

PHYTOTOXINS PRODUCED DURING DECOMPOSITION OF RICE STUBBLES IN PADDY SOIL AND THEIR EFFECT ON LEACHABLE NITROGEN^(1,2)

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(Received September 15, 1976; Accepted October 31, 1976)

Abstract

A field experiment was designed to determine the effect of different rotation systems in paddy fields on the growth and yield of rice crops. Soil extracts from experimental sites were determined for their phytotoxicity and the dynamic distribution of phytotoxins. The amount of phytotoxicity and phytotoxic phenolics were higher in the plots where residues were left in the soil than where the residues were removed, and the quantity of leachable soil nitrogen ions, NO_3^- and NH_4^+ , was lower in the former than the latter. Seven phytotoxic phenolics were identified, and the amount of compounds varied with culture treatments and sampling times. The results, therefore, indicate that the more rice stubble is left in the paddy soil, the higher will be the phytotoxic phenolic and the less amount of leachable nitrogen. This study confirms our previous findings that the growth of rice is affected by the phytotoxins produced during the decomposition of rice stubbles in soil.

Introduction

In cultivated fields, severe reduction of crop yield has been recognized as primarily due to a continuous monoculturing. The debris of crop plants in the soil is not always beneficial but may be harmful to the growth of succeeding crops (Börner, 1960; Chou and Lin, 1976; Chou and Patrick, 1976; McCalla, 1971; Patrick, 1971; Rice, 1974; Wang *et al.*, 1967a, 1967b). Chou and Lin (1976) demonstrated that the growth of rice seedlings was greatly suppressed when rice residues were mixed with soil and allowed to decompose for one month. They found that phytotoxicity reached a maximum at the early stage of decomposition (first month), and gradually declined thereafter. The aqueous extract from paddy soil also showed high phytotoxicity. However, due to the dynamic fluctuation of climatic conditions and the complexity of

(1) This study was financially supported by the National Science Council, Grant NSC-65B-0409-03(05), the Republic of China.

(2) Paper No. 191. of the Scientific Journal Series, Institute of Botany, Academia Sinica.

edaphic situation, the phytochemical nature of phytotoxins has not yet been fully understood, and the interaction of phytotoxins on available soil nitrogen has scarcely been studied. Thus, this study aims to elucidate the nature of phytotoxic substances under various culture treatments and to discover the interaction of rice stubbles with nitrogen ions, NH_4^+ and NO_3^- , in paddy soil.

Materials and Methods

Field experimental design

To understand the phytochemical nature of paddy soil, and the yield components and vegetative characters of rice, an experiment carrying field layout of a 4-fold split-plot design with 2 replications was conducted in Nankang, Taipei, Taiwan. The paddy field was planted for two seasons with rice; the seedlings of the first crop were transplanted on March 29, 1975 and those of the second crop on August 9, 1975. The first crop of rice was harvested between July 11-18, 1975, while the second one on November 26, 1975. During the first crop season, two plots were planted to beans (*Phaseolus vulgaris* L.), and two other plots were left unplanted and went to weeds, the remaining twelve plots were planted with two rice varieties (*Oryza sativa* var. Taichung 65 and Ai-chueh-wu-chien). Between the two rice crop seasons, the paddy field was allowed to lie fallow. During the fallow period, rice stubbles (straw plus root) were removed from 6 plots, and left on the plots in the rest. Unless otherwise stated, the treatment of the first crop season plots were as fallows; (1) bean, (2) control (weedy patch), (3) rice stubble removed, (4) rice stubble unremoved. During the second crop season, all 16 plots were planted with rice.

Vegetative characters of rice during growing stages

After rice was transplanted, the rice plants were sampled on the following dates: May 1 (33 days after transplanting), May 13 (45 days), May 20 (52 days), June (64 days), June 11 (74 days), June 21 (84 days), and July 1 (94 days), 1975 during the first crop season, and September 9 (31 days), September 23 (45 days), October 14 (66 days), November 4 (87 days), and November 25 (108 days), 1975 during the second crop season. Five rice plants were removed from each plot on each sampling day, and carefully washed with water to prevent root damage. The fresh weight, leaf dry weight, and number of tillers were measured.

Aqueous extraction of the paddy soil

For the bioassay of soil phytotoxins, aqueous extractions were made.

Thus, about 1 kg soil was collected from each plot every month. The sample was placed in a 2-liter plastic container, to which was added 1 liter of distilled water; after stirring, the soil-water mixture was centrifuged at 5000 rpm, and the supernatant was stored in a freezer for analysis. The residual soil was reserved for further use. The final volume of the extract was about 1000–1100 ml, in which one fourth of the extract was saved for chemical analysis, and the remainder was concentrated to one fifth of the volume (called 5X) for bioassay.

Alkaline alcohol extraction of soil toxins

The soil after aqueous extraction was further extracted to isolate phytotoxic phenolics by using the techniques described by Chou and Young (1975). The final clean ethanol solution obtained by ether fraction of alkaline alcoholic extraction was adjusted to 2 ml with 95% ethanol and stored in a 5 ml vial. This solution was further analyzed by paper chromatography for quantitative determination of phytotoxic phenolics.

Bioassay of the aqueous extracts of paddy soils

Three bioassay techniques were employed to determine the phytotoxicity of soil extracts. The sponge bioassay technique as described by Muller (1966) was conducted, using lettuce seeds as test material. The second bioassay technique, a modification of the first, using presoaked rice seed as test material as described by Chou (1976). These two bioassays were incubated at 25°C for 72 hr. The length of radicle and coleoptile were measured after incubation. The third bioassay was to determine the phytotoxic effect of extracts on the root initiation of hypocotyl cutting of mungbean seedling (7-day old). This bioassay technique was also described by Chou and Lin (1976). The number of root initiations from the hypocotyl cutting were counted after 72 hr incubation at 25°C.

Identification of phytotoxic phenolics in paddy soils

After the isolation of phytotoxic substances from paddy soil, the phytotoxins were identified by paper chromatography. About 50 μ l (1 μ l/0.05 g soil) of the ethanolic solution (obtained from the ether fraction of alkaline alcoholic soil extract) was chromatographed by a paper strip as one dimension and by 46×46 cm Whatman 3 MM chromatographic paper as two dimension chromatography. The synthetic chemicals were also simultaneously developed by a solvent of 2% glacial acetic acid and BAW (*n*-butanol: acetic acid: water, 4:1:5, v/v/v). The developed paper was then sprayed with three detecting reagents: (1) diazotized *p*-nitroaniline (DPNA), followed by 10% sodium

carbonate (Hais and Mecek, 1963); (2) 2,6-dichloroquinone chlorimide (DQC), followed by saturated sodium borate (Vázquez *et al.*, 1968), and (3) 0.3% ethanolic ninhydrin solution. The chromatograms were also examined under a short wavelength U. V. light, and to reveal absorption or fluorescence.

Determination of total soil phytotoxic phenolics

To make quantitative comparison of phytotoxic phenolics in the soil, a modified method of Kuwatsuka and Shindo (1973) was used. The synthetic phenolic compounds, *p*-hydroxybenzoic, *p*-coumaric, *o*-hydroxyphenylacetic, vanillic, ferulic, syringic, and *trans*-*o*-coumaric acids, all purchased from Wako Pure Chemical Industries, LTD, Japan, were prepared in a series of concentrations in 95% ethanol. To 50 μ l of each compound, 1 ml of 20% Na_2CO_3 and 150 μ l of Folin-Ciocalteu reagent (purchased from the same company) was thoroughly mixed, and allowed to stand for 20 min at room temperature, and the optical density was measured at 700 nm by using a Shimadzu Spectronic 20.

Determination of leachable soil nitrogen in the extracts

To measure the leachable soil nitrogen ions, NH_4^+ and NO_3^- , a method described by Bear (1964) was used. The concentrated 5X solution of the aqueous extract was determined.

Results

Effect of culture treatments on the vegetative characters of rice

The fresh weight of the rice was significantly differ in two crop seasons (Fig. 1A). In the first crop season, the rice grew slowly in the early stage (45 days after transplanting) and reached its maximum weight around the 90th day. However, in the second crop season the rice grew vigorously in the early stages and reached its maximum around the 6th day after transplanting, then the growth rate was significantly reduced. Fig. 1A shows that the leaf fresh weight of rice plants differed depending on the preculture treatment of the soil. Rice grown following beans grew differently than when following rice or weeds. The rice grown on plots with residues left on the soil grew better than when the residues were removed. These results seem contradictory to our hypothesis that the rice stubbles left in field would suppress the rice growth.

On the basis of leaf dry weight, the growth was similar to that shown for fresh weight as Fig. 1A. However, insignificant difference was found between the preculture treatments before the rice seedlings were transplanted (Fig. 1B).

Fig. 1A

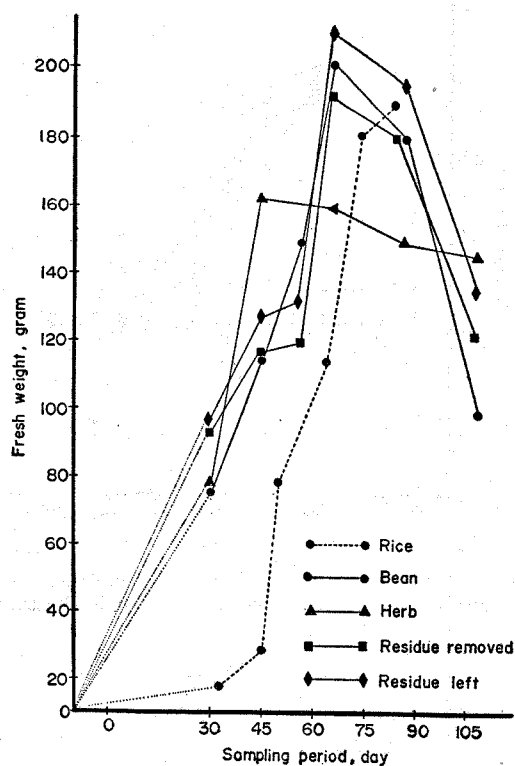


Fig. 1B

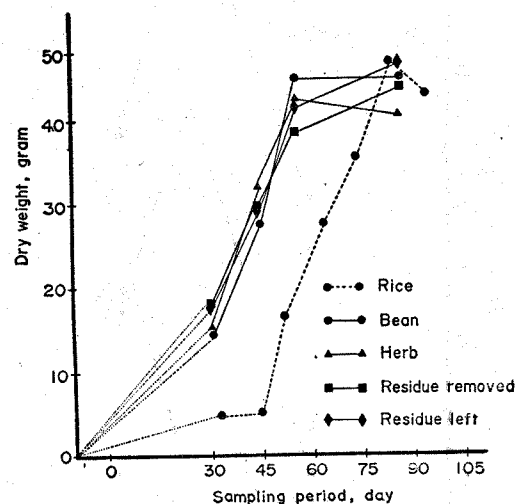


Fig. 1. The average fresh weight (Fig. 1A) and the average dry weight (Fig. 1B) of rice plants sampling during different growing times for various culture treatments. The dash line (●---● Rice) indicate the samples was obtained at the first crop season, and the solid lines indicate the samples from the second crop season. In the first crop season, 2 plots of the paddy field were planted with beans, 2 plots left unplanted and went to herbs, and 12 plots planted with rice; after rice harvesting, the rice residues were removed from 6 plots and left unremoved from the rest.

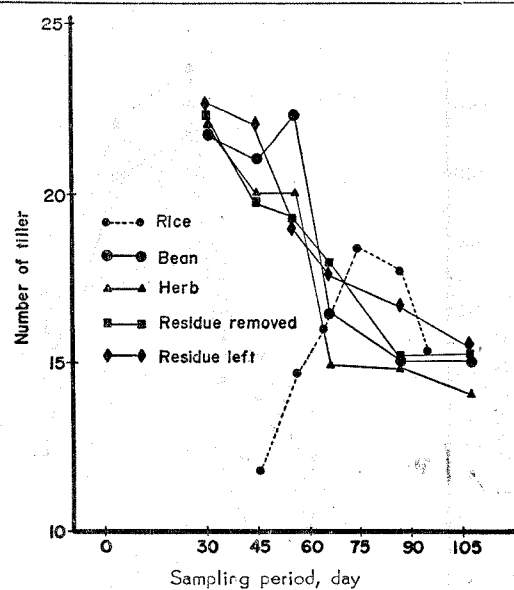


Fig. 2A. The average number of tiller of rice plants sampling during the growing times for various culture treatments. The description is the same as shown in Fig. 1.

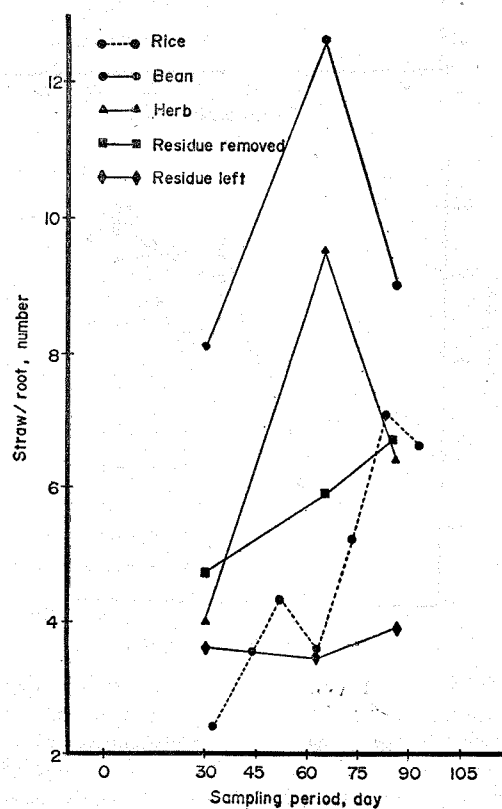


Fig. 2B. The average number of straw-root ratio of rice plants sampling during different growing times for various culture treatments. The description is the same as shown in Fig. 1.

The tillers of rice plant were also different between two crop seasons (Fig. 2A). The tillers in the early stage of the second crop were more than those in the first crop. Among the preculture treatment plots, the tiller number of rice plants was insignificant, except it was high in the treatment of bean plot on the 56th day after transplanting. The results of straw/root ratio are shown in Fig. 2B. The ratio differed with preculture treatment. Plants grew in the plot where was precultured with bean gave the highest ratio, while plots with stubbles the lowest.

Relative phytotoxicity of aqueous extracts in paddy soil

Soil extracts concentrated to 5X solution were bioassayed against rice and lettuce seeds. Bioassay results of soil extracts in the first crop are shown in Fig. 3. The extracts were found non-toxic to lettuce but were phytotoxic to the radicle growth of rice exhibiting 25% to 50% inhibition. Among the treatment plots, the herb-plot extract exhibited phytotoxicity significantly higher than the other plots. The phytotoxicities of soil extracts were variable during the sampling time.

The results of aqueous extracts (5X) obtained from the second crop season are shown in Fig. 4. Significant phytotoxicity was found in the soil extract, and rice seeds as the test material seemed more sensitive to the extracts. The results showed that the extracts obtained from the rice stubble left plots retarded the growth of rice radicle more severely than that obtained from the stubbles removed plots. The extract from plots precultivated with bean seems less phytotoxic to rice and lettuce. This indicated that the preculture treatments significantly affected the phytotoxicity of soil extracts.

An investigation, furthermore, was attempted to understand the inhibition of adventitious root initiation from the hypocotyl cuttings of mungbeans to see if they were affected by rice soil extracts. The results showed that root initiation of mungbeans was significantly retarded being 30% to 50% inhibition by the extracts mentioned. The inhibition was variable with sampling time and with the extracts obtained from various culture treatments as indicated above.

The dynamics and distribution of phytotoxins in paddy soil

Inasmuch as phytotoxicity was present in the aqueous extracts of paddy soil (Fig. 3 and 4), an attempt was made to identify the responsible phytotoxin. By means of paper chromatography, alkaline alcoholic extractable compounds were identified to be ferulic, *trans-p*-coumaric, vanillic, *p*-hydroxybenzoic, *cis-p*-coumaric, and *o*-hydroxyphenylacetic acids (Table 1) and several unidentified compounds. The dynamic distribution of the aforementioned com-

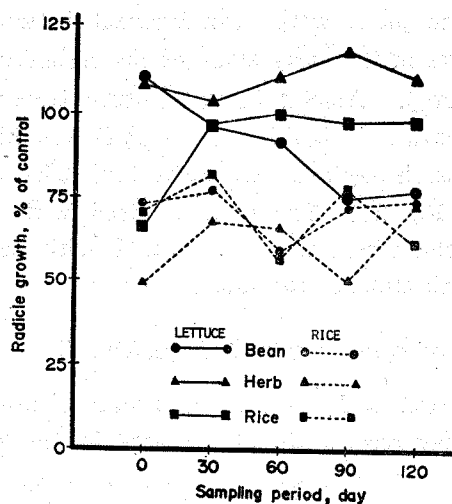


Fig. 3. The phytotoxic effects of aqueous soil extracts on the radicle growth of rice and lettuce. The extracts were made at the growing period of the first crop season.

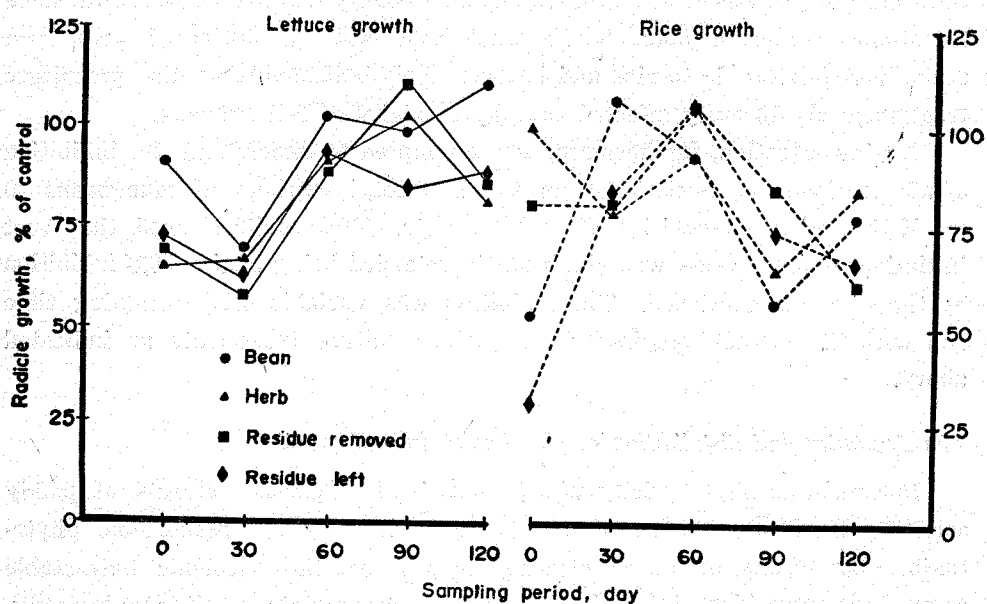


Fig. 4. The phytotoxic effects of aqueous soil extracts on the radicle growth of lettuce and rice. The extracts were made at the growing period of the second crop season.

Table 1. *The dynamics and distribution of phytotoxins in various soils*

The compounds were alkaline alcohol extractable, and the quantitative comparison of each toxin was given in order as

‡‡ > ‡: > ‡‡ > +: > +: > : > — (none).

Treatment plot	Extraction date (days) ⁽¹⁾	Compound ⁽²⁾					
		FA	<i>tp</i> CA	VA	<i>p</i> HBA	<i>c</i> <i>p</i> CA	<i>o</i> HPAA
Bean	0	+	‡‡	‡‡	+:	+:	:
	30	:	+:	+	+	+	:
	60	‡‡	‡‡	‡‡	:	:	:
	90	+	+:	+:	:	+:	:
	120	:	‡‡:	‡‡	+	:	:
Control (Herb)	0	+	‡‡	‡‡	+:	+:	+
	30	+	‡‡	+	+:	+:	:
	60	‡‡	‡‡	‡‡	+:	+:	:
	90	—	+	+	:	:	:
	120	:	‡‡:	‡‡:	+:	:	:
Rice stubbles removed	0	:	‡‡:	+:	+	+:	+
	30	+	‡‡	‡‡	+	+	+
	60	:	‡‡	+	:	+	:
	90	—	‡‡	+	:	+	:
	120	:	‡‡	‡‡	+	+	:
Rice stubbles unremoved	0	:	‡‡	‡‡	+	+:	+
	30	+	‡‡:	‡‡	+	+	:
	60	:	‡‡	+:	:	+	:
	90	—	‡‡:	:	:	+	:

(1) Date of soil sampling after rice seedlings were transplanted.

(2) Abbreviation of compound: FA=ferulic acid, *tp*CA=*trans* *p*-coumaric acid, VA=vanillic acid, *p*HBA=*p*-hydroxybenzoic acid, *c**p*CA=*cis* *p*-coumaric acid, *o*HPAA=*o*-hydroxyphenylacetic acid.

pounds seems irregular with the sampling time and with the preculture plots, being insignificantly different. However, the amount of toxin was significant between the compounds. Phytotoxins, *p*-coumaric and vanillic acids were the predominant compounds.

Determination of phenolic content in soil

Using the method described earlier, the linear regression equation of each standard curve of phenolics was obtained, and the curves are shown in Fig. 5. By using these equations (shown on Fig. 5) and curves, the total content of phenolics in the soil was obtained (Table 2). The data shown in Table 2

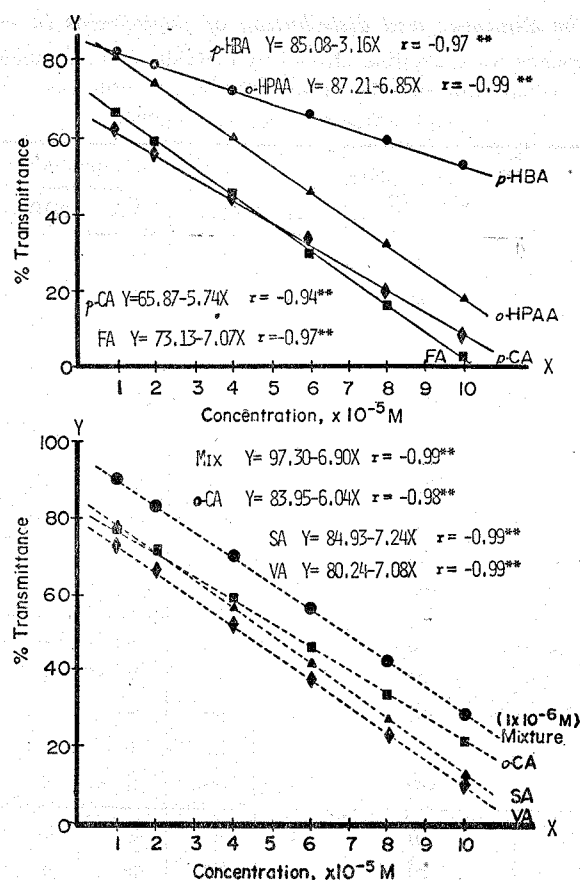


Fig. 5. The standard absorption curves of phenolic acids. The linear regression equation of each compounds and their mixture was shown on the figure. The symbol of Y expresses the transmittance of optical density, X expresses the concentration, r expresses the correlation coefficient. About $50 \mu\text{l}$ of each or mixed compound was added with 1 ml of 20% Na_2CO_3 and $150 \mu\text{l}$ of Folin-Ciocalteu reagent, and were measured at 700 nm.

was obtained from at least 4 replications. Thus, the amount of total phenolics in the soils of the first crop season was significantly higher than that in the second crop season. In the first crop season, although the quantity of phenolics in the preculture-bean plots was generally higher than the herb and rice plots, the statistical data showed insignificantly among them. In the second crop season, the herb and rice stubble unremoved plots exhibited higher phenolic content than the bean and rice stubbles removed plots. Particularly, the phenolic content on the 30th and 60th days extraction was significantly higher than that on the rest of extractions. The quantity of phenolics in soil seemed gradually decreasing but persisted until rice harvesting.

Table 2. *Quantitative comparison of total phenolics in various soil extracts made at different date after rice transplanting*

The data were obtained from the average of at least 4 replications.

Crop season	Treatment plot	Concentration, $\times 10^{-6}$ M					
		Soil extraction date, days ⁽¹⁾					Means
		0	30	60	90	120	
First	Bean	7.90	10.89	11.47	6.91	8.56	9.14
	Herb	9.28	7.84	8.89	6.10	5.14	7.41
	Rice	9.85	9.85	3.97	10.47	8.43	8.51
Second	Bean	1.49	7.59	6.27	6.30	5.37	5.40
	Herb	5.74	5.18	8.29	6.13	5.15	6.09
	Rice stubbles removed	5.11	7.68	6.24	3.83	4.50	5.47
	Rice stubbles unremoved	3.25	9.11	7.18	4.78	5.67	6.02
		Statistical analysis, LSD					
		5%			1%		
Between crop season		1.28			2.71		
Between treatment		1.83			3.54		
Between extraction date		1.42			2.60		

(1) The number indicated the days after rice transplanting.

Table 3. *The content of NH_4^+ in the aqueous extracts of soil collected from various plots at different days after rice transplanting⁽¹⁾*

Crop season	Treatment plot	Concentration, ppm					
		Soil extraction, days ⁽²⁾					Means
		0	30	60	90	120	
First	Bean	1.38	2.50	4.0	3.55	0.30	2.35
	Herb	0.01	0.01	4.0	0.88	1.05	1.19
	Rice	1.43	4.00	3.65	1.82	1.33	2.39
LSD between treatment		5%=1.04 1%=2.02,		between extraction days			5%=0.36 1%=0.65
Second	Bean	2.04	1.24	0.96	0.50	2.44	1.44
	Herb	1.89	1.41	0.95	0.92	2.98	1.63
	Rice stubbles removed	1.75	1.14	1.11	0.66	1.45	1.45
	Rice stubbles unremoved	1.28	1.09	1.09	0.94	2.30	1.20
LSD between treatment		5%=0.139 1%=0.236,		between extraction days			5%=0.045 1%=0.075

(1) The extracts for analysis were the original extracts for the first crop season, and the 5X concentrated extracts for the second, respectively.

(2) The number indicated the days after rice transplanting.

Quantitative distribution and dynamics of leachable nitrogen in soils

The leachable nitrogen ions, NH_4^+ and NO_3^- , in various soils sampled at different times were determined by Bear's method. The content of NH_4^+ in the soil collected from the first crop season was generally higher than that from the second. The difference was particularly significant in plots pre-culture with bean and rice. The original soil extracts in the first crop season revealed higher amount of NH_4^+ than the 5X concentrated soil extracts in the second crop (Table 3). When the extracts obtained from the first crop season were concentrated to 5X, the amount of NH_4^+ in the extracts was all above 4 ppm (Chou *et al.*, 1976 unpublished data).

The amount of NO_3^- ion in the aforementioned soils was also determined. The content of the ion was much higher in the first crop season than in the second (Table 4). Furthermore, soil collected from the bean treatment plot revealed the highest amount of nitrate nitrogen in both the two crop seasons, and the herb-treatment-plot soil was the lowest.

In conclusion, it is found that the concentration of leachable soil nitrogen, NH_4^+ and NO_3^- , was much higher in the first crop season than in the second crop. Plots grown with beans could produce a higher amount of nitrogen ions,

Table 4. *The content of NO_3^- in the aqueous extracts of soils collected from various plots at different date after transplanting*

The extracts were concentrated to one fifth of original volume (5X).

Crop season	Treatment plot	Concentration, ppm					
		Soil extraction, days ⁽¹⁾					Means
		0	30	60	90	120	
First	Bean	0.43	8.00	8.00	4.22	0.75	4.28
	Herb	8.00	0	0	0	0	1.33
	Rice	1.20	0	0.05	0	0	0.23
Second	Bean	0.64	0.33	0.02	0	0.02	0.20
	Herb	0.34	0.12	0	0	0.01	0.09
	Rice stubbles removed	0.30	0.21	0.15	0	0.01	0.13
	Rice stubbles unremoved	0.30	0.07	0.10	0.01	0.05	0.11
		Statistical analysis, LSD					
		5%			1%		
Between crop season		1.628			3.446		
Between treatment plot		2.388			4.630		
Between extraction day		1.880			3.430		

(1) The number indicated the days after rice transplanting.

which suggested that the available nitrogen could be developed when the field crops were rotated with beans. The certain amount of ammonium nitrogen would detoxicate the organic acids present in soil. The discussion on the interaction between phytotoxic organic acids and leachable nitrogen will be given in the later section.

Discussion

Chou and Lin (1976) reported that rice residues submerged in the soil revealed phytotoxicity and suppressed the growth of rice plant under waterlogged conditions. In addition, the soil extracts from paddy field also exhibited phytotoxicity. The present results confirmed their previous findings. The results of Fig. 1A, 1B, 2A, and 2B also agreed with those of Wu *et al.* (1975), in which they indicate that the yield of rice was significantly affected by of fallowing and rotation systems. The rapid decrease of tillers in the second crop season might be partly due to phytotoxins produced during the decomposition of rice stubbles in the soil. The phytotoxins were likely a variety of compounds, such as fatty acids, volatile organic acids, phenolics, and nitrogen containing compounds. In the present study, only the phytotoxic phenolics were determined, but the other phytotoxins mentioned may have also had some effects on the rice and they are not being reported in this paper. The concentration of the phytotoxic phenolics was somewhat higher on the 30th and 60th day of extraction during the second crop season (Table 2). During the early stage (first month) of the second crop season, the temperature was usually higher than 30°C, which could expedite the decomposition of rice stubbles in the soil, resulting in a large amount of organic acids produced (Chou *et al.*, 1976 unpublished data). If this was the case, the rapid decrease of tillers on the 45th day after rice transplanting in the second crop season would be logical. Furthermore, young rice seedlings are sensitive to toxic compounds. The retardation of rice plants in the second crop season is therefore clear.

Chandrasekaran and Yoshida (1973) reported that lack of available nitrogen did not cause the retardation of rice growth that occurred when green manure was added to the soil. They also indicated that ammonium sulfate effectively eliminated the injury caused by organic acids. The formation of methane in the rice field can be considered a detoxication of organic acids by methane bacteria. Thus, the phytotoxic organic acids, such as fatty acid and phenolics, can be detoxicated by ammonium ion, which was in higher amount in the first crop season than in the second crop (Table 3). Consequently, the phytotoxicity present in the paddy field could be reduced by ammonium ion in the first crop season. Chandrasekaran *et al.* (1973) also concluded that the

sulfate reducing bacteria may compete with propionic and butyric acid producing bacteria for carbohydrates in the soil. The change of microflora in soil may result in a change of toxic substances in soil, since some microorganisms are able to use phenolic acids as nutrient source, or convert the toxic organic acid to non-toxic form. Furthermore, microorganisms may produce toxic by-products during the decomposition of rice stubbles in soil. It is difficult to determine which phytotoxins come from the residues and which are produced directly by the microorganisms. Additionally, nitrogen-fixing and nitrifying bacteria may also be involved in the situation. Rice and his associates studied vegetation of abandoned fields in Oklahoma and found that plants in the first two successional stages produced substances that were very toxic to nitrogen fixing bacteria, whereas the climax plants produced substances inhibitory to nitrification (Rice 1965, 1974; Rice and Pancholy 1972, 1973, 1974; Blum and Rice 1969).

Turner and Rice (1975) reported that over 99% of the extractable ferulic acid was lost from decaying hackberry leaves in 300 days. They found microorganisms, *Rhodotorula rubra* and *Cephalosporium curtipis*, which actively metabolized ferulic acid. The reported pathway for ferulic acid breakdown is ferulic acid to vanillic acid to protocatechuic acid to β -keto-adipic acid. Wang *et al.* (1971) concluded that humic acid grows in soil which played a role of fixation and co-fixation polymerization of phenolic compounds. They indicated that ferulic and syringic acids were fixed in greater amounts than *p*-coumaric and vanillic acids. Fixation process may result phenolic acids in the soil becoming unextractable by the present extraction techniques. Thus, many studies involve the interaction between soil microorganisms, humic acid, and toxins need to be performed. We, furthermore, suggest that a crop rotation system should be developed to balance microorganism population in paddy soil which might be highly possible to eliminate the toxic substances produced during the decomposition of rice stubbles in soil.

Acknowledgment

The authors are indebted to Drs. S.C. Woo for his valuable criticisms and to H.P. Wu for his suggestion of the field experimental design. We also thank Dr. C.E. DeVol (Professor of Botany, National Taiwan University) for reading the manuscript. Deep appreciation is also due to Dr. Thomas S.C. Wang (Advisor, Taiwan Sugarcane Corporation) for his encouragement throughout the study.

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水稻殘株留於水田經分解後產生的植物毒性物質及其對土壤中可淋溶性氮的影響

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在南港的水稻實驗田中處以不同輪作系統。於第一期作間，兩方區種四季豆，兩方區讓其荒蕪雜草叢生，另十二方區種以水稻。第一期作收割後，其中有六方區中的水稻殘株被移去，而另六方區中的殘株則埋入土中讓其分解，於第二期作間上述十六方區均種以水稻。於期間，水稻植株及土壤均被分析。水稻第一期作與第二期作的生長情形不同，土壤中所含植物毒性亦異。除去水稻殘株的土壤，其具有植物毒性的酚酸類化合物含量較不移去者為高。但土壤中可淋溶性氮含量却相反。在種植豆類的土壤中所含氮量均較其他處理的土壤為高。土壤中已有七種植物毒物質被鑑定出，其中以 *trans-p-coumaric acid* 及 *vanillic acid* 的量最高，*ferulic*, *p-hydroxybenzoic* 及 *cis-p-coumaric acids* 次之，而 *o-hydroxy-phenylacetic acid* 最低。另外有數個植物毒物質仍未被鑑定出。上述化合物的量亦因處理及採樣時間的變化而異，但並無顯著區別。此研究再度證明作者過去的研究指出水稻殘留物分解的植物毒物質可抑制水稻生長，並進一步證明植物毒物質對水稻生育期間的影響。種植豆類的輪作系統提供較佳的土壤環境以利水稻生長。