duction in resting cell suspensions, but in batch cultures it sometimes increased and sometimes decreased evolution of N_2O. Growth in TSB often resulted in less N_2O release than in nutrient broth, but in a few cases (Table 4), more N_2O was produced in TSB. No facile explanation for these varying effects is at hand.

Most of the nondenitrifying N_2O producers were apparently capable of fermentative dissimilatory reduction of NO_2^- to NH_4^+ under the appropriate conditions. The *Bacillus* and *Citrobacter* isolates were NO_2^- accumulators in NB; in TSB they were NH_4^+ producers. This was also true for 48 of 58 soil isolates which were initially classified as NO_2^- accumulators in NB (Table 1). This suggests that, for many bacteria, reduction beyond the initial NO_3^- to NO_2^- step is limited more by the environment than by the genetic potential of the organism. In any case, more ionic N was produced than gaseous N, so fermentative NO_3^- reducers might be less likely than denitrifiers to cause significant volatile loss of fixed soil N. If NO_2^- reduction to NH_4^+ were competitive with denitrification, gaseous N loss could actually be reduced by these organisms.

It is difficult to evaluate the significance of nondenitrifying NO_3^- reducers as a source of soil N_2O with our present knowledge. These organisms did produce N_2O under a wide variety of conditons. From our survey it appears that they are more numerous than denitrifiers in soil. When added to autoclaved soil, fermentative NO_2^- reducers initially produced N_2O at a lower rate than denitrifiers but, because they also consumed N_2O, denitrifiers accumulated less N_2O as the incubation proceeded. Nondenitrifying NO_3^- reducers may thus contribute to N_2O evolution from soil.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Breitenbeck, G. A. , A. M. Blackmer, and J. M. Bremner. 1980. Effects of different nitrogen fertilizers on emission of nitrous oxide from soil. Geophys. Res. Lett. 7:85–88.
2. Bremner, J. M., and A. M. Blackmer. 1978. Nitrous oxide: emission from soils during nitrification of fertilizer nitrogen. Science 199:295–296.
3. Caskey, W. H., and J. M. Tiedje. 1979. Evidence for clostridia as agents of dissimilatory reduction of nitrate to ammonium in soils. Soil Sci. Soc. Am. J. 43:931–936.
4. Cole, J. A., and C. M. Brown. 1980. Nitrite reduction to ammonia by fermentative bacteria: a short circuit in the biological nitrogen cycle. FEMS Microbiol. Lett. 7:65–72.
5. Council for Agricultural Science and Technology (C.A.S.T.). 1976. Effect of increased nitrogen fixation on stratospheric ozone. Report no. 53. Iowa State University, Ames.
6. Crutzen, P. J., and D. Ehhalt. 1977. Effects of nitrogen fertilizer and combustion on the stratospheric ozone layer. Ambio 6:112–117.
7. Gamble, T. N., M. R. Betlach, and J. M. Tiedje. 1977. Numerically dominant denitrifying bacteria from world soils. Appl. Environ. Microbiol. 33:926–939.
8. Lowe, R. H., and J. L. Hamilton. 1967. Rapid method for determination of nitrate in plant and soil extracts. J. Agric. Food Chem. 15:359–361.
9. McElroy, M. B., S. C. Wolfsy, and Y. L. Yung. 1977. The nitrogen cycle: perturbations due to man and their impact on atomspheric N_2O and O_3. Philos. Trans. R. Soc. London 277B:159–181.
10. Nelson, D. W., and J. M. Bremner. 1970. Gaseous products of nitrite decomposition in soils. Soil Biol. Biochem. 2:203–215.
11. Payne, W. J. 1973. Reduction of nitrogenous oxides by microorganisms. Bacteriol. Rev. 37:409–452.
12. Sørenson, J. 1978. Capacity for denitrification and reduction of nitrate to ammonia in a coastal marine sediment. Appl. Environ. Microbiol. 35:301–305.
13. Yoshida, T., and M. Alexander. 1970. Nitrous oxide formation by *Nitrosomas europaea* and heterotrophic microorganisms. Soil Sci. Soc. Am. Proc. 34:880–882.

Measurement of Mycorrhizal Infection in Soybeans[1]

G. J. BETHLENFALVAY, R. S. PACOVSKY, AND M. S. BROWN[2]

ABSTRACT

Infection of soybean [Glycine max (L.) Merr.] roots by the vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatus* (Thaxt. *sensu* Gerd.) Gerd. and Trappe was assessed throughout the ontogeny of the symbiotic association. Degree of infection was evaluated by a histological method as percent infection and colorimetrically as mg chitin/g mycorrhiza. Correlation of data by the two methods was highly significant ($r = 0.99$) below 60% infection and not significant ($r = 0.62$) at higher levels. Assessment of infection by the histological method did not yield significantly different data above 60% infection. Data by the colorimetric method were statistically distinct at all levels of infection. The effects of biological and chemical contaminants on the colorimetric assay were determined. The fungal component of mycorrhizae was compared to purified chitin standards. Use of chitin for standard curves in the absence of degradation products from uninfected root materials significantly ($p < 0.05$) overstated the degree of infection. Colorimetric determination of fungal infection in mycorrhizae is recommended when high levels of infection are expected.

Additional Index Words: **chitin, *Glomus fasciculatus*, *Glycine max*, vesicular-arbuscular.**

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ASSESSMENT OF INFECTION by vesicular-arbuscular mycorrhizal (VAM) fungi (13, 17) is essential to establish their effect on the host plant (12), on other symbiotic (7) or parasitic (27) members of the association, or on the rhizosphere micorflora (3). Histological methods for calculating the levels of infection which are rapid and do not require specialized equipment have been recently reviewed (14). While such methods remain useful in estimating the level of fungal

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[3] Reference to a company or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

infection, they are subjective and prone to bias (14). The use of alternative or complementary methods for determining infection is therefore desirable.

A method to measure the endophyte quantitatively is based on the presence of chitin [poly-β-(1,4)-*N*-acetylglucosamine] or chitosan [poly-β-(1,4)-glucosamine] in fungal cell walls (4) and on the virtual absence of chitin (11) in higher plant tissues (19). Such methods make use of acid (29) or base (25) hydrolysis of hexosamine polymers and of subsequent chromophore development. These relatively simple colorimetric procedures (24, 30) permit quantitative determination of the fungal component in plant tissues. The chitin-based measurement of the extent of fungal contamination of plant matter has found widespread use (9, 16, 21) and was first adapted to VA mycorrhizae by Hepper (18) and Becker and Gerdemann (6). A limitation of the colorimetric assay lies in its sensitivity to plant and fungal degradation products (8, 26). Cell walls, therefore, which differ in chemical composition among plant groups (15) and with plant age (2) and, due to the presence of infection (10) or pigments (1,6), may interfere with the colorimetric assay, even though vigorous alkaline digestion recommended by Ride and Drysdale (25) reduces the possibility of interference. For these reasons, the use of internal controls [uninfected plant materials in conjunction with chitin or glucosamine (GlcN) standards] in the construction of standard curves for the calculation of fungal infection appears essential.

Table 1—Interference by plant and fungal degradation products with the colorimetric assay.

Contaminant†	A‡	GlcN-HCl equivalent§ (mg)
Cell-Wall Polymers		
Cellulose	0.06	2.0
Chitin	10.50	654.2
Cutin	0.04	1.4
Hemicellulose	0.05	1.5
Lignin	0.03	1.1
Pectin	0.04	1.3
Suberin	0.06	2.0
Monosaccharides		
N-acetylglucosamine	0.04	1.4
N-acetylgalactosamine	0.06	2.0
Arabinose	0.02	0.7
Galactose	0.02	0.7
Glucose	0.01	0.4
Glucuronic acid	0.03	1.1
Xylose	0.03	1.1
Proteins		
Serum albumin	0.02	0.7
Lysozyme	0.01	0.4
Amino Acids		
Alanine	0.02	0.7
Glutamate	0.01	0.4
Glycine	0.03	1.1
Proline	0.01	0.4
Serine	0.02	0.7
Heterocyclic Compounds		
Adenine	0.24	7.5
Nicotinic acid	0.03	1.1

† Alkaline hydrolysis was carried out on 1 mg of the cell-wall polymers as described in MATERIALS AND METHODS section. Other components were not degraded and were assayed at the 100-μg level.
‡ Absorbance in optical density units at 650 nm.
§ Glucosamine-HCl (GlcN-HCl) equivalent was calculated as μg of GlcN-HCl required to yield the same concentration of chromophore as 100 μg or 1 mg (cell wall polymers) of contaminant.

A comparison of methods, describing infection throughout the development of a specific plant-fungus association, is of interest to assess not only degrees of fungal infection, but the validity of each of the methods as well. The objective of this study was to compare and evaluate a histological and a colorimetric method for the assessment of VAM fungal infection throughout soybean plant ontogeny.

MATERIALS AND METHODS[3]

Growth Conditions

Soybean [*Glycine max* (L.) Merr. var. 'Kent'] plants were grown in 1.5-liter plastic pots in a greenhouse at Albany, Calif., from June to September 1980. Temperature and relative humidity varied seasonally and from day to day within the day/night ranges of 32/15 °C and 50/95%, respectively. Photosynthetic photon flux density (PPFD) averaged 500 μeinstein•m^{-2}•sec^{-1} (μE) at noon on sunny days and 300 μE on overcast days. Daylength was extended to 18 hours by Sylvania 1000 W metal halide lamps mounted vertically in parabolic reflectors and arranged to provide supplementary PPFD of 400 μE at plant emergence level. The growth medium in each pot consisted of 1.25 liters of perlite/sand mixture (2:1, v/v) covered by a 2.5-cm layer of perlite. This was flushed five days a week with a nutrient solution equivalent to 0.25-strength Johnson's solution (20) except for the concentration of P. Macronutrients consisted of 1 m*M* $CaCl_2$, 0.05 m*M* KH_2PO_4, 0.75 m*M* K_2SO_4, 0.25 m*M* $MgSO_4$, and 2 m*M* NH_4NO_3. Once a week the pots were flushed with deionized water. The concentration of available P was limiting to soybean growth (7) and varied daily between a known replenishment level and a 24-hour depletion level. Seeds were germinated for 2 days at 28 °C. Seedlings were selected for uniformity and either inoculated at planting with 10 g of soil containing approximately 50 spores of *Glomus fasciculatus* (Thaxt. *sensu* Gerd.) Gerd. and Trappe or left uninoculated as controls. The inoculum (obtained from S. Woodhead, Abbott Laboratories, Long Grove, IL 60047), originally grown on onions (*Allium cepa* L.), also contained approximately 110 root segments partially infected with *G. fasciculatus*. Control plants were initially watered with root- and spore-free washings (43 μm sieve) of the inoculm. Pots were rotated regularly to minimize positional effects. Plants were harvested at two-week intervals.

Histological Assay

At harvest, roots were washed throughly to remove the external mycelium. Lateral roots were excised from the taproot, aligned, cut into 1-cm segments, and dispersed in water. Randomly selected segments were stained according to Phillips and Hayman (22). Fifty segments were inspected for the presence of infection by *G. fasciculatus* under a dissecting microscope. Values of 1, 5, or 10 were assigned to segments showing trace, 50%, or 100% infection, respectively. Percent infection per plant was calculated by adding the numerical values assigned to individual segments and dividing the sum by the maximum value of 500.

Colorimetric Assay

Mycorrhizae and uninfected roots were dried at 80 °C for one day and ground in a Wiley mill (40 mesh). Ten mg of ground root material was suspended in 4 ml of a KOH solution (120 g KOH/100 ml H_2O) and autoclaved at 120 ° for 1 hour. Alkaline hydrolysis of chitin and centrifugation of the resulting chitosan in successive ethanol and water washes was carried out according to Ride and Drysdale (25). A wash with 0.01*N* HCl was substituted for the first water wash (9). Roots of uninfected control plants were assayed simultaneously with the mycorrhizae. The pellet containing plant and fungal degradation products was resuspended in water to a volume of 1.5 ml, followed by deamination, complete hydrolysis of the chitosan to 2,5-anhydromannose, and chromophore development (30). Absorbance at 650 nm was measured on a Bausch and Lomb Spectronic 20 spectrophotometer. Root material from the uninfected controls was added to chitin standards in amounts equal to the mycorrhiza used in the assays of fungal infection. This accounted for plant hexosamines (23) and compensated for chance contamination of root systems by chitin-containing

Table 2—Comparison of absorbance due to chitin with or without uninfected root material.

Chitin	Standard	
	Chitin without root	Chitin with root
μg	A†	
133.3	1.40 ± 0.05	1.60 ± 0.03*
66.7	0.74 ± 0.02	0.84 ± 0.04*
33.3	0.38 ± 0.01	0.47 ± 0.03*
0.0	0.0	0.12 ± 0.01‡

* Indicates significant differences ($P < 0.05$) between corresponding A values.
† Absorbance (A) in optical density units at 650 nm. Numbers represent means and SD of at least four replications.
‡ Values derived from plant glucosamine and degradation products.

organisms other than *G. fasciculatus*. The procedure was repeated for each level of infection at each successive two-week assay interval.

Standards and Contaminants

Purified chitin (derived from crab shells, Sigma Chemicals, Inc.) was used as the standard for the evaluation of fungal infection. Standards were determined by autoclaving chitin in the presence or absence of 10 mg of uninfected roots, according to Ride and Drysdale (25). Different times of autoclaving were used to determine the progress of chitin hydrolysis. A standard curve based on GlcN-HCl (not shown) was prepared using the same procedure as for chitin, but starting with the deamination step. Since colorimetric treatment (30) of GlcN-HCl results in complete chromophore development, the GlcN-HCl standard was used to calculate the degree of chitin hydrolysis by comparing absorbances. To account for the difference in the molecular weights of the chitin monomer and GlcN-HCl, the chitin/GlcN-HCl conversion ratio of 0.94 was applied to express GlcN-HCl in terms of chitin. All values obtained from the chitin standard curve were recalculated to account for the incomplete (37.2%; Table 3) hydrolysis of chitin. Organic materials expected to result from plant or fungal degradation were screened to determine their effect on the colorimetric assay. The amount of interference by 100 μg of contaminant was calculated as the amount of GlcN-HCl required to yield the same concentration of chromophore.

RESULTS AND DISCUSSION

Colorimetric Assay Conditions

Chitin is hydrolyzed by autoclaving in concentrated alkali to a partially de-acetylated mixture of chitosaccharides (11, 25). Complete depolymerization and deacetylation of chitin to GlcN, essential for quantitave determination, was not obtained. An autoclaving time of 1 hour was used, as this appeared to be the optimum. Further autoclaving (2 hours) resulted in less color development, indicating the destruction of the chitosaccharide monomers during prolonged autocalving. The effect of trace amounts of the ethanol used in precipitating chitosan on the consistency of colorimetric results was noted by Hepper (18). We also tested acetone and ethyl acetate and found that ethanol interfered the least. Interference by major plant (2) or fungal (5) cell wall constituents with the colorimetric assay was tested. Interference in all cases was < 0.2% of the color due to chitin alone (Table 1). The levels of GlcN found in the uninfected root material (Table 2) were similar to the values obtained by Racusen and Foote (23) for leaf hexosamines.

Calculations of Chitin in Mycorrhiza

Fungal infection of plant materials expressed in terms of chitin (9) or GlcN (18, 25) have been based on GlcN

Table 3—Reactivity, in the colorimetric determination, of glucososamine-HCl (GlcN-HCl) and chitin hydrolyzed by autoclaving.

Absorbance	Chitin	GlcN-HCl†	Apparent Chitin‡
optical density at 650 nm	μg		%
1.40	133.3	66.9	36.6
0.74	66.7	34.0	37.3
0.38	33.3	17.2	37.8
			37.2§

†Amount of GlcN-HCl that produced the same chromophore concentration as corresponding amounts of chitin autoclaved for 1 hour.
‡ Percentage of chitin that reacted in the colorimetric assay after being autoclaved for 1 hour. Amounts of GlcN-HCl used in this computation were adjusted by dividing by the theoretical chitin/GlcN-HCl conversion factor of 0.94. Percentages were based on four replications and were not significantly different ($P > 0.05$).
§ Mean of apparent chitin percentages. This value was used to calculate amounts of chitin obtained from chitin standard curves.

as the standard. This practice may need reconsideration. A GlcN standard would be applicable to direct calculation of fungal chitin only if complete, but nondestructive, conversion of chitin to GlcN were achieved. Our results showed, however, that a GlcN standard would severely understate the amount of chitin present. Almost twice the theoretical amount of chitin was required to obtain the colorimetric results produced by a given amount of GlcN-HCl (Table 3). Chitin is perferable as a standard because it must undergo the same hydrolysis procedure as the biological materials. Plant materials of the same age, species, and tissue as those being tested for infection, however, must be added to the purified standard to take into account interference by plant degradation products and non-VAM chitin. The resulting mixture provides an internal standard from which the chitin content of infected tissue may be accurately calculated. Comparison of the standard curves based on pure chitin or its combination with root material thus showed significant differences (Table 2). Use of standard curves based on chitin alone would therefore significantly overstate the amount of chitin contained in mycorrhiza compared to root-containing standards (Table 4). Uninfected root materials from control plants were used in separate standard curves at each growth stage at which soybean plants were assayed for fungal infection. Differences in absorbance due to plant age were not statistically significant. When uninfected materials are not available, such as in field studies, the colorimetric assay is of limited usefulness as

Table 4—Comparison of chitin content of mycorrhizae in plants of different age calculated from different standards.

Plant age	Mycorrhizal chitin†	
	Chitin‡ without root	Chitin‡ with root
weeks	mg chitin/g mycorrhiza	
4	4.0 ± 0.5	1.7 ± 0.4*
6	7.4 ± 0.6	4.9 ± 0.5*
8	8.5 ± 0.6	5.8 ± 0.3*
10	22.5 ± 0.8	19.0 ± 0.7*
12	27.2 ± 0.3	23.4 ± 0.5*
14	38.8 ± 1.0	26.8 ± 0.9*

* Indicates significant ($P < 0.5$) differences between corresponding values by *t*-test.
† Numbers represent means and SE of five replications.
‡ Standards, from which mycorrhizal chitin was calculated.

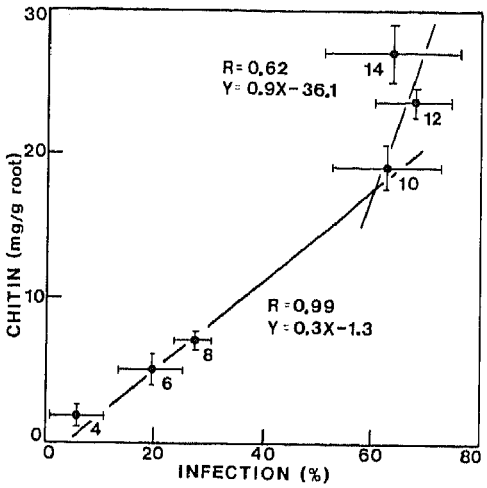

Fig. 1—Correlation of change in fungal infection in soybean mycorrhizae based on data from a histological (percent infection) and a colorimetric (mg chitin/g root) method. Numbers below data points indicate plant age (weeks). Mycorrhizae from plants aged 4 through 10 weeks were used to calculate the lower set of correlation data ($r=0.99$). The upper set of correlation data ($r=0.62$) was based on mycorrhizae 10 to 14 weeks old. Means and SD were based on five replicates.

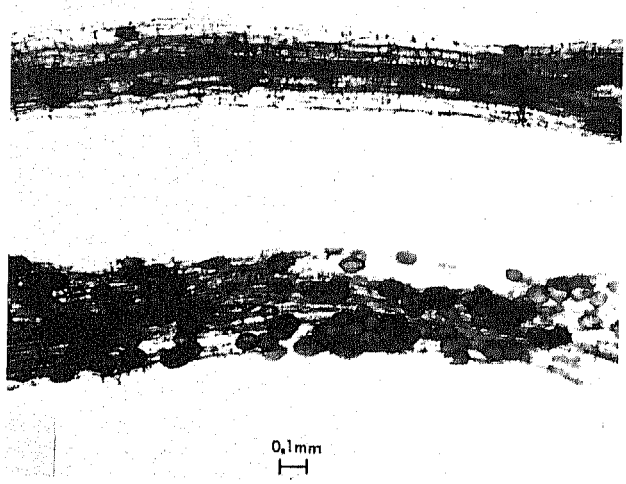

Fig. 2—Comparison of two mycorrhizal segments evaluated to be fully infected (arbitrary scale of 10) by the histological method.

it does not discriminate between VAM and non-VAM chitin.

Correlation of Histological and Colorimetric Data

Assessment of infection of soybean roots by *G. fasciculatus* by a histological and a colorimetric method showed a highly significant ($p<0.01$) correlation ($r=0.99$) up to approximately 60% infection as rated by the histological method (Fig. 1). Above this level, histological observations were not statistically different. Colorimetric measurements above 60% infection, however, clearly showed a significant increase in mycorrhizal chitin content with increasing plant age. Thus the chitin assay showed that three statistically different levels of chitin were present in VAM roots that were rated by histological procedures as being approximately 60% infected. Data from the two methods of assessment did not correlate significantly at an infection level above 60% ($r=0.62$). This is due to the difficulty in distinguishing between different intensities (number of fungal structures) at high levels of infection. Completely infected segments were scored a 10, although their intensity of infection differed (Fig. 2).

CONCLUSIONS

Differences in infection of soybeans by the VAM fungus *G. fasciculatus* were not significantly different at levels of infection higher than 60% by the histological method used. This is ascribed to the lack of an objective measure for the intensity of infection within a mycorrhizal segment. The usefulness of the histological method as a measure of percent infection thereby breaks down at some level of intensity of infection unless time-consuming counts of each fungal structure and cross-sectional observations (28) of mycorrhizal segments are made. The colorimetric assay yielded statistically distinct data at all levels of infection. It should therefore be considered as an alternative to the histological method when high levels of infection are expected. At low levels of infection, both methods appear to estimate infection satisfactorily. The extent of the external mycelium, which may be the crucial factor in determining growth enhancement of the host plant by VAM fungi, cannot, however, be inferred from either method.

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LITERATURE CITED

1. Abbott, L.K., and A.D. Robson. 1978. Growth of subterranean clover in relation to the formation of endomycorrhizas by introduced and indigenous fungi in a field soil. New Phytol. 81:575-585.
2. Albersheim, P. 1976. The primary cell wall. p. 225-274. *In* J. Bonner and J.E. Varner (ed.) Plant biochemistry. Academic Press, New York.
3. Bagyaraj, D.J., and J.A. Menge. 1978. Interaction between a VA mycorrhiza and *Azotobacter* and their effects on rhizosphere microflora and plant growth. New Phytol. 80:567-573.
4. Bartnicki-Garcia, S. 1968. Cell wall chemistry. Morphogenesis, and taxonomy of fungi. Annu. Rev. Microbiol. 22:87-108.
5. Bartnicki-Garcia, S. 1970. Cell wall composition and other biological markers in fungal phylogeny. P. 81-103. *In* J.B. Harborne (ed.) Phytochemical phylogeny. Academic Press, London.
6. Becker, W.N., and J.W. Gerdemann. 1977. Colorimetric quantification of vesicular-arbuscular mycorrhizal infection in onion. New Phytol. 78:289-295.
7. Bethlenfalvay, G.J., and J.F. Yoder. 1981. The *Glycine-Glomus-Rhizobium* symbiosis. I. Phosphorus effect on nitrogen fixation and mycorrhizal infection. Physiol. Plant. 52:141-145.
8. Boas, N.F. 1953. Method for the determination of hexosamines in tissues. J. Biol. Chem. 204:553-563.
9. Donald, W.W., and C.J. Mirocha. 1977. Chitin as a measure of fungal growth in stored corn and soybean seed. Cereal Chem. 54:466-474.
10. Esquerre-Tugaye, M.T., and D.T.A. Lamport. 1979. Cell surfaces in plant-microorganism interaction. I. Structural investigation of cell wall hydroxyproline-rich glycoproteins which accumulate in fungus-infected plants. Plant Physiol. 64:314-319.
11. Forester, A.B., and J.M. Webber. 1960. Chitin. Adv. Carbohydr.

Chem. 15:371-393.
12. Gerdemann, J.W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. Annu. Rev. Phytopathol. 6:397-419.
13. Gerdemann, J.W., and J.M. Trappe. 1974. The Endogonanceae in the Pacific Northwest. Mycol. Mem., no. 5.
14. Giovanetti, M., and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84:489-500.
15. Goodwin, T.W., and E.I. Mercer. 1972. p. 52-73. *In* Plant biochemistry. Pergamon Press, Oxford.
16. Hadwiger, L.A., and J.M. Beckman, 1980. Chitosan as a component of pea-*Fusarium solani* interactions. Plant Physiol. 66:205-211.
17. Hall, I.R., and B.J. Fish. 1979. A key to the Endogonaceae. Trans. Br. Mycol. Soc. 73:261-270.
18. Hepper, C.M. 1977. A colorimetric method for estimating vesicular-arbuscular mycorrhizal infection in roots. Soil Biol. Biochem. 9:15-18.
19. Jeuniaux, C. 1971. Chitinous structures. Compr. Biochem. 260:595-632.
20. Johnson, C.M., P.R. Stout, T.C. Boyer, and A.B. Carlton. 1957. Comparative chlorine requirements of different plant species. Plant Soil 8:337-353.
21. Mayama, S., D.W. Rehfeld, and J.M. Daly. 1975. A comparison of the development of rust fungi in resistant and susceptible wheat based on glucosamine content. Physiol. Plant Pathol. 7:243-257.
22. Phillips, J.M., and D.S. Hayman. 1970 Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment in infection. Trans. Br. Mycol. Soc. 55:158-160.
23. Racusen, D., and M. Foote. 1974. The hexosamine content of leaves. Can. J. Bot. 52:2111-2113.
24. Reissig, J.L., J.L. Strominger, and L.F. Leloir. 1955. A modified colorimetric method for the estimation of *N*-acetylamino sugars. J. Biol. Chem. 217:959-966.
25. Ride, J.P., and R.B. Drysdale. 1972. A rapid method for the chemical estimation of filamentous fungi in plant tissue. Physiol. Plant Pathol. 2:7-15.
26. Roseman, S., and I. Daftner. 1956. Colorimetric method for determination of glucosamine and galactosamine. Anal. Chem. 28:1743-1746.
27. Ross, J.P., and R. Ruttencutter. 1977. Population dynamics of two vesicular endomycorrhizal fungi and the role of hyperparasitic fungi. Phytopathology 67:490-496.
28. Strzemska, J. 1975. Mycorrhiza in farm crops grown in monoculture. p. 545-560. *In* F.E. Sanders, B. Mosse, and P.B. Tinker (ed.) Endomycorrhizas. Academic Press, London.
29. Toppan, A., M.T. Esquerre-Tugaye, and A. Touze. 1976. An improved approach for the accurate determination of fungal pathogens in diseased plants. Physiol. Plant Pathol. 9:241-251.
30. Tsuji, A., T. Kinoshita, and M. Hoshino. 1969. Analytical chemical studies on amino sugars. II. Determination of hexosamines using 3-methyl-2-benzothiazalone hydrazone hydrochloride. Chem. Pharmaceut. Bull. 17:1505-1510.

DIVISION S-4—SOIL FERTILITY AND PLANT NUTRITION

Seasonal Patterns of Growth and Soil Nitrogen Uptake by Rice[1]

P. A. MOORE, JR., J. T. GILMOUR, AND B. R. WELLS[2]

ABSTRACT

In order to model growth and nitrogen (N) uptake by the rice plant as well as improve nitrogen fertilizer management, a description of the temporal behavior of nitrogen in a flooded soil cropped to rice is needed. The objective of this study was to characterize the seasonal changes in soil, water, and plant nitrogen in a flooded rice culture. Lebonnet rice (*Oryza sativa* L.), a short-season variety, was grown on a Crowley silt loam soil (a fine, montmorillonitic, thermic Typic Albaqualf). Twelve plots were established utilizing a randomized complete block design with six controls, and six receiving 130 kg N/ha as urea in a three-way split. Amounts of inorganic soil nitrogen, as well as nitrogen uptake and dry matter production by the different plant fractions, were monitored at weekly intervals for 13 weeks.

Nearly 90 % of the preflood application and 95 % of all subsequent inorganic N peaks were exchangeable NH_4-N until the flood was removed. Although the preflood nitrogen application was accounted for in the soil, the midseason applications were virtually undetected. An increase in inorganic soil nitrogen occurred later in the season which was thought to be due to the decomposition of rice straw or algae. In the fertilized plants, 35% of the total dry matter production at harvest was found in the dead tissue, with 31% in the active-vegetative tissue and 34% in the reproductive tissue. Dry matter production for control plants was 23, 34, and 43% of the total, respectively, for the dead, active-vegetative, and reproductive tissues. Nitrogen concentrations in fertilized plants were significantly higher than controls until after tillering. Active-vegetative tissues in fertilized and control plants showed decreases in N concentration with time, while reproductive and dead tissue nitrogen concentrations remained relatively constant after the initial sample dates. Patterns of N uptake were similar to dry matter production. Sixty kg of N per hectare accumulated in control plants and 150 kg of N per hectare in fertilized plants. At harvest, 23, 28, and 49% of the total nitrogen uptake was in the active-vegetative, dead, and reproductive tissues of the fertilized plants, respectively. In control plants at harvest, 25, 16, and 59% of the uptake was in the active-vegetative, dead, and reproductive tissues, respectively. In a plot of plant N vs. soil N, the efficiency of N fertilizer use was 55% for the preflood fertilizer application. A relationship was found to exist whereby N in the reproductive tissue increased as the inverse of the N in the active-vegetative tissue increased.

Additional Index Words: **exchangeable ammonium, dry matter production.**

Moore, P.A., Jr., J.T. Gilmour, and B.R. Wells. 1981. Seasonal patterns of growth and soil nitrogen uptake by rice. Soil Sci. Soc. Am. J. 45:875-879.

MANY RESEARCHERS have studied the behavior of native and applied nitrogen (N) in submerged soils planted to rice (*Oryza sativa* L.) Several studies with varying degrees of emphasis on the soil and plant components have described the fate of nitrogen in this system (Mikkelsen and Finfrock, 1957; Patrick and

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