No. 7500063

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

Iowa Agriculture and Home Economics Experiment Station

Whereas, THERE HAS BEEN PRESENTED TO THE

Secretary of Agriculture

AN APPLICATION REQUESTING A CERTIFICATE OF PROTECTION FOR AN ALLEGED NOVEL VARIETY OF SEXUALLY REPRODUCED PLANT, THE NAME AND DESCRIPTION OF WHICH ARE CONTAINED IN THE APPLICATION AND EXHIBITS, A COPY OF WHICH IS HEREUNTO ANNEXED AND MADE A PART HEREOF, AND THE VARIOUS REQUIREMENTS OF LAW IN SUCH CASES MADE AND PROVIDED HAVE BEEN COMPLIED WITH, AND THE TITLE THERETO IS, FROM THE RECORDS OF THE PLANT VARIETY PROTECTION OFFICE, IN THE APPLICANT(S) INDICATED IN THE SAID COPY, AND WHEREAS, UPON DUE EXAMINATION MADE, THE SAID APPLICANT(S) IS (ARE) ADJUDGED TO BE ENTITLED TO A CERTIFICATE OF PLANT VARIETY PROTECTION UNDER THE LAW.

NOW, THEREFORE, THIS CERTIFICATE OF PLANT VARIETY PROTECTION IS TO GRANT UNTO THE SAID APPLICANT(S) AND THE SUCCESSORS, HEIRS OR ASSIGNS OF THE SAID APPLICANT(S) FOR THE TERM OF seventeen YEARS FROM THE DATE OF THIS GRANT, SUBJECT TO THE PAYMENT OF THE REQUIRED FEES AND PERIODIC REPLENISHMENT OF VIABLE BASIC SEED OF THE VARIETY IN A PUBLIC REPOSITORY AS PROVIDED BY LAW, THE RIGHT TO EXCLUDE OTHERS FROM SELLING THE VARIETY, OR OFFERING IT FOR SALE, OR REPRODUCING IT, OR IMPORTING IT, OR EXPORTING IT, OR USING IT IN PRODUCING A HYBRID OR DIFFERENT VARIETY THEREFROM, TO THE EXTENT PROVIDED BY THE PLANT VARIETY PROTECTION ACT. THE UNITED STATES SEED OF THIS VARIETY (1) SHALL BE SOLD BY VARIETY NAME ONLY AS PART OF CERTIFIED SEED AND (2) SHALL CONFORM TO THE NUMBER OF GENERATIONS [illegible] BY THE OWNER OF THE RIGHTS. (84 STAT. 1542, AS AMENDED, 7 U.S.C. 2321 ET SEQ.)

REED CANARYGRASS

'Vantage'

In Testimony Whereof, *I have hereunto set my hand and caused the seal of the* Plant Variety Protection Office *to be affixed at the City of* Washington *this* 18th *day of* March *in the year of our Lord one thousand nine hundred and* seventy-seven

Attest:

Commissioner
Plant Variety Protection Office
Grain Division
Agricultural Marketing Service

Secretary of Agriculture

FORM GR-470
(12-15-72)

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL MARKETING SERVICE
GRAIN DIVISION
HYATTSVILLE, MARYLAND 20782

FORM APPROVED
OMB NO. 40-R3712

APPLICATION FOR PLANT VARIETY PROTECTION CERTIFICATE

INSTRUCTIONS: *See Reverse.*

1. VARIETY NAME OR TEMPORARY DESIGNATION Vantage	2. KIND NAME Reed canarygrass	FOR OFFICIAL USE ONLY PV NUMBER 7500063
3. GENUS AND SPECIES NAME Phalaris arundinacea	4. FAMILY NAME *(Botanical)* Gramineae	FILING DATE 3.4.75 / TIME 2:00 P.M.
	5. DATE OF DETERMINATION June 23, 1972	FEE RECEIVED $ 250.00 / $ 250.00 / $ 250.00 / BALANCE DUE $ — / $ — / $ —
6. NAME OF APPLICANT(S) Iowa Agriculture and Home Economics Experiment Station	7. ADDRESS *(Street and No. or R.F.D. No., City, State, and ZIP Code)* R24 Curtiss Hall Iowa State University Ames, IA 50010	8. TELEPHONE AREA CODE AND NUMBER (515) 294-1736
9. IF THE NAMED APPLICANT IS NOT A PERSON, FORM OF ORGANIZATION: *(Corporation, partnership, association, etc.)* An agency of the State of Iowa	10. STATE OF INCORPORATION	11. DATE OF INCORPORATION

12. Name and mailing address of applicant representative(s), if any, to serve in this application and receive all papers:

Ralph Bean
Room 24, Curtiss Hall
Iowa State University
Ames, IA 50010

13. CHECK BOX BELOW FOR EACH ATTACHMENT SUBMITTED:

[X] 13A. Exhibit A, Origin and Breeding History of the Variety *(See Section 52 of the Plant Variety Protection Act.)*

[X] 13B. Exhibit B, Botanical Description of the Variety

[X] 13C. Exhibit C, Objective Description of the Variety

[X] 13D. Exhibit D, Data Indicative of Novelty

[X] 13E. Exhibit E, Statement of the Basis of Applicant's Ownership

14A. Does the applicant(s) specify that seed of this variety be sold by variety name only as a class of certified seed? *(See Section 83(a). (If "Yes," answer 14B and 14C below.)* [X] YES [ ] NO

14B. Does the applicant(s) specify that this variety be limited as to number of generations? [X] YES [ ] NO

14C. If "Yes," to 14B, how many generations of production beyond breeder seed? [X] FOUNDATION [ ] REGISTERED [X] CERTIFIED

The applicant declares that a viable sample of basic seed of this variety will be deposited upon request before issuance of a certificate and will be replenished periodically in accordance with such regulations as may be applicable.

The undersigned applicant(s) of this sexually-reproduced novel plant variety believes that the variety is distinct, uniform, and stable as required in Section 41 and is entitled to protection under the provisions of Section 42 of the Plant Variety Protection Act.

Applicant is informed that false representation herein can jeopardize protection and result in penalties.

Nov. 10, 1976 (DATE) — Ralph Bean (SIGNATURE OF APPLICANT)

(DATE) — (SIGNATURE OF APPLICANT)

00001

INSTRUCTIONS

GENERAL: Send an original copy of the application, exhibits and $250.00 fee to U.S. Dept. of Agriculture, Agricultural Marketing Service, Grain Division, 6525 Belcrest Road, Hyattsville, Maryland 20782. (See Section 180.175 of the regulations and rules of practice.) Retain one copy for your files. All items on the face of the form are self-explanatory unless noted below.

ITEM

5 Insert the date the applicant determined that he had a new variety based on the definition in Section 41 (a) of the Act and decision is made to increase the seed.

13a First, give the genealogy, including public and commercial varieties, lines, or clones used, and the breeding method. Second, give the details of subsequent stages of selection and multiplication. Third, indicate the type and frequency of variants during reproduction and multiplication and state how these variants may be identified. Fourth, provide evidence on stability.

13b First, give any special characteristics of the seed and of the plant as it passes through the seedling stage, flowering stage and the fruiting stage. Second, describe the mature plant and compare it with a similar commercial variety grown under the same conditions, and indicate the differences.

13c A supplemental form will be furnished by the PVPO to describe in detail a variety for each kind of seed.

13d Provide complete data indicative of novelty. Seed and plant specimens or photographs of seed and plant comparisons clearly indicating novelty may be submitted. Seeds submitted may be sterile.

13e Indicate whether applicant is the actual breeder, the employer of the breeder, the owner through purchase or inheritance, etc.

Exhibit A

Origin and Breeding History of the Variety

1. 'Vantage' is a synthetic variety developed from six clones that originated from seed collected in Iowa and southern Minnesota. The parent clones were selected on the basis of individual plant performance, clonal evaluation, and topcross progeny performance.

2. Selection was mainly for high seed yield and seed retention with some attention given to other traits including maturity, forage yield, disease reaction, and palatability. Steps in the development of Vantage were as follows:

   (a) Collection of germplasm in 1954.

   (b) Evaluation of over 6,000 plants in a space-planted source nursery during 1955-57.

   (c) Selection of 240 plants for seed retention, winter hardiness, vigor, leafiness, disease resistance, and heading ability.

   (d) Clonal evaluation of the 240 selections in a topcross nursery during 1958-59.

   (e) Selection of 53 plants, first for seed retention and then for superior performance for other agronomic traits.

   (f) Progeny testing of 53 plants for forage and seed traits during 1960-64.

   (g) Selection of 6 parent clones mainly for high seed yield and seed retention for incorporation into Vantage. Maturity, forage yield, disease reaction, and palatability were considered also in selection of parent clones.

   (h) Production of Syn. 1 seed in 1966.

   (i) Production of Syn. 2 seed in 1968.

(j) Testing in comparison with other varieties, 1969-74.

Syn. 1 (breeder) seed is produced by the Iowa Agricultural Experiment Station in isolated crossing blocks in which the six parent clones are allowed to interpollinate naturally. The parent clones are replicated and randomized to promote random crossing. Equal quantities of seed are composited from the six parent clones to form Syn. 1 seed. Syn. 2 (foundation) seed is produced by the Committee for Agricultural Development in isolated plantings established by seeding Syn. 1 seed in 40-inch rows. The certified class of seed will be the second advanced generation from breeder seed and will not be eligible for producing any class of certified seed.

3. No unusual variants have been noticed in Vantage.

4. The Syn. 1 and Syn. 2 generations have performed similarly (see attached data indicative of novelty).

Exhibit B

Botanical Description of the Variety

'Vantage' averaged 3 days earlier in panicle emergence than Rise at Ames, Iowa in 1970-72. It was ready to harvest for seed a day or two before Rise. Vantage is an excellent seed producer and it does not shatter its seed as readily as Rise and Ioreed. The seeds of Vantage are heavier than those of Rise and Ioreed.

No botanical characteristics atypical of *Phalaris arundinacea* have been noticed in Vantage. Similar to other cultivars of reed canarygrass, Vantage is a rhizomatous, tall-growing perennial.

Exhibit C - 'Vantage' reed canarygrass

All required data concerning the novelty of 'Vantage' reed canarygrass have been presented in Exhibits A, B, and D.

Revised Exhibit D (November 9, 1976)

Data Indicative of Novelty

The attached data are presented to show that Vantage is a novel variety. It has the unique combination of the following characteristics:

1. Early heading
2. Early seed maturity
3. High seed yield
4. Good seed retention
5. High weight per 100 seeds
6. High forage yield
7. The forage contains only the indole alkaloid gramine.

Vantage is differentiated from other varieties as follows:

Contrasting Variety	Distinguishing Characteristics of Vantage
Rise	Contains only gramine; earlier maturity; greater seed retention, seed yield and 100-seed weight.
Ioreed	Much greater seed retention; greater seed yield and 100-seed weight.
Frontier	Contains only gramine; much greater seed retention; greater seed yield.
Grove	Contains only gramine.
Castor	Greater forage yield.

A copy of an article (Crop Science 15:705-707, 1975) on alkaloid content of reed canarygrass cultivars is attached.

Exhibit D

Data Indicative of Novelty

The attached data are presented to show that Vantage is a novel variety. It has the unique combination of the following characteristics:

1. Early heading
2. Early seed maturity
3. High seed yield
4. Good seed retention
5. High weight per 100 seeds
6. High forage yield
7. The forage contains only the indole alkaloid gramine

In tests at the University of Minnesota, Vantage was the only variety that contained only gramine. Three-hundred-eleven of 312 Syn. 1 plants of Vantage contained only gramine. The other Syn. 1 plant contained a tryptamine type of indole alkaloid. That plant was probably an outcross or a contaminant. The parent clones and plants of the Syn. 2 generation of Vantage contained only gramine. All other varieties contained gramine plus tryptamine alkaloids.

Exhibit D, Data Indicative of Novelty (continued)

Table 1. Average performance of Vantage, Rise, and Ioreed reed canarygrass cultivars in Iowa tests.

Trait	Year	Cultivar: Vantage Syn. 1	Vantage Syn. 2	Rise	Ioreed	LSD (.05)[1/]
Days to heading after April 30 at Ames	1970	25.2	25.8	28.5	28.0	0.7
	1971	32.2	32.2	35.0	34.5	0.7
	1972	31.5	31.5	35.0	34.0	1.1
Date in June when seed was ready to harvest at Ames	1970	17.0	17.2	18.5	16.2	0.6
	1971	23.2	23.0	24.2	22.8	0.8
	1972	23.5	23.5	25.5	23.2	0.7
Yield of seed in pounds per acre at Ames, early harvest[2/]	1970	357	334	269	270	68
	1971	556	621	404	481	102
	1972	278	333	162	200	85
Yield of seed in pounds per acre at Ames, late harvest[3/]	1970	116	125	57	21	24
	1971	37	46	19	5	12
	1972	93	118	47	22	25
Late harvest seed yield in percent of early harvest seed yield at Ames	1970	32.5	37.5	21.2	7.7	--
	1971	6.6	7.4	4.8	1.0	--
	1972	33.5	35.6	28.9	10.8	--
Weight per 100 seeds of late-harvested seed from Ames (mg)	1970	90.3	86.4	82.9	74.3	1.4
	1971	79.0	77.0	71.8	66.8	--
	1972	84.8	82.0	80.2	71.2	--

1/Least significant difference at the 0.05 probability level, a dash indicates data were not analyzed statistically, ns indicates differences among cultivars are not significant statistically.

2/Timed to maximize yield of mature seed.

3/Taken in late June or early July to measure seed shattering resistance.

00001

7500063

Table 2. Average performance of Vantage, Rise, Ioreed and Castor reed canarygrass cultivars in Iowa tests

Trait	Year	Cultivar					
		Vantage					
		Syn. 1	Syn. 2	Rise	Ioreed	Castor	LSD (.05)[1/]
Days to heading after April 30 at Beaconsfield	1970	28.0	28.0	30.2	28.0		--
	1971	33.2	33.8	36.5	33.5		--
	1972	35.8	33.2	38.0	35.2		--
Seed yield in pounds per acre at Beaconsfield	1970	272	270	171	153		53
	1971	377	418	345	230		61
	1972	305	370	259	148		73
Forage yield in tons per acre at Ames	1970	5.62	5.72	5.57	5.71		ns
	1971	5.58	5.80	5.26	5.47		0.53
	1972	5.11	5.29	4.91	4.91		0.35
Forage yield in tons per acre at Independence	1970		5.53	5.50	5.75		ns
	1971		4.89	4.75	5.23		0.43
	1972		5.39	5.01	5.36		ns
Forage yield in tons per acre at Beaconsfield	1973		5.47	5.14	5.14	4.67	0.52
	1974		3.93	3.70	3.78	3.65	ns

1/Least significant difference at the 0.05 probability level, a dash indicates data were not analyzed statistically, ns indicates differences among cultivars are not significant statistically.

7500063

Exhibit D, Data Indicative of Novelty (continued)

Table 3. Seed yield of reed canarygrass cultivars at Roseau, Minnesota, 1970-71[1/].

	Seed yield in pounds per acre on 3 harvest dates[2/]					
	Early		Intermediate		Late	
Cultivar	1970	1971	1970	1971	1970	1971
Vantage[3/]	555	384	203	160	128	132
Rise	480	335	171	143	117	94
Frontier	480	362	75	81	21	11

1/Data supplied by L. J. Elling, Department of Agronomy and Plant Genetics, University of Minnesota.

2/Dates of harvest in July:

	1970	1971
Early	10	8
Intermediate	21	14
Late	23	22

3/Syn. 2 generation.

Table 4. Forage yield of reed canarygrass cultivars in Minnesota, Wisconsin and Illinois

	Yield of dry matter in tons per acre						
	Minnesota 1/				Wisconsin 2/		
Cultivar	Rosemount 1973-74	Grand Rapids 1973-74	Morris 1974	Lamberton 1974	Arlington 1972-73	Lancaster 1972-73	Spooner 1972-73
Vantage, Syn. 2	4.99	4.92	2.67	2.05	4.08	5.82	0.88
Rise	5.04	4.66	2.77	2.12	3.90	5.82	1.02
Ioreed		4.62					
Grove	4.60	4.12			3.68	5.66	0.90
Frontier	4.90	4.60	2.78	1.86	4.00	5.96	1.12
Castor			2.64	1.77			

	Illinois 3/			
	DeKalb 1971-73	Urbana 1971-73	Brownstown 1971-72	Carbondale 1972
Vantage, Syn. 2	3.45	3.42	1.56	1.60
Rise	3.72	3.54	1.41	1.44

1/Data supplied by Arne Hovin, Department of Agronomy and Plant Genetics, University of Minnesota.

2/Data from the Department of Agronomy, University of Wisconsin.

3/Data from the Department of Agronomy, University of Illinois.

7500063

Exhibit D, Data Indicative of Novelty (continued)

Table 5. Alkaloid concentration and alkaloid type of parent clones of Vantage sampled in the field at Rosemount and in the greenhouse at St. Paul, Minnesota[1/].

Parent clone	Field samples 1974 #1 % alkaloids[2/]	Field samples 1974 #1 Type[3/]	Field samples 1974 #2 % alkaloids[2/]	Field samples 1974 #2 Type[3/]	Mean	Greenhouse 1974 % alkaloids[2/]	Greenhouse 1974 Type[3/]
6-3	0.10	G	0.09	G	0.095	0.02	G
15-1	0.04	G	0.02	G	0.030	0.01	G
16-7	0.11	G	0.12	G	0.115	0.03	G
37-1	0.14	G	0.08	G	0.110	0.04	G
47-2	0.06	G	0.05	G	0.055	0.02	G
48-5	0.02	G	0.03	G	0.025	0.02	G

1/Data supplied by Arne Hovin, Department of Agronomy and Plant Genetics, University of Minnesota.

2/Alkaloid concentration in percent of dry weight.

3/Alkaloid type: G = gramine

Table 6. Frequency of types of alkaloids and total alkaloid concentration in three reed canarygrass cultivars including the Syn. 1 and Syn. 2 generations of Vantage[1/].

Cultivar	No. of plants	Percentage of plants with alkaloid type[2/]: G	G + D	G + M	D + M	D	M	Total alkaloids in percent of dry weight
Vantage, Syn. 1 components[3/]								
6-3	42	100						
15-1	43	98					2	
16-7	49	100						
37-1	48	100						
47-2	43	100						
48-5	44	100						
Vantage, Syn. 2	47	100						0.17
Grove	49	63		4	23		10	0.15
Rise	47	66		4	19	2	9	0.19

1/Data supplied by Arne Hovin, Department of Agronomy and Plant Genetics, University of Minnesota.

2/G = gramine (3-dimethylaminomethylindole)
D = N,N-dimethyltryptamine
M = 5-methoxy-N, N-dimethyltryptamine

3/Identified by maternal parent of seed harvested from crossing block used for production of Syn. 1 seed

7500063

Table 7. Total indole alkaloids and alkaloid types of 4 cultivars of reed canarygrass, Rosemount, Minnesota[1/]

	Total alkaloids in percent of dry weight					Alkaloid type[2/]			
	1973		1974			1973		1974	
Cultivar	Aug. 4	Aug. 20	July 15	Aug. 28	Mean	May 29	Sept. 4	July 15	Aug. 28
Vantage, Syn. 2	0.25	0.10	0.12	0.12	0.148	G	G	G	G
Rise	0.27	0.14	0.11	0.13	0.162	G + T	G + T	G + T	G + T
Grove	0.18	0.10	0.09	0.10	0.118	G + T	G + T	G + T	G + T
Frontier	0.19	0.09	0.10	0.13	0.128	G + T	G + T	G + T	G + T
LSD (.05)	0.04	0.03	0.03	0.04					

1/Data supplied by Arne Hovin, Department of Agronomy and Plant Genetics, University of Minnesota.

2/G = gramine, T = tryptamine

750006 3

7500063

Distribution of Specific Alkaloids in Reed Canarygrass Cultivars[1]

A. W. Hovin and G. C. Marten[2]

ABSTRACT

Alkaloids in reed canarygrass (*Phalaris arundinacea* L.) are negatively associated with palatability and sometimes have undersirable effects on ruminant animals. Our objectives were to characterize several reed canarygrass cultivars with respect to alkaloid concentration and distribution patterns of specific alkaloids. Because current cultivars are difficult to describe morphologically, chemical characterization may aid in identifying or describing them.

We determined frequency distribution of three primary indole alkaloids: 3-dimethylaminomethylindole (gramine); N,N-dimethyltryptamine (DMT); and 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT), among spaced plants of selected reed canarygrass cultivars and experimental strains grown in the field in 1973. Alkaloid composition and concentration were also determined from solid-stand plots in 1973 and 1974.

'Vantage' was the only cultivar containing exclusively gramine. The presence of gramine was verified using forage from the six parent clones, plants from syn 1 generation and syn 2 generation seed, and from first growth and regrowth of solid-stand plots. 'Frontier,' 'Grove,' 'Rise,' and three experimental strains contained both gramine and tryptamines. The experimental strain MN-72 was differentiated from Grove, Rise, and two experimental strains by frequency distribution of gramine, DMT, and 5-MeO-DMT.

The 47 to 49 plants used for alkaloid typing of each entry were possibly too few to characterize other cultivars and strains. Grove, MN-72, and Frontier had significantly lower total alkaloid concentration (% dry wt) than did RC-2, Vantage, and Rise when determined on 4-week-old second regrowth in 1973 but not on 4-week-old first or second regrowth in 1974. Differences among entries were also smaller and seldom significant when based on 6-week-old first regrowth in 1973. The 2-year mean alkaloid values over all sampling dates were significantly lower for Grove and MN-72 compared to Vantage, Rise, and RC-2. Concentration of total indole alkaloids appears to distinguish some reed canarygrass cultivars, but only at specific conditions of growth.

Additional index words: Indole alkaloids, Forage quality, *Phalaris arundinacea* L.

ALKALOIDS are present in 10 to 15% of all vascular plants, and over 2,000 different alkaloids have been identified (7). According to a recent review (3) at least eight alkaloids occur in reed canarygrass (*Phalaris arundinacea* L.), and the majority of these appears to be indole compounds (gramine and tryptamines) and their β-carboline analogues. The simplest indole alkaloid is gramine, which is sometimes included in the tryptamine classification because it is biosynthetically related to tryptophan (9). Oram (6) surveyed 33 strains of 14 *Phalaris* spp. and found none to be entirely free of tryptamines.

Many cultivars of reed canarygrass cannot be distinguished on the basis of gross morphological characters. Because primary indole alkaloids in reed canarygrass appear to be controlled by very few genes (11; Hovin et al., unpublished), it is desirable to determine if these alkaloids may be used to characterize or describe reed canarygrass cultivars. Also, the relative concentration of total alkaloids may be a useful distinguishing trait, because of a negative relationship in this species between alkaloid concentration and its palatability to sheep and cattle (3, 4, 8). Knowledge about the relative concentration of total alkaloids in reed canarygrass cultivars and breeding materials would be of interest to plant breeders concerned with breeding for improved forage quality.

Our objectives were to determine i) the frequency distribution of primary alkaloids among spaced plants and ii) the total concentration of alkaloids in selected reed canarygrass cultivars, breeding materials, and experimental strains.

MATERIALS AND METHODS

Seed of 'Frontier,' 'Grove,' 'Rise,' and 'Vantage' was obtained from the originating agencies and organizations. Clonal propagules of the six parent clones of Vantage, the syn 1 generation seed of each parent (breeder seed), and syn 2 seed (foundation seed) were obtained from I. T. Carlson, Iowa State Univ., Ames. The experimental strains included Happy Valley, RC-2, and MN-72 from the breeding programs at the Alaska, Iowa, and Minn. Agric. Exp. Stn., respectively. Happy Valley is a local collection, and RC-2 is a five-clone synthetic selected for forage and seed yield. MN-72 is a 12-clone synthetic based on parents selected at Minnesota for low-to-intermediate levels of total alkaloid concentration.

In 1972 a spaced-plant trial and a solid-stand trial were established at Rosemount, Minn. The spaced-plant trial was established with transplanted seedlings in July. Each entry was represented by one 10-plant row in each of five replicates, with plants spaced on 0.9-m centers and evaluated in a randomized complete block design. Entries included in the solid-stand trial were seeded in August with a nine-row seeder, at a rate of 11 kg/ha in plots (1.8 m wide and 6.1 m long) arranged in a randomized complete block with four replicates. The soil was Typic Hapludous, fine-silty over sandy or sandy skeletal, mixed mesic (Waukegan) silt loam. It was treated with methyl bromide to control weeds and remained weed free during the trial. Soil tests showed 25 kg/ha P and 145 kg/ha K. Each year, plots of both trials received 90 kg/ha N in the early spring and shortly after each harvest. Annual applications of 35 kg/ha P and 130 kg/ha K exceeded or maintained the levels, respectively, removed by the harvested crops.

In 1973 forage samples for alkaloid determination of the spaced-plant trial were obtained from 5-week-old (September 4) second regrowth. The forage from the solid-stand trial was cut on May 29, July 12, and August 20. Samples for alkaloids were taken from first growth (May 29) and from second regrowth at 4 weeks (August 8) and 6 weeks (August 20) following the July 12 cut. In 1974 the solid-stand trial was cut on June 13, July 29, and August 29 and alkaloid samples were taken from 4½-week-old (July 15) first regrowth and from 4-week-old (August 28) second regrowth.

Forage samples were clipped from the upper 1/3 of each plant in the spaced-plant trial and from the upper 1/3 of the canopy at random within each plot of the solid-stand trial, to obtain the highest and most uniform alkaloid concentration (2). Between 25 and 50 g fresh forage were placed in a plastic bag and frozen at −17 C. The frozen samples were chopped into 1 to 2-cm segments.

[1] Joint contribution, Dep. of Agron. and Plant Genet., and ARS, USDA. Paper no. 8967, scientific journal series, Minn. Agric. Exp. Stn. Received Feb. 7, 1975.

[2] Professor; and research agronomist, ARS, USDA, and professor, respectively, Dep. of Agron. and Plant Genet., Univ. of Minn., St. Paul, MN 55108.

00014

7500063

Three major alkaloids (gramine; N,N-dimethyltryptamine; and 5-methoxy-N,N-dimethyltryptamine) were distinguished by ascending paper chromatography as outlined by Simons and Marten (8). Alkaloid concentration (% dry wt) was determined by the total base titration method of Simons and Marten (8).

RESULTS AND DISCUSSION

All but one of the 269 Vantage (syn 1) plants contained gramine and no tryptamines (Table 1). The exceptional tryptamine-containing plant may have been the result of out-crossing, a mutant, or a seed contaminant. All plants of Vantage (syn 2) contained gramine and no tryptamines. We examined the six parent clones of Vantage in 1974 and identified only gramine in all clones grown in both the greenhouse and the field. The data support the hypothesis by Woods and Clark (11) that the gene controlling inheritance of gramine appears recessive to the genes controlling inheritance of tryptamines.

All other cultivars and experimental lines contained both tryptamines and gramine (Table 1). Chi-square analysis of frequency distribution of the three primary alkaloid types and combinations of these among spaced plants showed that MN-72 could be distinguished from Grove, Rise, and RC-2 (range of $\chi^2 = 20.56$, 4 df, $P < 0.001$; 13.56, 4 df, $P < 0.005$) and from Happy Valley ($\chi^2 = 7.84$, 3 df, $P = 0.02$ to 0.05). It may have been possible to obtain differences in alkaloid typing among other cultivars if larger plant populations had been used.

Alkaloid analysis of first growth (May 29, 1973) and of regrowth (Sept. 4, 1973 and July 15 and Aug. 28, 1974) from each solid-stand plot of cultivars showed without exception that Vantage contained only gramine, whereas Frontier, Grove, MN-72, RC-2, and Rise always contained mixtures of gramine and tryptamines. Our observations suggest that Vantage can be distinguished from Frontier, Grove, and Rise because it contains only the primary indole alkaloid gramine. From previous experiments, we believe that growing these cultivars in other climates or under different management regimes does not alter the pattern of occurrence of gramine or tryptamines (4, 5).

The mean alkaloid concentration of spaced plants of Grove was slightly lower than that of Rise (Table 1).

Data from the solid-stand plots also indicated that Grove, MN-72, and Frontier had lower alkaloid concentrations than did RC-2, Vantage, and Rise, when determined from 4-week-old second regrowth forage in 1973 (Table 2). Mean alkaloid concentrations were generally only half as high in the 6-week growth of 1973 and in both 4-week growths in 1974 as that of the 4-week growth in 1973, and differences among entries were not as apparent. When the 2-year mean alkaloid values of 1973 and 1974 samples were considered, Grove and MN-72 had significantly lower alkaloid concentrations than Vantage, Rise, and RC-2. Alkaloid concentration appeared to distinguish reed canarygrass cultivars only at times of high leaf production at regrowth stages. We have shown elsewhere (2) that maximum concentration of alkaloids occurred in the early regrowth stage and diminished with advanced maturity. The lack of differences in alkaloid concentration between Frontier and Rise at some times agreed with an earlier Minnesota report (3).

Table 1. Proportion of plants containing particular alkaloids or combinations and mean total alkaloid concentration of spaced plants of reed canarygrass at Rosemount, Minn. Sept. 4, 1973.

Entry	Primary indole alkaloids*						Total plants	Mean alkaloid conc.
	G	G+D	G+M	D+M	D	M		
	%							% dry wt
Vantage (Syn 1):								
6-3	100	0	0	0	0	0	42	0.172
15-1	98	0	0	0	0	2	43	0.132
16-7	100	0	0	0	0	0	49	0.192
37-1	100	0	0	0	0	0	48	0.184
47-2	100	0	0	0	0	0	43	0.136
48-5	100	0	0	0	0	0	44	0.135
Mean (Syn 1)	99.6	0	0	0	0	0.4	45	0.159
Vantage (Syn 2)	100	0	0	0	0	0	47	0.168
Grove	63	0	4	23	0	10	49	0.148
Happy Valley	85	0	0	11	0	4	47	0.192
MN-72	92	0	0	2	6	0	48	0.162
RC-2	74	2	0	13	0	11	47	0.202
Rise	66	0	4	19	2	9	47	0.190
L. S. D. 0.05								0.045

* G-gramine; D = N, N-dimethyltryptamine; M = 5-methoxy-N, N-dimethyltryptamine.

Table 2. Total indole alkaloids of solid stands of reed canarygrass as affected by sampling date and length of regrowth period at Rosemount, Minn.

Entry	Sampling dates				2-year Mean
	1973		1974		
	8/4	8/20	7/15	8/28	
	Length of regrowth period, weeks				
	4	6	4	4	
	total alkaloids, % dry wt				
Grove	0.180	0.095	0.093	0.105	0.118
MN-72	0.190	0.105	0.097	0.100	0.123
Frontier	0.193	0.090	0.103	0.132	0.129
Vantage	0.245	0.103	0.115	0.118	0.145
Rise	0.272	0.137	0.110	0.132	0.163
RC-2	0.240	0.153	0.123	0.165	0.170
Mean	0.220	0.113	0.107	0.125	0.141
L. S. D. 0.05	0.038	0.035	0.026	0.047	0.019

The tryptamines in *P. aquatica* L. (*P. tuberosa* L.) have been reported to be potentially harmful to grazing sheep (1). Research at St. Paul, Minn. (Marten, Jordan, and Hovin, unpublished), has shown that lambs grazing tryptamine-containing reed canarygrass clones had higher incidence of diarrhea (17.5% and 50.8% of lambs on low and high tryptamine-containing clones, respectively) than those grazing low (3.3%) and high (16.6%) gramine-containing clones. The mean alkaloid concentrations of the low (0.085%) and high (0.235%) tryptamine clones were nearly identical to the corresponding low (0.075%) and high (0.235%) gramine clones. The more frequent appearance of diarrhea in lambs grazing tryptamine-containing clones agrees with an observation by Woods (10) in Canada.

We have yet to determine whether reed canarygrass pastures seeded to Vantage or similar gramine-containing cultivars, when grazed by livestock, will result in an improved state of animal health, compared to cultivars with tryptamines or a mixture of tryptamines and gramine. In the forage-breeding program at the Univ. of Minn., we have identified several clones with low concentrations of gramine that are being progeny tested for alkaloid concentration and agronomic characteristics.

REFERENCES

1. Gallagher, G. H., J. H. Koch, R. M. Moore, and J. D. Steel. 1964. Toxicity of *Phalaris tuberosa* for sheep. Nature 204: 542-545.

00015

7500063

2. Hagman, J. L., G. C. Marten, and A. W. Hovin. 1975. Alkaloid concentration in plant parts of reed canarygrass of varying maturity. Crop Sci. 15:41-43.
3. Marten, G. C. 1973. Alkaloids in reed canarygrass. p. 15-31. *In* A. G. Matches (ed.) Antiquality components of forages. Crop Sci. Soc. Am. Special Publ. 4, Madison, Wis.
4. ———, R. F. Barnes, A. B. Simons, and F. J. Wooding. 1973. Alkaloids and palatability of *Phalaris arundinacea* L. grown in diverse environments. Agron. J. 65:199-201.
5. ———, A. B. Simons, and J. R. Frelich. 1974. Alkaloids of reed canarygrass as influenced by nutrient supply. Agron. J. 66:363-368.
6. Oram, R. N. 1970. Genetic and environmental control of the amount and composition of toxins in *Phalaris tuberosa* L. Int. Grassl. Congr., Proc. 11 (Surfers Paradise, Queensland) p. 785-788.
7. Pelletier, S. W. 1970. The plant alkaloids - general information. p. 1-10. *In* S. W. Pelletier (ed.) Chemistry of the alkaloids. Van Norstrand Reinholdt Co., N.Y.
8. Simons, A. B., and G. C. Marten. 1971. Relationship of indole alkaloids to palatability of *Phalaris arundinacea* L. Agron. J. 63:915-919.
9. Smeckus, V. 1968. The distribution of indole alkaloids in plants. p. 1-40. *In* R. H. F. Manske (ed.) The alkaloids. Vol. XI. Academic Press, N.Y.
10. Woods, D. L. 1973. Evaluating reed canarygrass clones for palatability. p. 34-35. *In* Proceedings XXII Western Grass Breeders Work Planning Conference (Swift Current, Saskatchewan, Canada).
11. ———, and K. W. Clark. 1971. Genetic control and seasonal variation of some alkaloids in reed canarygrass. Can. J. Plant Sci. 51:323-329.

Growth of Cotton Plants on Nitrate and Ammonium Nitrogen[1]

J. W. Radin and C. R. Sell[2]

ABSTRACT

Vegetative and reproductive growth of cotton (*Gossypium hirsutum* L.) were evaluated in the greenhouse on media containing nitrate or ammonium or both, to test for interactions between the two N sources on growth and yield. An inhibitor of nitrification was included in all treatments. Both N sources supported vegetative growth, although there was little response to increments of nitrate above meq/liter under the conditions of the test. Combinations of the two N sources did not support better growth than either one alone, except at low total N levels, perhaps because ammonium at $\geqslant$ 2 meq/liter inhibited *in vivo* nitrate reductase activity.

Reproductive growth of the plants was enhanced by N; however, 9 meq/liter nitrate was less effective than lower concentrations of either source, or their combination, in promoting fruitfulness and N accumulation relative to the vegetative parts. The failure to find a synergistic interaction in response to the two sources suggests that cotton responds to N source differently from some other crop species.

Additional index words: Leaf area, Relative Growth rate, Nitrate reductase, *Gossypium hirsutum* L., N-Serve®.

IN recent years some investigators have shown that wheat (*Triticum aestivum* L.) and corn (*Zea mays* L.) grow and yield better on combinations of ammonium and nitrate N than on equivalent amounts of either source alone (4, 8, 18). In addition, many other crops, including cotton (*Gossypium hirsutum* L.), are believed to grow better on nitrate alone than on ammonium alone (9, 20, 5). Nitrate must be reduced by an energy-consuming process before it can be assimilated. Thus, from an energetic standpoint, one might predict lower yields to the extent that photosynthetic products are diverted for nitrate reduction.

We sought to determine, in greenhouse experiments, the responses of cotton to nitrate and ammonium N and to combinations of the two. Although it is common field practice to fertilize cotton with either nitrate or reduced N, complex microbial transformations in the soil alter the spectrum of available forms and make interpretation of responses difficult. In our experiments, these difficulties were largely obviated by the use of an inhibitor of nitrification.

MATERIALS AND METHODS

Seeds of 'Deltapine 16' cotton were germinated in pots with vermiculite and grown on deionized water in the greenhouse for 2 weeks. Plants were then thinned to two/pot and watered twice weekly with test nutrient solutions. An experiment typically lasted until flowering. The solutions were all based upon a modified half-strength Hoagland's solution, in which KNO_3 was present at 1 meq/liter and $Ca(NO_3)_2$ at 4 meq/liter. For nitrate levels $<$ 5 meq/liter, K_2SO_4 and $CaCl_2$ were substituted for the nitrate salts to give the desired concentration. For levels $>$ 5 meq/liter, both nitrate salts were increased proportionately. Ammonium was added as $(NH_4)_2SO_4$. The pH of the nutrient solution was about 6.0, and the pH of eluates from the pots was not $<$ 5.1 except when the ammonium concentration was 20 meq/liter. Dow M-3322[3], a liquid formulation of N-Serve®, 2-chloro-6-(trichloromethyl)pyridine, (6) was included in all solutions at concentrations $\leqslant$ 10 ppm and effectively inhibited nitrification with no observable effects on the plants. Each treatment was replicated at least six times.

Leaf area was determined weekly by the method of Ashley, Doss, and Bennett (1). The first determination was made 1 week after treatments were begun. All leaves of length $>$ 2 cm were included. This nondestructive technique allowed the monitoring of individual plants over an extended interval, and thus minimized the error inherent in such experiments. Plant height and stem diameter were also measured, but were less satisfactory as indicators of growth. The relative growth rate R_A (14) was calculated from leaf area measurements during the period of exponential growth by the relationship:

$$R_A = \frac{\ln A_2 - \ln A_1}{t_2 - t_1} = \frac{0.693}{t_d}$$

where A is leaf area, t is time, and t_d is the time for leaf area to double. Doubling times were determined graphically from semilogarithmic plots of leaf area vs. age.

Nitrate reductase activity was determined in a separate group of 3-week-old plants (1 week after first watering with nutrient solution). The shoots were excised just below the cotyledonary node and were weighed and assayed by an *in vivo* method described previously (15). Activity is expressed as μmol/g fresh wt/hour.

In separate experiments, the influence of N source in reproductive growth was studied. Plants were grown, one/pot, on a standard high-nitrate (9 meq/liter) nutrient solution of the composition given by Guinn (7). After 6 weeks, when flower

[1] Contribution of the ARS, USDA, in cooperation with the Ariz. Agric. Exp. Stn., journal paper no. 2,405. Received Jan. 10, 1975.

[2] Plant physiologist and physical sciences technician, respectively, ARS, Western Cotton Res. Lab., 4135 E. Broadway Rd., Phoenix, AZ 85040.

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

00016

Exhibit E

Statement of the Basis of Applicants Ownership

The Committee for Agricultural Development, Iowa State University, Ames, Iowa, increases and distributes foundation seed of varieties developed by the Iowa Agriculture and Home Economics Experiment Station. Employees of the Iowa Agriculture and Home Economics Experiment Station were the first and only breeders of the 'Vantage' variety of reed canarygrass for which a certificate of protection is solicited.

7300007	OAT	ABANDONED
7300090	TREF	EXTEND TO
7700050	SOY	CERT
7700051	SOY	I
7500063	CANG	I

51 IOWA A. E. S.

Issued to Iowa State but request was to have it issued to "Committee for agricultural development"

Check this out!