Nitrate Content of Seeds of Certain Crop Plants, Vegetables, and Weeds

Ann S. McNamara,[1] Lowell A. Klepper,[2] and Richard H. Hageman*

The nitrate content of 113 seed samples of 37 different plant speceis was determined on the water extract of dry ground material by two nitrate assay procedures. The method that utilizes an enzymatic reduction of nitrate to nitrite was found to be superior to the chemical reduction procedure. Nitrite was determined by the standard diazotization procedure for both methods. A third method (chromotropic acid) was tested on several samples and proved unsuitable for assay of nitrate of untreated water extracts of seeds. Nitrate content in wheat and oat seeds ranged from nondetectable to a maximum of 1.9 μg NO_3—N/g dry wt. The nitrate content of corn grain ranged from 1.1 to 3.8 μg of NO_3—N/g of dry wt. Nitrate was not detectable in the one sample of rice tested. Soybean seeds had higher levels of nitrate (8.6 to 22.9 μg of NO_3—N/g of dry wt) than the seeds of the cultivated crop plants tested. As a group, weed seeds had consistently higher nitrate content than seeds from other plant species.

Although the literature is quite limited (Wright and Davison, 1964), most reports indicate that the nitrate content of seeds and grains is negligible or nil. Because of the limited amount of information of nitrate content of seeds, the concern over nitrate as a toxic component of foods and feeds, and the fear that the enhanced use of nitrogenous fertilizers could have increased the nitrate content of seeds used for foods and feeds, it was considered desirable to assay a collection of seeds and grains of several plant species for nitrate content. Nitrate assays were made on the seeds by three different nitrate assay procedures to demonstrate the superiority of the rapid, sensitive, and specific procedure developed by Lowe and Hamilton (1967).

EXPERIMENTAL

Plant Material. Oats, wheat, soybean, and weed seeds were obtained from various sections within the department. Corn and sorghum seeds were supplied by Pioneer Hybrid Corn, Co., Champaign, Ill. The remaining material was purchased from local grocery and seed stores. The limited information available as to locale or conditions used in production of the various seeds is given with the data.

The seeds were ground in an intermediate Wiley mill (60-mesh screen) or a Spex mill (Spex Industries, Scotch Plains, N.J.). The ground material was redried (100° C for 24 hr) and stored in sealed bottles in desiccators for assay. The nitrate was extracted by mixing 1 g of dried ground material with 10 ml of deionized water. The slurry was agitated at intervals for 180 min prior to placing in a water bath at 45° C for 1 hr. The material was then centrifuged (15 min at 10,000 *g*) and the supernate, after decanting through glass wool, was used for assay.

Nitrate Assay Procedures. The enzymatic reduction procedure (Lowe and Hamilton, 1967) was used as described. The dissimilatory enzyme used to reduce nitrate quantitatively to nitrite can also be obtained from *E. coli* (McNamara *et al.*, 1971).

METHODS

The powder method was a modification of the procedure developed by Woolley *et al.* (1960). Since the modifications were drastic, the changes are detailed.

Mix intimately by grinding in a mortar and pestle 5 g of sulfanilic acid [sulfanilic acid (J. T. Baker Chem. Co., or recrystallize from hot water with ethanol to remove Cu^{2+})], 0.2 g of zinc dust [zinc dust (99% pure) Mallinckrodt Chem. Co.], and 0.8 g of 1-naphthylamine. The 6 g of mixture is then added to 45 g of sulfanilic acid and mixed thoroughly by tumbling in a brown wide-mouthed bottle. The powder is not stable and should be mixed just prior to use.

For assay, the samples or standards (0.5 to 50 μg of NO_3—N ml^{-1}) are pipetted into test tubes and the volume adjusted to 1 ml with deionized water. To each tube add 9 ml of acetic acid-copper solution [acetic acid (20% v/v) that also contains 50 ppm Cu^{2+} added as $CuSO_4$]. The samples are then placed in an ice bath until chilled to 0–3° C. The 0.5 g of powder mixture is then added to each tube. A small scoop of plastic tubing can be made that will accurately deliver the required amount of powder. After adding the powder, each tube is gently inverted once. The inversion is repeated three times at 2–3 minute intervals. After the final inversion the samples are allowed to incubate for 20 min at 0–3° C. Aliquots of the clear supernate are removed, placed in clean tubes, allowed to warm to room temperature, and absorbance determined at 520 mμ. Precautions: Excess of Cu^{2+}, Pb^{2+}, or high levels of nucleotides, inexact timing of incubation, or vigorous agitation during incubation are sources of error.

Department of Agronomy, University of Illinois, Urbana, Ill. 61801

[1] Present address: Pathology Department, Northwestern Medical School, Chicago Campus, Ill. 60611

[2] Present address: International Center for Improvement of Corn and Wheat, Londres 40, Mexico 6, D.F.

Table I. Nitrate Content of Seeds of Several Plant Species

Plant Species (with location and treatment when known)	Assay Method: Enzymic — Varieties No.	Enzymic — NO_3^-—N μg/g dry wt[a]	Enzymic — Recovery %	Powder — NO_3^-—N μg/g dry wt[a]	Powder — Recovery %
Barley (*Hordeum vulgare*)	1	4.6	101	5.6	88
Corn (*Zea mays*)	11	2.6 (1.1–3.5)	98 ± 5	5.1 (0.5–18.2)	69 ± 34
Cotton (*Gossypium hirsutum*)	1	59.6	94	303.2	99
Oats (*Avena sativa*)					
DeKalb, Ill.	10	0.03 (ND[b]–0.3)		ND	64 ± 6
Urbana, Ill.	10	0.8	100 ± 3	ND	76 ± 7
Peanuts (*Arachis hypogaea*) Athens, Ga.	2	0.7 (ND–1.4)	97 ± 6	ND	83 ± 4
Rice (*Oryza sativa*)	1	ND	101	7.4	112
Sorghum (*Sorghum vulgare*)	1	ND	97	ND	60
Soybeans (*Glycine max*)					
Illinois	4	16.5 (11.9–22.1)	95 ± 4	220.0 (139.3–301.2)	113 ± 22
Indiana	3	7.2 (8.6–23.8)	98 ± 2	7.2 (4.3–9.1)	99 ± 14
Iowa	1	15.6	99	219.5	155
Minnesota	2	19.0 (15.1–22.9)	97 ± 6	16.1 (4.3–27.9)	111 ± 6
Sunflower (*Helianthus annus*)	1	3.6	99	6.6	76
Sweet clover (*Melilotus* spp.)	1	9.2	90	36.2	134
Wheat (*Triticum aestivum*)					
DeKalb, Ill.	10	0.08 (ND–0.8)	97 ± 5	0.09 (ND–1.0)	114 ± 15
Urbana, Ill.	10	0.11 (ND–0.4)	100 ± 4	ND	112 ± 15
Atlas X Commanche Urbana, Ill.					
A-Control (no nitrogen)		1.4	...	...	...
B-Control (no nitrogen)		2.5	...	...	...
A 90 lb N/A applied April 29		1.2	...	...	...
B 90 lb N/A applied April 29		1.2	...	...	...
A 90 lb N/A applied May 13		1.0	...	...	...
B 90 lb N/A applied May 13		1.4	...	...	...
A 90 lb N/A applied May 27		0.8	...	...	...
B 90 lb N/A applied May 27		1.2	...	...	...
Vegetable Crops					
Beans (*Phaseolus vulgaris*)					
Great Northern (white)		3.4	91	11.9	64
Yellow wax		11.3	96	58.5	75
Green stringless		3.8	91	55.9	103
Beans (*Phaseolus lunatis*)		1.3	70[a]	...	...
Beets (*Beta vulgaris*)					
Burpees Red Ball (with hulls)		346.8	0	...	...
Detint (with hulls)		349.5	0	699.5	0
Detint (without hulls)		81.1	100	42.2	55
Carrot (*Dacus carota*)		9.8	98	55.3	146
Cucumber (*Cucumis sativus*)		16.6	90	14.2	88
Lettuce (*Lactuca sativa*)		15.5	82[a]	104.0	231
Pea (*Pisum sativum*)		2.4	101	14.2	57
Pepper (*Capsicum* spp.)		13.0	100	56.0	86
Pumpkin (*Curcubita pepo*)		19.7	98	25.9	143
Radish (*Raphanus sativus*)					
Cherry Belle		16.6	95	42.2	55
Icicle		10.2	94	19.4	63
Squash (*Cucurbita moschata*)		14.7	101	84.0	79
Tomato (*Lycopersicon esculentum*)		6.9	96	33.1	56
Turnip (*Brassica rapa*)		5.5	96	79.6	47
Watermelon (*Citrullus vulgaris*)		8.5	96	82.5	15
Weeds					
Foxtail (*Setaria faberii*)		8.9	93	6.4	102
(*Setaria lutescens*)		14.1	51[a]	3.8	37
Jimsonweed (*Datura stramonium*)		19.7	80[a]	...	...
Lamb's quarters (*Chenopodium album*)		33.0	101	34.5	65
Milkweed (*Ampelamus albidus*)		25.8	97	44.4	100
Morningglory (*Ipomoea purpurea*)		35.0	88[a]	48.1	0
(*Convolvulus* spp.)		42.1	90	...	...
Panicum (*Panicum dicotomiflorum*)		15.6	96	13.9	80
Pigweed (*Amaranthus retroflex*)		21.1	87[a]	...	...
Smartweed (*Polygonum pensylvanicum*)		122.3	82	112.9	124
Spina sida (*Sida spinosa*)		46.9	86[a]	...	...
Ragweed (*Ambrosia artemisiifolia*)		25.1	93	117.7	52
(*Ambrosia trifida*)		13.6	94	64.1	155
Velvetleaf (*Abutilon theophrasti*)		98.7	13[a]	...	...
Wild cucumber (*Echinocystis lobata*)		78.0	98	278.6	172
Miscellaneous					
Apple (*Malus domestica*)		6.4	16	82.4	68
Castor bean (*Ricinus communis*)		7.6	97	18.3	131

[a] Average and range. [b] ND = none detected.

The chromotropic acid method was used as described by West and Ramachandran (1966).

The size of sample aliquots of ground seed extracts used for the enzymic, powder, and chromotropic acid methods were 0.2, 1.0, and 1.4 ml, respectively. Each sample was assayed in duplicate by each procedure and repeated if agreement between duplicates was not reasonable. For all methods a final assay volume of 10.0 ml was used. Recovery of added nitrate was done by adding 1.25, 10.0, and 6.78 μg of NO_3—N to the sample (assay) aliquots for the enzymic, powder, and chromotropic acid methods, respectively.

All three procedures gave reproducible, straight line plots with standard nitrate solutions.

RESULTS AND DISCUSSION

The results obtained with the enzymic and powder methods are given in Table I. Since the chromotropic acid procedure gave excessively high values (200 to 500 μg of NO_3—N/g of dry wt) in initial trials (for wheat, corn, milkweed, squash, apple) and low recoveries of the added nitrate (0 to 85%), the results of these assays are not presented. It was concluded that the chromotropic acid assay was not suited to assay of nitrate of untreated water extracts of seeds.

Based upon recovery of nitrate added to the sample extracts and greater variation in nitrate content within a species (Table I) (especially soybeans and corn) it is concluded that the enzymic assay is the more reliable assay. The greater variation in assay values within a species and in recoveries of added nitrate with the powder method is attributable to the fact that the chemical, catalytic reduction by Zn and Cu^{2+} of nitrate to nitrite are not quantitative (Woolley *et al.*, 1960). Slight changes in concentrations of components in the experimental material and experimental techniques alter the degree of reduction to and beyond nitrite in this method. In the enzymatic procedure the conversion of nitrate to nitrite is quantitative, and errors in this method arise from components in the extract that would inhibit the enzyme or interfere with the diazotization reaction. The reason for including the recovery values is to indicate which plant species have inhibitors or components that interfere with the assay. Although the enzymic method is more sensitive, either method could be used on purified extracts, as both methods give excellent results with standard solutions.

The comments made concerning the data (Table I) will be based on the results obtained with the enzymic method. Of the cereals, corn had appreciably higher levels of nitrate than either wheat or oats. However, the level of nitrate in the corn seed was low (maximum of 3.8 μg of NO_3—N/g of dry wt).

With respect to data from wheat and oats, there is no apparent reason why the seed produced at DeKalb should have a lower level of nitrate than that produced at Urbana, Ill. Seed age, management, and harvest practices were comparable. Based on the data obtained with the Atlas X Commanche wheat, it does not appear that late spring supplemental nitrogen causes increases in nitrate in the grain.

Of the major crop plants, soybean seeds contained the highest level of nitrate. Values ranged from 8.6 to 22.9 μg of NO_3—N/g of dry wt for the various genotypes. In the production of soybeans, initial and supplemental nitrogen fertilizer applications are usually minimal. Thus it was anticipated that the nitrate content of soybean seeds would have been lower than for the cereal grains. Since this was not the case, the data raise the question: Are there factors other than species differences related to the accumulation of nitrate in seeds?

As a group, the seeds of weed plants had the highest average nitrate content. One is tempted to speculate that the high level of nitrate found in the weed seeds is in some way related to dormancy, germination, and/or survival. However, much more work is needed to establish such a relationship or to show that this higher seed nitrate arose by natural selection.

LITERATURE CITED

Lowe, R. H., Hamilton, T. L., J. AGR. FOOD CHEM. **15,** 359 (1967).
McNamara, A. S., Meeker, G., Shaw, D. P., Hageman, R. H., J. AGR. FOOD CHEM. **19,** 229 (1971).
West, P. W., Ramachandran, T. R., *Anal. Chim. Acta* **35,** 317 (1966).
Woolley, J. T., Hicks, G. P., Hageman, R. H., J. AGR. FOOD CHEM. **8,** 481 (1960).
Wright, M. J., Davison, K. L., *Advan. Agron.* **16,** 197 (1964).

Received for review August 24, 1970. Accepted January 7, 1971. Supported in part by USDA Cooperative Agreement 12-14-100-8455 and Hatch Funds.