Bot. Bull. Academia Sinica **21**: 111-117 (1980)

EFFECTS OF DECAYING RICE STRAW ON GROWTH AND NITROGEN FIXATION OF A BLUE GREEN ALGA[1]

ELROY L. RICE

Department of Botany and Microbiology
University of Oklahoma, Norman, 73019 *U. S. A.*

CHU-YUNG LIN and CHI-YING HUANG

Department of Botany, National Taiwan University
Taipei, Taiwan 107, Republic of China

(Received April 3, 1980; Accepted April 23, 1980)

Abstract

Four of five phenolic compounds produced by decomposing rice straw were very inhibitory to the growth of *Anabaena cylindrica,* a nitrogen fixing blue-green alga. All except one inhibited nitrogen fixation (acetylene reduction) by this alga also. A combination of all five compounds was particularly effective in inhibiting growth and nitrogen fixation. This seems particularly significant because the five compounds always occur together in decomposing rice residues. Nitɹogen fixation by bluegreen algae is so important in rice culture that these experiments should be extended to field tests, with and without rice residues.

Introduction

It has been commonly observed that productivity of the second rice crop in a paddy is less than that of the first crop, so Chou and Lin (1976) studied the effects of decomposing rice residues in soil on the growth of rice plants. They found that aqueous extracts of decomposing rice residues in soil inhibited radicle growth of rice (*Oryza sativa* L.) and lettuce (*Lactuca sativa* L.) seedlings and growth of rice plants. Maximum toxicity occurred in the first month of decomposition and declined thereafter. Extracts of the soil in rice fields were also inhibitory to rice and lettuce, and the toxicity was persistent for 4 months. Five phytotoxins, *p*-hydroxybenzoic, *p*-coumaric, vanillic, ferulic, and *o*-hydroxyphenylacetic acids were identified from decomposing rice residues under waterlogged conditions; and several unknowns were isolated. Chou and

(1) This work was supported in part by grants from the National Science Council of the Republic of China.

Lin concluded that the decline in productivity of rice subsequent to the first crop is due chiefly to the allelopathic effects of decaying rice residues in the paddy soil.

Large amounts of chemical fertilizer including nitrogen, are required to maintain good productivity of rice (Huang, 1978). It would obviously be economically important if a considerable portion of the nitrogen could be furnished by biological nitrogen fixation. This could conceivably be accomplished either by rotating legume crops with rice or by inoculation of paddies with appropriate bluegreen algae, if conditions conducive to nitrogen fixation could be maintained. Chou *et al.* (1977) found that the quantities of leachable nitrate (NO_3^-) and ammonium-nitrogen (NH_4^+) were lower in paddies where the stubble was removed. No research was done, however, on effects of decaying rice straw on nitrogen fixation.

Filamentous bluegreen algae possessing heterocysts often have vigorous nitrogen fixing activity. It has been estimated that free-living bluegreen algae add from 13 to 70 pounds of nitrogen per acre per year to rice paddy fields (De and Sulaiman, 1950; Singh, 1961; Watanabe and Yamamoto, 1971). Galston (1975) reported that bluegreen algae associated with *Azolla* in rice paddies gave yields 50 to 100% greater than those obtained in adjoining rice paddies where *Azolla* was absent. Huang (1978) reported that inoculation of pots of rice with bluegreen algae increased grain production of the rice plants by 34 to 41% depending on the rice cultivar used.

Parks and Rice (1969) found that decomposing residues of many plants were very inhibitory to growth of several genera and species of nitrogen fixing bluegreen algae. Moreover, several phenolic compounds known to be produced by allelopathic plants significantly inhibited growth of several nitrogen fixing bluegreen algae also. One of these phenolics was *p*-coumaric acid which is produced by decomposing rice straw also. The goal of this project, therefore, was to determine effects of decomposing rice straw on growth and nitrogen fixation of a selected bluegreen alga.

Materials and Methods

Effects of Known Phenolics from Decomposing Rice Straw on Growth and Nitrogen Fixation by Anabaena cylindrica

Stock cultures of a pure strain of filamentous bluegreen algae, *Anabaena cylindrica*, were grown in a controlled environment room on a 16-hr photoperiod (3,000 lux-cool white fluorescent lights) at 28 C and a night temperature of 22 C. The algae were cultured with continuous aeration in 5 liter flasks which contained 2 liters of nitrogen-free nutrient medium, and 500 ml of sterized fresh medium were added to each flask each week after an equivalent amount of used medium was removed. This was to keep the algae growing continuously and vigorously. The medium selected for use after considerable testing with the algal strain involved, consisted of a combination of the macronutrients of Bold's Basal Medium and the trace elements of the Woods Hole Marine Biological Station Medium (Nichols, 1973) plus 248 mg per liter of H_3BO_3. The pH was set at 7.0, and the buffering was so effective that the pH was never found to vary any even after many weeks of growth.

The effects of the five phenolics, known to be produced by decomposing rice straw, were determined by adding them to 150 ml aliquots of the stock culture of *Anabaena* which were withdrawn after thorough agitation of the stock culture and added to sterilized 500 ml Erlenmeyer flasks. Each phenolic was tested in 10^{-3} M and 10^{-4} M concentrations and each concentration of each phenolic was run in quadruplicate. In addition, combinations of all five phenolics each in a 10^{-3} M or a 10^{-4} M concentration were tested similarly. Four control flasks were run with each phenolic tested and with each combination of phenolics. The test flasks were aerated and kept under the same environmental conditions as the stock cultures. After 5 days, the algal suspensions were filtered and the algal filaments were washed several times with distilled water. The filaments were transferred to a 150 ml serum bottle to determine the nitrogen fixing activity of the algae by the acetylene reduction method (Huang, 1978). Acetylene was injected into each bottle to give an acetylene partial pressure of 0.1 atmosphere. Exactly 1 hour after injection of the acetylene, 3 ml of 5N H_2SO^4 were injected into the serum bottle to terminate the acetylene reduction reaction. Samples of the gas phase were then withdrawn and the acetylene and ethylene were separated and quantitated by means of a gas chromatograph equipped with a glass column 0.8 m long and 3 mm i.d. packed with Porapak R, and a hydrogen ionization detector. The oven temperature was 65 C and the carrier gas was N_2 flowing at 30 ml per minute.

After measuring the acetylene reduction activity of the algae, the filaments were dried overnight in an oven at a temperture of 75 C and weighed.

Effects of Extracts of Decomposing Rice Straw on Growth and Nitrogen Fixation by Anabaena cylindrica

Rice straw consisting of a mixture of several cultivated varieties and hybrids was chopped into pieces 2–3 cm long and incorporated into soil in the ratio of 0, 25, and 100 g per 3 kg of air-dry soil. Each mixture was placed in a large plastic container and 2 l of distilled water were added. The containers with the mixtures were placed in a greenhouse room and the straw was allowed to decompose for 2 weeks. At the end of the decomposition period, an aqueous extract was obtained by squeezing the residue-soil mixture in several layers of cheesecloth and centrifuging for 20 min at 16,000×g. The extract was concentrated to one-tenth the original volume by flash evaporation and then was filter sterilized with 0.45 μ Millipore pads.

Five milliliters of concentrated sterile extract were added to 95 ml aliquots of the stock culture of *Anabaena* in sterile 250 ml Erlenmeyer flasks, making the final concentration of the extract only half the actual concentration in the soil. Culture conditions were the same as previously described. After 5 days, the nitrogen fixing activity and weights were determined. Three replicates of each extract were measured.

Results and Discussion

All the phenolics, except *p*-hydroxybenzoic acid, significantly inhibited growth of *A. cylindrica* in a 10^{-3} M concentration (Table 1). The combined phenolics, each in a 10^{-3} M concentration, significantly inhibited growth also. Ferulic acid was the only compound to significantly inhibit algal growth in a 10^{-4} M concentration. A 10^{-4} M concentration of *p*-hydroxybenzoic acid significantly stimulated growth of *Anabaena*. The combined phenolic phytotoxins (10^{-3} M) caused *Anabaena* to be very chlorotic, virtually white in appearance.

Ferulic and *p*-hydroxybenzoic acids, in 10^{-3} M concentrations, significantly inhibited N_2-fixation (acetylene reduction) by *Anabaena* (Table 1). A 10^{-4} M concentration of *p*-hydroxybenzoic acid significantly inhibited N_2-fixation also. *p*-Coumaric acid appeared to inhibit N_2-fixation but the difference from the control mean was not statistically significant. On the other hand, *o*-hydroxyphenylacetic and vanillic acids in 10^{-3} M concentrations significantly stimulated N_2-fixation in *Anabaena*, whereas 10^{-4} M concentrations had no effects. The combination of phenolic phytotoxins, each in a 10^{-3} M concentration, completely eliminated N_2-fixation in *Anabaena*. This seems particularly significant, because these compounds always occur together in decaying rice straw. Moreover, it indicates that the inhibitory effects of some of the phytotoxins override the stimulatory effects of others on N_2-fixation.

The extract of the highest straw-soil concentration significantly stimulated growth and nitrogen fixation (acetylene reduction) (Table 2). These results were somewhat surprising initially in view of the fact most known phenolics inhibited growth and nitrogen fixation by *A. cylindrica*. On the other hand, the final concentration of each extract tested in this experiment was only half that if the soil. Moreover, most of the 10^{-4} M concentrations of the known phenolics did not inhibit nitrogen fixation significantly either. Another factor which could have affected results is that the sterile extracts were allowed to

Table 1. *Effects of phenolics produced by decomposing rice straw on growth and acetylene reduction (N_2-fixation) in* ***Anabaena cylindrica*** *(mean±S.E.), D.F.=6*

Treatment		Dry Weight of Algae (mg)	C_2H_4 (μmoles/g/hr)
	Control	120.0±12.4	1.36±0.59
p-Coumaric Acid	10^{-4} M	142.4± 7.9	1.03±0.17
	10^{-3} M	62.3± 1.6[a, b]	0.61±0.09
	Control	70.6± 0.9	0.13±0.01
Ferulic Acid	10^{-4} M	60.8± 0.8[a]	0.15±0.01
	10^{-3} M	51.2± 2.5[a, b]	0.07±0.02[a, b]
	Control	76.5± 3.8	6.65±0.49
p-Hydroxybenzoic Acid	10^{-4} M	136.5± 7.6[a]	1.64±0.36[a]
	10^{-3} M	77.0± 5.8	0.75±0.31[a]
	Control	130.4± 5.1	0.18±0.07
o-Hydroxyphenyl-acetic Acid	10^{-4} M	141.6± 3.5	0.14±0.01
	10^{-3} M	79.6± 9.9[a, b]	1.21±0.43[b]
	Control	142.3±21.3	0.35±0.06
Vanillic Acid	10^{-4} M	134.9± 5.1	0.36±0.02
	10^{-3} M	89.9± 7.3[a, b]	1.49±0.53[a]
	Control	123.0± 8.2	2.28±0.51
All of Above[c]	10^{-4} M	118.1± 7.9	5.36±2.88
	10^{-3} M	63.1± 2.6[a, b]	0.00±0.00[a]

[a] Significantly different from respective control at 0.05 level or better.
[b] Significantly different from 10^{-4}M at 0.05 level or better.
[c] The indicated molarity is for each of the five phenolics in the mixture.

Table 2. *Effects of extracts of decomposing rice straw on growth and acetylene reduction (N_2-fixation) in* ***Anabaena cylindrica*** *(mean±S.E.)*

Ratio of Rice Straw to Soil	Dry Weight of Algae (mg)	C_2H_4 (μmoles/g/hr)
0 g/3 kg (Control)	79.5±9.7	0.50±0.01
25 g/3 kg	92.3±5.8	0.59±0.07
100 g/3 kg	111.2±3.7[a]	0.91±0.16[a]

[a] Difference from respective control significant at 0.05 level or better (D.F.=4).

set in the refrigerator for several weeks prior to using them. Some allelopathic agents are known to break down rather rapidly even when stored in a refrigerator, and their effective action depends on a constant production in the natural habitat. It is possible, therefore, that a concentration of fresh soil extract equivalent to that in the soil might inhibit nitrogen fixation by *Anabaena*.

It appears likely that concentrations of the known phenolic phytotoxins become high enough at times in decomposing rice straw in soil to slow the rate of nitrogen fixation by bluegreen algae in rice paddies. Obviously, more research is needed, however, before a definite conclusion can be made. Such research should include measurements under actual field conditions, with and without rice straw residues.

Acknowledgements

This research was done while the senior author was a visiting professor in the Department of Botany at the National Taiwan University. We are grateful to Dan-Wen Wang who was our fulltime research assistant, and to faculty and staff members who furnished space, equipment and suggestions.

Literature Cited

CHOU, C.H. and H.J. LIN. 1976. Autointoxication mechanism of *Oryza sativa* L. Phytotoxic effects of decomposing rice residues in soil. J. Chem. Ecol. **2**: 353-367.

CHOU, C.H., T.J. LIN and C.I. KAO. 1977. Phytotoxins produced during decomposition of rice stubbles in paddy soil and their effect on leachable nitrogen. Bot. Bull. Academia Sinica **18**: 45-60.

DE, P.K. and M. Sulaiman. 1950. Influence of algal growth in the rice fields on the yield of crops. India J. Agr. Sci. **20**: 327-342.

GALSTON, A.W. 1975. The water fern-rice connection. Natural Hist. **12**: 10.

HUANG, C.Y. 1978. Effects of nitrogen fixing activity of blue-green algae on the yield of rice plants. Bot. Bull. Academia Sinica **19**: 41-52.

NICHOLS, H.W. 1973. Growth media-frdshwater. pp. 7-24. **In** J.R. Stern (ed.) Handbook of Phycological Methods. Cambridge University Press, London.

PARKS, J.M. and E.L. RICE. 1969. Effects of certain plants of old-field succession on the growth of blue-green algae. Bull. Torrey Bot. Club **96**: 345-360.

SINGH, R.N. 1961. Roles of blue green algae in nitrogen economy of Indian agriculture. Indian Council of Agric. Res., New Delhi. p.157.

WATANABE, A. and Y. YAMAMOTO. 1971. Algal nitrogen fixation in the tropics. pp. 403-413. **In** T.A. Lie and E.G. Mulder (eds.) Biological Nitrogen Fixation in Natural and Agricultural Habitats. Special Volume of Plant and Soil.

稻幹腐化對藍綠藻的生長及其固氮作用的影響

萊斯 (E. L. Rice)

美國奧克拉荷馬大學植物及微生物學系

林 秋 榮　　黄 啓 穎

國立臺灣大學植物學系

念珠藻（Anabaena Glindrica）的固氮作用對水稻的生長及產量有促進的作用，惟稻幹腐化過程中所釋放出來的5種酚類化學物質，*p*-hydroxybenzoic acic, *p*-coumaric acid, vanillic acid, ferulic acid, 以及 *o*-hydroxyphenylacetic acid, 除了 *p*-hydroxybenzoic acid 對念珠藻的生長及固氮作用沒有抑制現象外其他4種化學物質均可產生毒害作用。尤其是上述5種化學物質混合存在時其所產生的毒害作用更爲嚴重，而此種多類化學物質混合一起所引起的毒害加强作用則是於稻幹腐化過程常發生的現象。