1972, for Ca and Fe in 1973, and for yield in both years (Tables I, II, and III). The wheat varieties with significantly high values for the variables in both years were as follows: for total P, Ciano and Jolgeh; for nonphytate P, Ciano and Akowa; and for protein, Inia and Tobari. The wheat varieties with significantly low values for the variables in both years were as follows: for total P, Koohrang, Navid, and Derakhshan; for nonphytate P, Derakhshan; for phytate P as percent of total P, Akowa; for Mg, Jolgeh; for Zn, Roshan and Derakhshan; and for protein, Navid, Deihim, Ommid,

Table III. Yields and Concentrations of Protein in Grains of Wheat (*Triticum aestivum* L.) Varieties during Two Successive Years

Varieties	Yield,[a] kg/ha		Protein,[a] %	
	1972	1973	1972	1973
Akowa	2350a	1291a	13.60a	10.36abc
Ciano	1863a	1495a	12.45a	10.03abc
Deihim	2552a	1630a	10.00c	9.05bc
Derakhshan	2066a	2287a	10.46bc	9.20bc
Inia	2862a	1261a	12.21ab	11.67a
Jolgeh	2557a	1979a	10.22c	10.03abc
Koohrang	2722a	2287a	9.91c	9.33bc
Navid	1987a	1953a	9.25c	9.07bc
Ommid	2279a	2001a	8.53c	8.55c
Penjamo	2700a	1693a	9.83c	10.84ab
Roshan	2425a	1849a	8.97c	9.39bc
Tobari	3077a	1406a	13.27a	11.16ab
Av	2453	1761	10.73	9.89
C.V.,[b] %	26.4	19.4	10.7	9.8

[a] Means in each column followed by the same letter are not significantly different at the 1% probability level (Duncan's test). [b] Coefficient of variability.

Table IV. Correlation Coefficients among Concentrations of Total P (TP), Phytate P (PP), Nonphytate P (NP), Phytate P as Percent of Total P (PP % TP), Mg, Ca, Zn, Fe, Protein, and Yield of Wheat (*Triticum aestivum* L.) Grains[a]

	TP	PP	NP	PP % TP	Mg	Ca	Zn	Fe	Protein
PP	0.93a								
NP	0.58a	0.24							
PP % TP	0.35a	0.65a	−0.55a						
Mg	0.03	0.07	−0.10	0.12					
Ca	0.42a	0.37a	0.29b	0.08	0.07				
Zn	0.55a	0.53a	0.29b	0.21	0.15	0.22			
Fe	0.28c	0.19	0.26c	−0.05	0.22	0.66a	0.17		
Protein	0.36a	0.36a	0.14	0.23	−0.05	−0.07	0.29c	−0.01	
Yield	0.12	0.14	−0.01	0.09	−0.27c	0.09	0.21	−0.19	0.21

[a] Letters a, b, and c following numbers indicate statistically significant at $P < 0.001$, $P < 0.005$, and $P < 0.01$ level, respectively.

Derakhshan, and Koohrang.

Data on correlation coefficients of the variables are shown in Table IV. There was a highly significant positive correlation between the following characteristics: between total P and phytate P, nonphytate P, phytate P as percent of total P, Ca, Zn, Fe, and protein; between phytate P and total P, phytate P as percent of total P, Ca, Zn, and protein; between nonphytate P and total P, Ca, Zn, and Fe; between phytate P as percent of total P and total P and phytate P; between Ca and total P, phytate P, nonphytate P, and Fe; between Zn and total P, phytate P, nonphytate P, and protein; between Fe and total P, nonphytate P, and Ca; between protein and total P, phytate P, and Zn. There was a highly significant negative correlation between nonphytate P and phytate P as percent of total P and between Mg and yield.

DISCUSSION

The present data clearly indicate that environmental factors could exert significant changes on concentration of most the variables under study. Higher concentrations of total P, phytate P, and nonphytate P in wheat grains harvested in 1972 might thus be due to better environmental factors causing higher availability of P to the grain in that year. This conclusion is further confirmed by a highly significant correlation of total P with both phytate P ($r = 0.93$) and nonphytate P ($r = 0.58$).

Simple regression equations were calculated to fully understand the relationships between the total P and the phytate P. They were $Y = 139.18 + 0.88X$ and $Y = 85.54 + 0.98X$ in 1972 and 1973, respectively. In these equations, Y is the concentration of total P in mg/100 g and X is the concentration of phytate P in mg/100 g. The regression lines obtained for such a relationship in individual years are shown in Figure 1. It can be noted that regression coefficients close to unity could be obtained between the two characters in each year. The t test performed showed no significant difference between the two values of the regression coefficients of each year. The closeness of the regression lines shown in Figure 1 clearly demonstrates that in spite of great differences between the years in both the total P and the phytate P, the relationship between these two characters is similar and does not seem to be affected by seasonal differences. Since phytate P determinations are rather complicated and time consuming, the implications of the linear relationships found in this study can be quite important. In other words, they can be used to estimate the phytate P from total P in wheat. Lolas and Markakis (1975) have similarly obtained a linear relationship between the two characters in beans.

The significant yearly variations observed in yield, phytate, and concentrations of minerals in the wheat varieties might be similarly due to the better environmental conditions and better availability of the minerals to the grain in the 1972 growing season.

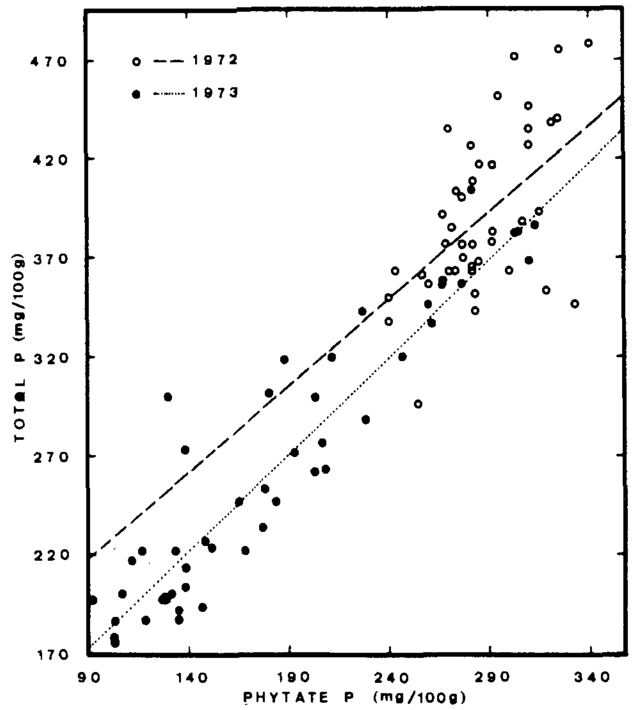

Figure 1. Regression lines for the relationship between concentrations of total P and phytate P in wheat (*Triticum aestivum* L.) grains in 2 years.

The high correlation found between phytate P and Ca ($r = 0.37$) was in agreement with the value reported earlier for mature wheat heads (Nahapetian and Bassiri, 1975). In contrast, the significant relationship reported between phytate P and Mg in wheat heads (Nahapetian and Bassiri, 1975) was not observed in the wheat grains used in the present study.

The positive relationship between phytate P and protein was highly significant ($r = 0.36$). However, Lolas and Markakis (1975) could not find a significant correlation between the two variables in beans.

A survey of wheat growing conditions made by Bassiri (1974) on 112 villages of the Fars region revealed that 65.0% of the land under wheat cultivation was planted with Roshan. Ommid was found to be the second most common wheat variety grown in this area (19.2% of wheat fields). Data in the present study suggest that Roshan, although a suitable variety for the region, can be replaced by other varieties with as well or better performance. The protein contents of almost all Iranian varieties used in this study were lower than Inia and Tobari. Grain yields of all varieties were statistically equal in both years. This is not surprising since the varieties used in this experiment were actually selected as high yielders from several variety trials each conducted for several years in the area (Bassiri, 1974). In general there were great varietal differences with respect to the concentrations of the minerals (Mg, Ca, Zn, and Fe) determined in this study. It should be pointed out, however, that the choice of agricultural practices might prove to be much more effective in decreasing the phytate content of the grain than the choice of variety. There was no significant difference in phytate P concentrations among the varieties in 1972, when average phytate P concentration was high, most probably due to higher availability of P to the grains. In fact average phytate P concentration of the wheat varieties in 1972 (284 mg/100 g) was about one and a half times that in 1973 (177 mg/100 g).

Reinhold (1971) has attributed the difference in phytate concentrations of Iranian breads in Fars area mainly to the method of baking. The present study indicates that the differences might also be attributed to the environmental conditions under which wheat is grown. Thus, it can be expected to find significant yearly variations of phytate concentration in the village breads.

LITERATURE CITED

Abernethy, R. H., Paulsen, G. M., Ellis, R., Jr., *J. Agric. Food Chem.* **21**, 282 (1973).
Asada, K., Tanaka, K., Kasai, Z., *Plant Cell Physiol.* **9**, 185 (1968).
Association of Official Analytical Chemists, "Official Methods of Analysis", Washington, D.C., 1970.
Averill, H. P., King, C. G., *J. Am. Chem. Soc.* **48**, 724 (1926).
Bassiri, A., Research Bulletin No. 2 of the Agricultural Research Center of the College of Agriculture, Pahlavi University, 1974, in Persian.
Berlyne, G. M., BenAri, J., Nord, E., Shainkin, R., *Am. J. Clin. Nutr.* **26**, 910 (1973).
Booth, R. G., Carter, R. H., Jones, C. R., Moran, T., *J. Soc. Chem. Ind. (London)* **60**, 903 (1941).
Cullumbine, H., Basnayake, V., Lemottee, J., Wickamanayake, T. W., *Br. J. Nutr.* **4**, 101 (1950).
Duncan, D. B., *Biometrics* **11**, 1 (1955).
Haghshenass, M., Mahloudji, M., Reinhold, J. G., Mohammadi, N., *Am. J. Clin. Nutr.* **25**, 1143 (1972).
Hall, J. R., Hodges, T. K., *Plant Physiol.* **41**, 1459 (1966).
Halsted, J. A., *Am. J. Clin. Nutr.* **21**, 1384 (1968).
Halsted, J. A., Ronaghy, H. A., Abadi, P., Haghshenass, M., Amirhakimi, G. H., Barakat, R. M., Reinhold, J. G., *Am. J. Med.* **53**, 277 (1972).
Harison, D. C., Mellanby, E., *Biochem. J.* **33**, 1660 (1939).
Hoff-Jorgensen, E., Andersen, U., Nielsen, G., *Biochem. J.* **40**, 555 (1946).
Knowles, F., Watkins, J. E., *J. Agric. Sci.* **22**, 755 (1932).
Krebs, H. A., Mellanby, K., *Biochem. J.* **37**, 466 (1943).
Likuski, H. J. A., Forbes, R. M., *J. Nutr.* **85**, 230 (1965).
Lolas, G. M., Markakis, P., *J. Agric. Food Chem.* **23**, 13 (1975).
McCance, R. A., Widdowson, E. M., *J. Physiol.* **101**, 304 (1942).
Nahapetian, A., Bassiri, A., *J. Agric. Food Chem.* **23**, 1179 (1975).
Nelson, T. S., Ferrara, L. W., Storer, N. L., *Poultry Sci.* **47**, 1372 (1968).
O'Dell, B. L., *Am. J. Clin. Nutr.* **22**, 1315 (1969).
O'Dell, B. L., deBoland, A. R., Koirtyohann, S. R., *J. Agric. Food Chem.* **20**, 718 (1972).
O'Dell, B. L., Savage, J. E., *Proc. Soc. Exp. Biol. Med.* **103**, 304 (1960).
Prasad, A. S., Halsted, J. A., Nadimi, M., *Am. J. Med.* **31**, 532 (1961).
Prasad, A. S., Miale, A., Farid, Z., Sandstead, H. H., Schulert, A. R., Darby, W. J., *Arch. Int. Med.* **111**, 407 (1963).
Reinhold, J. G., *Am. J. Clin. Nutr.* **24**, 1204 (1971).
Reinhold, J. G., *Ecol. Food Nutr.* **1**, 187 (1972).
Reinhold, J. G., *Clin. Chem.* **21**, 476 (1975a).
Reinhold, J. G., *Iran. J. Agric. Res.* **3**, 1 (1975b).
Reinhold, J. G., Faradji, B., Abadi, P., Ismail-Beigi, F., "Trace Elements and Human Disease", Prasad, A. S., Ed., Academic Press, New York, N.Y., in press.
Reinhold, J. G., Hedayati, H., Lahimgarzadeh, A., Nasr, K., *Ecol. Food Nutr.* **2**, 157 (1973a).
Reinhold, J. G., Nasr, K., Lahimgarzadeh, A., Hedayati, H., *Lancet* **1**, 283 (1973b).
Roberts, A. H., Yudkin, J., *Nature (London)* **185**, 823 (1960).
Ronaghy, H. A., *Pahlavi Med. J.* **1**, 29 (1970).
Ronaghy, H. A., Caughey, J. E., Halsted, J. A., *Am. J. Clin. Nutr.* **21**, 488 (1968).
Sandegren, E., *J. Inst. Brew.*, 200 (1948).
Sharpe, L. M., Peacock, W. C., Cooke, R., Harris, R. S., *J. Nutr.* **41**, 433 (1950).
Steel, R. G. D., Torrie, J. H., "Principles and Procedures of Statistics", McGraw-Hill, New York, N.Y., 1960.
Williams, S. G., *Plant Physiol.* **45**, 376 (1970).

Received for review January 5, 1976. Accepted May 12, 1976. This study was supported in part by grants from Pahlavi University Research Council (51-AG-25) and the College of Agriculture Research Center (ARC-43-52).

Atomic Absorption Spectrometric Determination of Eight Trace Metals in Orange Juice following Hydrolytic Preparation

James A. McHard,* James D. Winefordner, and Sik-Vung Ting

The further application of acid hydrolysis as a preparative procedure for orange juice is described. The hydrolysate is a useful matrix for the flame atomic absorption determination of eight metals, Ca, Cu, Fe, K, Mg, Mn, Na, and Zn. Precision studies are described, and the contents of these eight elements in eight different orange juice samples from Florida are given.

A recent publication by the authors (McHard et al., 1976) describes a hydrolysis procedure for the preparation of orange juice for the atomic absorption spectrometric determination of calcium. It was predicted that the procedure would be useful for the determination of several other elements as well. This prediction has been verified by the work presented in this paper.

Of the principal elements found in the analysis of plants, only eight—calcium, copper, iron, magnesium, manganese, potassium, sodium, and zinc—are readily determinable at the levels encountered in orange juice by common flame atomic absorption or flame emission methods. A recent

Department of Chemistry, University of Florida, Gainesville, Florida 32611 (J.A.M., J.D.W.), and the Research and Educational Center, Lake Alfred, Florida 33850 (S.V.T.).