Effect of Nitrogen Fertilization on Free Amino Acid and Soluble Sugar Content of Poa pratensis and on Infection and Disease Severity by Drechslera sorokiniana

Philip W. Robinson and Clinton F. Hodges

Research and Development Representative, American Hoechst Corporation, Agricultural Division, Somerville, NJ 08876, and Professor of Horticulture and Botany and Plant Pathology, Department of Horticulture, Iowa State University, Ames, IA 50011.

Journal Paper No. J-8736 and Project 2001 of the Iowa Agriculture and Home Economics Experiment Station, Ames.

Portion of a Ph.D. thesis submitted by the senior author to the Graduate College, Iowa State University, Ames.

Accepted for publication 25 March 1977.

ABSTRACT

ROBINSON, P. W., and C. F. HODGES. 1977. Effect of nitrogen fertilization on free amino acid and soluble sugar content of Poa pratensis and on infection and disease severity by Drechslera sorokiniana. Phytopathology 67:1239-1244.

Preinfection germination and growth of conidia of *Drechslera sorokiniana* on leaf surfaces of *Poa pratensis* and subsequent postinfection lesion and disease development were influenced by soil applications of $(NH_4)_2SO_4$, NH_4NO_3, and $Ca(NO_3)_2$ to *P. pratensis*. All nitrogen sources increased bipolar germination, germ-tube length, and germ-tube branches on leaf surfaces; appressoria formation was increased by NH_4NO_3, and penetrations without appressoria were not influenced by any of the nitrogen sources. Number of lesions per unit leaf area was not significantly changed by any of the nitrogen sources, but lesion size increased in response to all nitrogen sources, and disease severity increased significantly in response to $(NH_4)_2SO_4$. Total soluble sugars in leaf tissue were decreased by $(NH_4)_2SO_4$ and NH_4NO_3, but did not change significantly in response to $Ca(NO_3)_2$. Individual soluble sugars decreased were fructose, glucose, and sucrose. Correlations between individual sugars and preinfection growth characteristics of *D. sorokiniana* (bipolar germination, germ-tube growth, appressoria formation) on leaf surfaces showed that decreases in fructose, glucose, and sucrose were negatively correlated with increased growth of the pathogen; decreases in sugars showed essentially no correlations with postinfection disease development (lesion number, lesion type, disease severity) suggesting that decreasing sugar levels in response to nitrogen fertilization may induce growth of the pathogen on leaf surfaces, but have little direct influence on disease severity. Total free amino acids in leaf tissue increased in response to all nitrogen sources; individual amino acids, however, increased, decreased, or remained unchanged in response to the nitrogen sources. Increases in aspartic and glutamic acids were negatively correlated with various preinfection characteristics of *D. sorokiniana* (germ-tube growth, germ-tube branching, appressoria formation) on leaf surfaces; increases in proline were positively correlated with almost all preinfection germination and growth characteristics of *D. sorokiniana* conidia on leaf surfaces of *P. pratensis*. Glutamic acid and proline increases were positively correlated with most postinfection disease development characteristics (lesion number, lesion type, disease severity), suggesting that increases in these and other amino acids in response to nitrogen fertilization may contribute more directly to disease severity after infection than the decreases in soluble sugars.

Additional key words: aspartic acid, *Bipolaris sorokiniana*, etiology, exosmosis, exudates, fructose, glucose, glutamic acid, guttation, *Helminthosporium sorokinianum*, *H. sativum*, proline, sucrose.

Nitrogen fertilization often increases the severity of diseases caused by *Drechslera sorokiniana* (Sacc.) Subram. and Jain (= *Helminthosporium sativum* P.K. and B) and other species of *Drechslera* on *Poa pratensis* L. (3, 7, 11, 14, 30). Physiological explanations for increased disease severity on nitrogen-fertilized *P. pratensis* are incomplete. It is probable, however, that the soluble sugar and free amino acid content of the host tissue is involved in the etiology of diseases caused by *Drechslera* species and that nitrogen influences the quantity of these substances present (2, 20, 26, 33). The application of some nitrogen sources to *P. pratensis* reduces the quantity of carbohydrates present in various tissues (24, 34). The relationship of soluble sugar content to severity of diseases caused by *Drechslera* species is not clear; the incidence of melting-out caused by *Drechslera poae* (*H. vagans*) increases as the soluble sugar content of *P. pratensis* leaf tissue decreases (23), suggesting that melting-out due to *D. poae* is a low-sugar disease (19). Other studies show no significant relationship between the sugar content of *P. pratensis* leaves and disease severity resulting from infection by *D. dictyoides* (*H. dictyoides*) (15, 16) and *D. sorokiniana* (9). Nitrogen fertilization also induces guttation in perennial grasses and influences the free amino acid content of the plants (10, 17, 29). Glutamine and asparagine increase markedly in response to NH^+_4 nitrogen (10, 17, 27), but glutamine is the predominant amino acid in guttation fluid of perennial grasses. In the presence of glutamine, germination and germ-tube development of *D. sorokiniana* conidia is accelerated (12, 13, 18).

The various observations on the effects of nitrogen on soluble sugars and free amino acids and their subsequent influence on diseases caused by species of *Drechslera* have been assembled from several species of Gramineae and

Drechslera under widely diverse experimental conditions. It is evident that nitrogen influences the soluble sugars and free amino acids in grasses and that such physiological changes may influence diseases caused by *Drechslera* species. The research to date, however, has dealt with nitrogen, soluble sugars, and free amino acids as separate entities that influence diseases caused by various species of *Drechslera*. It is, therefore, difficult to arrive at any final conclusions because of the probable interactions of these various factors. The research herein was initiated to determine the effect of several nitrogen sources on the soluble sugar and free amino acid content of *P. pratensis* and to determine if potential changes in these substances are related to infection and disease development by *D. sorokiniana* on *P. pratensis*.

MATERIALS AND METHODS

Treatments.—*Poa pratensis* cv. 'Newport' was asexually propagated for 90 days in 7.6-cm (3-inch diameter) pots in a steamed loam, peat, perlite (1:1:1, v/v) mix. Plants then were placed in growth chambers at 22 C with an 18-hr daylength of 18,500 to 23,000 lux. Plants were treated with 50 ml of 0.01 M $(NH_4)_2SO_4$, NH_4NO_3, or $Ca(NO_3)_2$, or with distilled water (control) applied to the soils. Each treatment was applied to 24 plants. The leaf blades of six plants of each treatment were evaluated for soluble sugars, free amino acids, preinfection development of *D. sorokiniana* on leaf surfaces, or for lesion development after infection. Treatments were evaluated for their effect on sugars and amino acids for 72 hr after application of the nitrogen sources; the leaf blades of one shoot was sampled from each plant at 24 hr and 72 hr (between 0900 and 1000 hours) and combined for analysis. Inoculations for preinfection evaluations of *D. sorokiniana* growth on leaf surfaces were timed to coincide maximum appressorial formation on leaf surfaces with the 24-hr and 72-hr sampling periods for sugars and amino acids. Postinfection lesion and disease development on leaves was evaluated 10 days after inoculation (31). The data presented are the means of three replicates of each treatment.

Sugar and amino acid analyses.—Leaves of each treatment were collected and freeze-dried at −50 C for 48 hr, ground in a Wiley mill, and passed through a 635-μm sieve. Sugars were extracted with 80% ethanol for 4 hr. The extract was evaporated, and ethyl ether added to partition the chlorophyll. The aqueous phase was prepared for thin-layer chromatography (8). Sugars were absorbed onto Kieselguhr G (250-μm thick) impregnated with 0.02 M sodium acetate and then separated with n-propanol, ethyl acetate, and water (40:50:50, v/v). Sugars were visualized with aniline-diphenylamine-phosphoric spray reagent (22) and referenced to standard sugar concentrations.

Free amino acids were extracted five times from ground leaf tissue of each treatment in 95% and 80% ethanol for 24 hr each. Extracts were combined and evaporated to dryness in a hood. Amino acids were partitioned from chlorophyll in ethyl ether and water (1:1, v/v) and demineralized by passing them through columns of analytical grade ion-exchange resins (Bio-Rad Laboratories, Richmond, CA 94800). AG50W-X8, 74- to 38-μm (200- to 400-mesh) (H^+ form) was used to collect basic amino acids, and AG2-X10, 74- to 38-μm (200- to 400-mesh) (OH^- form) was used to collect neutral and acidic amino acids (5). The eluate was evaporated to dryness and analyzed on an amino acid analyzer.

Inoculations.—The four youngest visible leaves of a single shoot of plants from each treatment were inoculated at five positions on the upper epidermis in a specially constructed inoculation apparatus (31). The five inoculation points on each leaf received about five conidia in 0.05 ml of sterile distilled water applied with a standard tuberculin syringe. Inoculations were timed to coincide maximum appressoria formation on leaf surfaces with the 24- and 72-hr sampling periods for sugar and amino acid analysis. Appressoria formation by *D. sorokiniana* occurs between 18 and 30 hr after conidia germination (25); under conditions of this study, appressoria formation was maximal at 24-hr. Therefore, nitrogen treatments were applied to the soil and conidia to the leaf surfaces simultaneously for the 24-hr analysis and conidia were applied to the leaf surfaces 48 hr after the nitrogen treatments for the 72-hr analysis.

Preinfection development of germinating conidia was evaluated on leaf surfaces at the 24- and 72-hr sampling periods. Leaves were removed from the inoculation apparatus, cleared in Carnoy's solution for 1-3 days, placed in lactophenol for 24 hr, stained with 0.1% acid fuchsin in lactophenol, and then destained in fresh lactophenol. Mono- and bipolar germination, germ-tube length (including primary branches), number of primary branches, and leaf penetration by hyphae with and without appressoria were recorded for each germinating conidium.

Postinfection lesion development on leaves was evaluated 10 days after inoculation. Leaves were removed from the inoculation apparatus, cut into pieces 10 cm long, and cleared in 95% ethanol. Number of lesions per square centimeter of leaf surface was determined, and lesions were given a numerical rating according to

TABLE 1. Effect of nitrogen sources on the soluble sugar content of *Poa pratensis* leaves

Treatments[z]	Sugar content (μmol/g fresh weight)[y]				
	Fructose	Glucose	Sucrose	Raffinose	Total Sugars
None (control)	13.66 a	11.58 a	15.44 a	0.51 a	41.19 a
$(NH_4)_2SO_4$	8.91 b	7.85 b	14.14 b	0.50 a	31.40 b
NH_4NO_3	10.48 b	9.11 b	14.72 bc	0.70 b	35.01 b
$Ca(NO_3)_2$	15.16 a	13.28 a	16.02 ac	0.77 b	45.23 a

[y]Between-treatment means followed by the same letter are not significantly different. Duncan's multiple-range test ($P = 0.05$).
[z]Each nitrogen treatment applied to soil in 0.01 M concentrations.

development: fleck-type lesions = 1; lesion without halos = 2; lesions with halos = 4; streaking lesions = 8; and lesions that coalesced between points of inoculation = 16 (31). An estimate of disease severity was determined by dividing the sum of lesion ratings per leaf by the leaf area.

RESULTS

Sugars.—Fructose, glucose, sucrose, and raffinose were the soluble sugars recovered from *P. pratensis* leaf tissue (Table 1). Total soluble sugar content decreased in the leaves of plants treated with $(NH_4)_2SO_4$ and NH_4NO_3; in plants treated with $Ca(NO_3)_2$, total sugar content increased. The significant decrease in total soluble sugars in leaves of plants treated with $(NH_4)_2SO_4$ and NH_4NO_3 was due primarily to lower levels of fructose and glucose (Table 1). Increases in all soluble sugars contributed to the increase in total soluble sugars in plants treated with $Ca(NO_3)_2$; but only raffinose increased significantly (Table 1).

Amino acids.—All nitrogen sources increased the total free amino acid content of the leaf tissue, but increases were significant only in plants treated with NH_4NO_3 and $Ca(NO_3)_2$ (Table 2). The content of individual amino acids in leaves varied with the specific nitrogen source. Threonine, serine, glutamic acid, proline, and lysine increased in response to all nitrogen sources, with most significant increases in this group occurring in leaves of plants treated with NH_4NO_3 and $Ca(NO_3)_2$ (Table 2). Proline also was increased significantly by $(NH_4)_2SO_4$. Glycine, isoleucine, and leucine remained the same or decreased in response to all nitrogen sources; most significant decreases of these amino acids occurred in leaves of plants treated with $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ (Table 2). Isoleucine decreased significantly in response to all nitrogen sources. Aspartic acid, alanine, valine, tyrosine, and phenylalanine increased or decreased in response to the different nitrogen sources (Table 2). Aspartic acid and phenylalanine increased significantly in response to $Ca(NO_3)_2$, and tyrosine increased significantly in response to NH_4NO_3. Tyrosine and phenylalanine were significantly decreased by

TABLE 2. Effect of nitrogen sources on the free amino acid content of *Poa pratensis* leaves

	Amino acid content in μmol/g fresh weight[y]			
	Nitrogen treatments[z]			
Amino acids	None (control)	$(NH_4)_2SO_4$	NH_4NO_3	$Ca(NO_3)_2$
Aspartic acid	0.49 a	0.38 a	0.61 ab	0.66 b
Threonine	0.26 a	0.30 ab	0.35 b	0.45 c
Serine	0.47 a	0.52 a	0.72 b	0.85 b
Glutamic acid	1.34 a	1.61 ab	2.07 bc	2.62 c
Proline	0.10 a	0.24 b	0.28 b	0.12 a
Glycine	0.15 a	0.11 b	0.15 a	0.14 a
Alanine	1.53 ab	1.44 b	1.91 a	1.96 a
Valine	0.13 a	0.15 b	0.12 a	0.13 a
Isoleucine	0.15 a	0.08 b	0.07 b	0.11 b
Leucine	0.13 a	0.12 a	0.13 a	0.10 b
Tyrosine	0.07 a	0.06 b	0.09 c	0.07 ab
Phenylalanine	0.07 a	0.06 b	0.07 ac	0.08 c
Lysine	0.17 a	0.20 ab	0.26 c	0.23 bc
Totals	5.07 a	5.27 a	6.83 b	7.51 b

[y]Between-treatment means (across) followed by same letter are not significantly different according to Duncan's multiple-range test ($P = 0.05$).

[z]Each nitrogen treatment applied to soil in 0.01 M concentration.

TABLE 3. Preinfection germination and growth of *Drechslera sorokiniana* conidia on leaf surfaces of *Poa pratensis* treated with different nitrogen sources

	Characteristics observed (means)[v]				
Treatments[w]	Bipolar germination[x] (%)	Germ-tube length[y] (μm)	Germ-tube branches[z] (no.)	Appressoria formed[z] (no.)	Penetration w/o appressoria[z] (no.)
None (control)	37.5 a	962.0 a	4.9 a	1.4 a	0.02 a
$(NH_4)_2SO_4$	40.6 b	1114.2 b	5.0 a	1.4 a	0.02 a
NH_4NO_3	41.1 b	1166.6 c	6.7 b	1.7 b	0.02 a
$Ca(NO_3)_2$	38.5 a	1050.3 ab	5.9 ab	1.4 a	0.01 a

[v]Between-treatment means followed by the same letter are not significantly different according to Duncan's multiple-range test ($P = 0.05$).

[w]Each nitrogen treatment applied to soil in 0.01 M concentrations.

[x]Percentage based on germinating conidia only.

[y]Mean includes germ tubes and primary branches per geminating conidium.

[z]Mean number of germ-tube branches, appressoria, and penetrations without appressoria are expressed per germinating conidium.

$(NH_4)_2SO_4$. Changes in alanine and valine were not significant.

Preinfection germination and growth of conidia on leaves.—Percentage bipolar germination of conidia and length of germ tubes were significantly greater on leaves of plants treated with $(NH_4)_2SO_4$ and NH_4NO_3 than on leaves of control plants; these characteristics increased also on leaves of plants treated with $Ca(NO_3)_2$, but not significantly (Table 3). Number of primary branches on germ tubes and appressorium formation increased in response to all nitrogen sources, but the increases were significant only on leaves of plants treated with NH_4NO_3 (Table 3). Direct penetration by hyphae without appressoria did not change significantly on leaves in response to any of the nitrogen sources (Table 3).

Postinfection lesion and disease development on leaves.—No significant change occurred in the number of lesions per square centimeter on leaves of plants treated with any of the nitrogen sources (Table 4). Development of individual leaf lesions, as reflected by the lesion type rating, was significantly increased $(NH_4)_2SO_4$ and NH_4NO_3 (Table 4). Disease severity was significantly increased by $(NH_4)_2SO_4$; application of NH_4NO_3 and $Ca(NO_3)_2$ increased disease severity, but not significantly (Table 4).

Interactions.—No correlations existed between total soluble sugar (Table 1) or total free amino acid (Table 2) content of plants and any of the preinfection germination characteristics of *D. sorokiniana* (Table 3) or the postinfection lesion and disease development characteristics (Table 4) in any of the treatments. Negative correlations were established, however, between fructose, glucose, and sucrose and preinfection bipolar germination, germ-tube length and branching, and appressoria formation by conidia of *D. sorokiniana* on leaf surfaces (Table 5). Most negative correlations with fructose, glucose, and sucrose occurred among control, $(NH_4)_2SO_4$, and NH_4NO_3 treatments. The only positive correlations between sugars and preinfection characteristics of *D. sorokiniana* on leaf surfaces occurred between penetrations without appressoria and fructose and glucose (Table 5). Sucrose also showed a negative correlation with postinfection lesions per square centimeter (Table 5); no other correlations existed between sugars and postinfection lesion and disease development.

Correlations of aspartic and glutamic acids with preinfection germ-tube growth and branching, appressoria formation, and penetration without appressoria of *D. sorokiniana* were negative; glutamic acid, however, also showed positive correlations with postinfection lesion type and disease severity (Table 5).

TABLE 4. Postinfection development of lesions and disease severity caused by *Drechslera sorokiniana* on leaves of *Poa pratensis* treated with different nitrogen sources

Treatment[w]	Characteristics observed (means)[v]		
	Lesions per cm^2[x]	Lesion-type rating[y]	Disease severity[z]
None (control)	2.2 a	27.4 a	6.5 a
$(NH_4)_2SO_4$	2.8 a	32.2 b	7.5 b
NH_4NO_3	2.5 a	29.8 c	6.8 a
$Ca(NO_3)_2$	2.7 a	28.9 ac	6.9 a

[v]Between-treatment means followed by the same letter are not significantly different according to Duncan's multiple-range test ($P = 0.05$).
[w]Each nitrogen treatment applied to soil in 0.01 M concentrations.
[x]All leaf samples were 10 cm long. Leaf area was determined by multiplying the mean width of each leaf (cm) by the length of the leaf sample.
[y]Mean of the sum of the lesion-type ratings of each leaf.
[z]Lesion-type ratings divided by leaf area.

TABLE 5. Correlations[a] between soluble sugar and free amino acid content of *Poa pratensis* leaves and the pre- and post-infection characteristics of *Drechslera sorokiniana* on the leaves

Sugars and amino acids	Preinfection germination and growth characteristics					Postinfection lesion and disease characteristics		
	Bipolar germ.	Germ-tube length	Germ-tube branches	Appressoria formed	Penetration w/o appress.	Lesions per cm^2	Lesion-type rating	Disease severity
Fructose	(−)[b]	(−)[b]		(−)[b,c]	(+)[c]			
Glucose				(−)[b,c]	(+)[b]			
Sucrose	(−)[c,d]		(−)[c,d]			(−)[d]		
Aspartic acid				(−)[b]	(−)[e]			
Glutamic acid		(−)[e]	(−)[e]	(−)[d]			(+)[d]	(+)[d]
Proline		(+)[e]	(+)[d,e]	(+)[d]	(+)[d]	(+)[e]	(+)[d,e]	(+)[d,e]

[a]Correlation coefficient 0.55; significant at $P = 0.05$.
[b]Control treatment.
[c]$(NH_4)_2SO_4$ treatment.
[d]NH_4NO_3 treatment.
[e]$Ca(NO_3)_2$ treatment.

The amount of proline was positively correlated with almost all pre- and postinfection characteristics observed; the positive correlations of both proline and glutamic acid with postinfection characteristics occurred only in response to NH_4NO_3 and $Ca(NO_3)_2$ (Table 5).

DISCUSSION

Preinfection evaluations of germinating conidia on leaf surfaces established that the application of nitrogen to *P. pratensis* can influence germination of conidia and subsequent growth of germ tubes on the external surfaces of the plant (Table 3). Such reactions are believed to be related to exosmosis, or leaching, of inorganic and organic (primarily carbohydrates) substances from leaf surfaces (6, 32), and in perennial grasses to exudation of amino acids (primarily glutamine) from leaves (10, 17, 29). All nitrogen sources caused various degrees of increases in bipolar germination, germ-tube length, and germ-tube branching; appressoria formation was increased only by NH_4NO_3, but penetrations without appressoria were not influenced by any nitrogen source (Table 3). Stimulation of bipolar germination, germ-tube length, and branches on the leaf surface (Table 3) does not, however, result in more lesions per unit leaf area (Table 4). This is reflected by the absence of any great stimulation of appressoria, or penetrations without appressoria (Table 3), and by the absence of any significant change in number of lesions per unit leaf area (Table 4). It is of interest, however, that those nitrogen treatments [$(NH_4)_2SO_4$, NH_4NO_3] that showed the greatest general increases in preinfection germination, germ-tube growth, and branching of *D. sorokiniana* conidia on leaf surfaces were associated with the only significant increases in lesion development (rating) and disease severity after infection (Table 4).

Previous studies indicate that no relationship exists between soluble sugar content of *P. pratensis* and its infection by *D. sorokiniana* or *D. dictyoides* and subsequent disease development (9, 15, 16). Similarly no correlations could be established in this study between changes in the total soluble sugars of nitrogen-treated plants and pre- and postinfection characteristics of *D. sorokiniana*. A relationship may exist, however, between individual sugars (fructose, glucose, sucrose) and *D. sorokiniana* that is masked by the changes in total soluble sugars. The primarily negative correlations between the various soluble sugars and preinfection characteristics of the pathogen seem to support the concept that decreasing levels of soluble sugars may increase the germination and growth of conidia on the leaf surface (Table 5). This increased germination activity should not, however, be confused with the concept of low-sugar diseases (19). Although lesion-type ratings and disease severity were significantly increased by the $(NH_4)SO_4$ and NH_4NO_3 treatments (Table 4) and these treatments also caused the greatest decrease in soluble sugars (fructose, glucose) (Table 1), only one negative correlation was established between sucrose and postinfection disease characteristics (Table 5). Therefore, it is reasonable to conclude that decreasing soluble sugar levels associated with nitrogen application may enhance conidia germination and germ-tube growth on leaf surfaces, but the soluble sugar content has a negligible influence on disease severity.

Total free amino acids were increased by all nitrogen treatments (Table 2), but like total soluble sugars, no correlations were established between the total increases and pre- and postinfection characteristics of *D. sorokiniana*. Individually, however, correlations were established between aspartic acid, glutamic acid, and proline and pre- and postinfection characteristics of pathogen (Table 5). Aspartic and glutamic acids correlated negatively with various preinfection germination characteristics of the pathogen. This observation seems to be in conflict with reports that increases in glutamine in leaf exudates of nitrogen-fertilized grasses stimulates germination and growth of *D. sorokiniana* conidia (12, 13). The interpretation of these different observations is beyond the scope of the data presented, but such factors as the constituents of guttation fluids, the potential interactions of soluble sugars and free amino acids as substrate, and the isomers of glutamic acid could be involved in the differences. The positive correlation of proline with several preinfection growth characteristics of *D. sorokiniana* (Table 5) is in agreement with previous observations that L-proline in the presence of trace elements stimulates growth of this pathogen (28).

Glutamic acid and proline were positively correlated with postinfection lesion and disease development (Table 5). Other research shows that glutamic acid (also aspartic acid) increases lesion size on rice leaves infected by *Cochliobolus miyabeanus*, but does not increase the number of lesions (1). In the present study, no increase in the number of lesions occurred in relation to any of the treatments, but lesion ratings and disease severity was increased (Table 4). Thus, the positive correlation of glutamic acid and proline with postinfection lesion and disease development (Table 5) suggests that these amino acids play a significant role in increasing disease severity. The role of proline in postinfection disease development is unknown, but proline may be of special interest in future research because of its implications in the stress physiology of other perennial grasses (4, 21).

LITERATURE CITED

1. AKAI, S., J. SHISHIYAMA, and H. EGAWA. 1958. Studies on the pathological physiology of rice plants.IV. On the susceptibility of rice plants cultured with nutrient solution containing amino acid to the Helminthosporium blight caused by Cochliobolus miyabeanus. Ann. Phytopathol. Soc. Jpn. 23:165-168.
2. ALBERDA, T. 1965. The influence of temperature, light intensity, and nitrate concentration on dry matter production and chemical composition of Lolium perenne L. Neth. J. Agric. Sci. 13:335-360.
3. ALTMAN, J. 1965. Nitrogen in relation to turf diseases. Golf Course Rep. 5:16-30.
4. BARNETT, N. M., and A. W. NAYLOR. 1966. Amino acid and protein metabolism in bermuda grass during water stress. Plant Physiol. 41:1222-1230.
5. BRENNER, M., A. NIEDERWIESER, and G. PATAKI. 1969. Amino acids and derivatives. Pages 730-786 *in* E. Stahl. Thin-layer chromatography, a laboratory handbook,2nd ed. Springer-Verlag, New York. 1,041 p.
6. BROWN, W. 1922. Studies in the physiology of parasitism. VIII. On the exosmosis of nutrient substances from the

host tissue into the infection drop. Ann. Bot. 36:101-119.
7. CHEESMAN, J. H., E. C. ROBERTS, and L. H. TIFFANY. 1965. Effects of nitrogen level and osmotic pressure of the nutrient solution on incidence of Puccinia graminis and Helminthosporium sativum infected Merion Kentucky bluegrass. Agron. J. 57:599-602.
8. COLLINS, F. W., and K. R. CHANDORKAR. 1971. Thin-layer chromatography of fructo-oligosaccharides. J. Chromatogr. 56:163-167.
9. COUCH, H. B., and L. D. MOORE. 1971. Influence of nutrition and total nonstructural carbohydrate content on Helminthosporium sativum-incited leaf spot of Kentucky bluegrass. Phytopathology 61:888.
10. CURTISS, L. C. 1944. The exudation of glutamine from lawn grasses. Plant Physiol. 19:1-5.
11. ELLIOTT, E. S. 1962. The effect of soil fertility on the development of Kentucky bluegrass diseases. Phytopathology 52:1218.
12. ENDO, R. M., and R. H. AMACHER. 1964. Influence of guttation fluid on infection structures of Helminthosporium sorokinianum. Phytopathology 54:1327-1334.
13. ENDO, R. M., and J. J. OERTLI. 1964. Stimulation of fungal infection of bentgrass. Nature (Lond.) 201:313.
14. FUNK, C. R., and R. E. ENGEL. 1966. Influence of variety, fertility level, and cutting height on weed invasion of Kentucky bluegrass. Proc. Northeast. Weed Control Conf. 20:480-485.
15. GIBBS, A. F., and R. D. WILCOXSON. 1972. Effect of sugar content of Poa pratensis on Helminthosporium leaf disease. Physiol. Plant Pathol. 2:279-287.
16. GIBBS, A. F., R. D. WILCOXSON, and H. L. THOMAS. 1973. The effect of nitrogen fertilization and mowing on Helminthosporium leaf spot and sugar content of bluegrass leaves. Plant Dis. Rep. 57:544-548.
17. GREENHILL, A. W., and A. C. CHINBALL. 1934. The exudation of glutamine from perennial ryegrass. Biochem. J. 28:1422-1427.
18. HEALY, M. J., and M. P. BRITTON. 1968. Infection and development of Helminthosporium sorokinianum in Agrostis palustris. Phytopathology 58:273-276.
19. HORSFALL, J. G., and A. E. DIMOND. 1957. Interactions of tissue sugar, growth substances, and disease susceptibility. Z. Pflanzenkr. 64:415-421.
20. JONES, D. I. H., G. GRIFFITH, and R. J. K. WALTERS. 1961. The effect of nitrogen fertilizer on the water-soluble carbohydrate content of perennial ryegrass and cocksfoot. J. Br. Grassl. Soc. 16:272-275.
21. KEMBLE, A. R., and H. T. MACPHERSON. 1954. Liberation of amino acids in perennial ryegrass during wilting. Biochem. J. 58:46-49.
22. LEWIS, B. A., and F. SMITH. 1969. Sugar and derivatives. Pages 807-837 *in* E. Stahl. Thin layer chromatography, a laboratory handbook, 2nd ed. Springer-Verlag. New York. 1,041 p.
23. LUKENS, R. J. 1970. Melting-out of Kentucky bluegrass, a low-sugar disease. Phytopathology 60:1276-1278.
24. MONROE, C. A., G. D. COORTS, and C. R. SKOGLEY. 1969. Effects of nitrogen-potassium levels on the growth and chemical composition of Kentucky bluegrass. Agron. J. 61:294-296.
25. MOWER, R. G. 1961. Histological studies of suscept-pathogen relationships of Helminthosporium sativum P.K. and B., Helminthosporium vagans Drechs., and Curvularia lunata (Wakk.) Boed. on leaves of Merion and common Kentucky bluegrass. Ph.D. Thesis. Cornell University. 150 p.
26. NOWAKOWSKI, T. Z. 1962. Effects of nitrogen fertilizers on total nitrogen, soluble nitrogen, and soluble carbohydrate content of grass. J. Agric. Sci. 59:387-392.
27. NOWAKOWSKI, T. Z., and R. K. CUNNINGHAM. 1966. Nitrogen fractions and soluble carbohydrates in Italian ryegrass. II. Effects of light intensity, form, and level of nitrogen. J. Sci. Food Agric. 17:145-150.
28. PETERSON, E. A., and H. KATZNELSON. 1954. Studies of the nutrition of Helminthosporium sativum and certain related species. Can. J. Microbiol. 1:190-197.
29. RALEIGH, G. J. 1946. Glutamine from ryegrass. Science 103:206-207.
30. ROBERTS, E. C., and J. H. CHESSMAN. 1964. Effect of nitrogen and moisture stress on leaf spot infections in Merion bluegrass. Golf Course Rep. 5:18-20.
31. ROBINSON, P. W., and C. F. HODGES. 1976. An inoculation apparatus for evaluation of Bipolaris sorokiniana lesion development on progressively older leaves of Poa pratensis. Phytopathology 66:360-363.
32. TUKEY, H. B., JR. 1970. The leaching of substances from plants. Annu. Rev. Plant Physiol. 21:305-324.
33. WATSCHKE, T. L., R. E. SCHMIDT, and R. E. BLASER. 1970. Response of some Kentucky bluegrasses to high temperature and nitrogen fertility. Crop Sci. 10:372-376.
34. ZONONI, L. J., L. F. MICHELSON, W. G. COLBY, and M. DRAKE. 1969. Factors affecting carbohydrate reserves of cool season turfgrasses. Agron. J. 61:195-198.