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Effect of Acetic, Propionic, and Butyric Acids on Young Rice Seedlings' Growth[1]

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ABSTRACT

Anaerobic bacteria in flooded soils degrade various types of organic matter, including rice straw and green manure crops, with the production of low carbon, monobasic aliphatic acids. The major fermentation products are formic, acetic, propionic, butyric, and lactic acids. Organic acids produced in flooded soils may adversely affect the growth of rice seedlings.

Rice seedlings, *Oryza sativa* L. 'Earlirose', were grown in sterile nutrient solution containing 1, 5, and 10 m*N* concentrations of acetic, propionic, and butyric acid adjusted to variables of pH 3.0, 5.0, and 7.0. The seedlings were grown in treated nutrient solutions for 7 days before harvesting for plant growth measurements.

Root elongation, shoot height, and weight were retarded by all organic acids, the severity increasing with concentration and decreasing pH. Root elongation was affected most. Residual seed reserves were greater with higher acid concentrations, corresponding to decreased growth of the plant root and shoot suggesting that the translocation of stored seed reserves was inhibited. The magnitude of organic acid effects was in the order of butyric > propionic > acetic. At 1 m*N* concentration, all organic acids evaluated, reduced root length by 40 to 50%, but did not inhibit root initiation. At 5 m*N* concentration, root elongation was severely inhibited and most root tips showed severe root injury. At 10 m*N* concentration, both elongation and initiation of roots were completely inhibited. An increase in pH from 3 to 7 decreased the inhibiting effects of all organic acids at all concentrations, indicating that the concentration of undissociated acid was more critical than total acid concentration in inhibiting seedling growth. Anaerobic fermentation products, particularly organic acids, are potential problems in rice stand establishment in flooded soils. A proper time interval for their decomposition prior to planting rice must be observed to avoid seedling damage.

Additional index words: **Organic acids, acetic, butyric, propionic, shoot elongation, root elongation, pH interactions, *Oryza sativa* L.**

INTERMEDIARY anaerobic decomposition products of organic matter accumulated under flooded conditions have toxic effects on the growth of rice (*Oryza sativa* L.) plants. Among the anaerobic products frequently observed to cause injury under flooded conditions are the lower-carbon aliphatic monobasic acids, formic, acetic, propionic, butyric, and lactic acids. The toxic effects of these ecids depend on the type of organic acid present and its concentration. Among the monobasic aliphatic acids, an increase in molecular weight tends to increase the inhibitory effects on rice seedlings [Takijima (7, 8, 9); Takijima et al. (11)]. Inhibition of root elongation by the acids studied is in the following order: > propionic > acetic > formic.

Rice roots are the plant parts shown to be generally most affected by harmful substances, including organic acids [Takijima (7, 8, 9, 10); Takijima et al. (11); Patrick et al. (5); Chandrasekaran and Yoshida (1)]. Studies on organic acid effects have been done in both a soil medium and in nutrient solution culture. Chandrasekaran and Yoshida (1) added 0.5 to 0.1 mmole of organic acids (formic, acetic, propionic, and butyric) per 100 g soil, transplanted 14-day-old rice seedlings, and grew them for 23 days. Their results show that, even at a concentration of 0.5 mmole/100 g soil, organic acids were harmful to rice seedlings, reducing the dry weight yields of plants. However, it is rather difficult to separate the effects of organic acids directly on the plants and their secondary effects (through effects on soil) in experiments where soil is used as a medium. Nutrient solution techniques are perhaps better suited since they minimize secondary effects. Tanaka and Navasero (12) grew 20-day-old rice seedlings in nutrient solutions containing acetic and butyric acids at concentrations of 0, 10, 20, and 40 m*N*. The pH was maintained at 4 and 6. Plant growth was retarded at 10 m*N* when pH was 4.0, whereas at pH 6.0 the toxicity of the acids with an increase in pH was due to a decrease in the concentration of undissociated acid at a higher pH. This seems to be the main factor controlling the toxicities of these acids, rather than total concentration. The organic acid concentrations used by these workers were rather high and there may be a function of varietal tolerance. Takijima (10), on the other hand, suggested that much lower concentrations of organic acids were toxic. He defined toxic level as the concentration that reduced root length 25%. The suggested toxic levels of some common organic acids are: formic, 3.2 m*N*; acetic, 4.6 m*N*; and butyric, 0.7 m*N*. Most of Takijima's experiments were conducted with much younger seedlings than used by Tanaka and Navasero (12), which may account for the differences.

Several investigators found nutrient uptake reduced at higher concentrations of organic acids. Takijima

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Table 1. Effects of organic acid concentration on 7-day-old rice seedlings.

	Root length				Plant ht.				Root dry wt.				Shoot dry wt.				Residual seed wt.			
	mN				mN				mN				mN				mN			
Acid	0	1	5	10	0	1	5	10	0	1	5	10	0	1	5	10	0	1	5	10
	cm								mg											
Acetic	10.98	8.90	7.58	6.24	9.78	6.67	5.98	5.71	3.71	3.15	2.92	2.39	4.66	3.81	3.72	3.56	15.50	14.20	16.43	17.68
Propionic	10.98	9.17	5.58	3.57	9.78	6.64	6.06	4.77	3.71	3.81	3.09	2.18	4.66	3.88	3.72	3.45	15.50	15.00	16.50	18.90
Butyric	10.98	7.63	4.04	2.48	9.78	6.08	5.67	4.77	3.71	3.62	2.88	2.10	4.66	3.64	3.56	3.87	15.50	13.92	17.43	17.90
L.S.D. 0.05	0.81				0.54				0.24				0.54				1.60			

Table 2. Effect of solution reaction and organic acids on 7-day-old rice seedlings.

	Root length			Plant ht.			Root dry wt.			Shoot dry wt.			Residual seed wt.		
	pH			pH			pH			pH			pH		
Acid	3	5	7	3	5	7	3	5	7	3	5	7	3	5	7
	cm						mg								
Control	9.15	11.15	12.63	7.25	9.85	12.25	2.49	3.83	4.80	4.23	4.43	5.31	15.14	16.85	14.25
Acetic	3.68	9.25	9.79	5.73	6.50	6.12	2.38	2.94	3.14	3.85	3.70	3.53	16.74	15.03	16.54
Propionic	2.66	6.21	9.44	5.24	6.31	5.92	2.41	3.25	3.42	3.75	3.80	3.52	17.61	16.09	16.70
Butyric	2.81	4.50	6.83	4.94	5.90	5.81	2.08	3.20	3.30	3.66	3.62	3.78	16.45	16.36	16.44
L.S.D. 0.05		0.70			0.46			0.21			ns			ns	

et al. (11) reported that concentrations above 1 mN reduced P, K, and Si in the rice plant. Tanaka and Navasero (12) reported a reduction of K with butyric acid but not with acetic acid, even up to 40 mN concentrations. Mitsui et al. (4) found that butyric acid (0 to 0.2 mole/liter) reduced uptake of K, P, NH_4-N, Mn, Mg, and Ca much more than did other physiological inhibitors studied, namely NaCN and NaN_3.

This reduction in nutrient uptake may be a result of organic acid effects on respiration. Robinson and Taylor (6) reported that acetic acid concentrations, 0.02 to 0.086 mg/ml, inhibited respiration of oat (*Avena sativa* L.) root tips up to 50% of the control at the maximum concentration. Tanaka and Navasero (12) studied respiration rates of rice roots with acetic and butyric acids at 10 and 40 mN and pH 4 and 7. At pH 4.0 both the acids at both concentrations inhibited respiration, while at pH 7.0 respiration rates were not affected much.

With the increased practice of soil incorporation of rice straw and other crop residues, as an alternative to burning, a greater incidence of seedling problems in rice stand establishment has been observed. The cause of seedling injury in a number of instances has been traced directly to the accumulation of low carbon-volatile fatty acids. In this research the toxic effects of acetic, propionic, and butyric acid were studied in respect to seedling height, shoot weight, root weight and elongation, and seed residues. Sterile nutrient cultures with seedling rice plants were used to minimize the effects of acids on nutrient uptake and secondary soil interactions.

MATERIALS AND METHODS

The present experiment observed the toxic effects of acetic, propionic, and butyric acids at 1, 5, and 10 mN as modified by solution pH's of 3, 5, and 7. Young rice (*Oryza sativa* L. 'Earlirose') seedling, still largely dependent on seed reserves for their energy source, were used to observe treatment effects.

Two sprouted rice seeds were transferred to a sterile blotting paper cut to fit 28-mm test tubes. The seeds were properly oriented, radical down, and then covered with a layer of wet cellulose tissue to hold them on the blotters. The blotters were then lowered into test tubes with 60 ml of nutrient solution containing the following nutrients: 7 ppm N, 3.9 ppm P, 9.5 ppm K, 2.5 ppm Ca, 6.1 ppm Mg, 12.1 ppm S, 3.5 ppm Si, 5.8 ppm Na, 4.0 ppm Fe, 0.25 ppm B, 0.25 ppm Mn, 0.025 ppm Zn, 0.01 ppm Cu, and 0.005 ppm Mo. The seeds were at least 2 to 3 cm above the level of the nutrient solution.

Before the nutrient solution was distributed to the test tubes, it was autoclaved at 6.81 kg (15 lb) of pressure at 107 C (225 F) for 1 hour to prevent microbial growth. Sufficient nutrient solutions and proper amounts of the respective organic acids (acetic, propionic, and butyric) were added to obtain the desired organic acid concentrations (1, 5, and 10 mN). The nutrient solution was thoroughly mixed, and any adjustment of pH was done with either NaOH or H_2SO_4. Then the nutrient solution was distributed into test tubes.

All possible combinations of the following factors were examined:

a) organic acids; acetic, propionic, and butyric.
b) concentrations; 1.0, 5.0, and 10.0 mN.
c) pH of the nutrient solution; 3.0, 5.0, and 7.0.

In addition, proper controls were established at all three pH's with all treatments replicated four times.

The test tubes, with treated nutrient solution and germinated seeds, were sealed with parafilm during the test period. The test tubes were held upright, in a growth chamber with controlled light and temperature. The conditions were as follows: temperature, day 23.9 C; night 21.1 C; day length 12 hours; light intensity 21,528 lux.

The seedlings were grown for 7 days and then harvested. Maximum root length and shoot height were measured. Later the shoots, roots, and seeds (residual) were dried in an oven at 75 C for 48 hours before the dry weights were recorded.

RESULTS AND DISCUSSION

Tables 1 and 2 present the growth responses of all the plant characters measured: root length, plant height, and dry weights of shoot, root, and residual seed with appropriate statistical analyses. Of all the characters studied, root elongation was the most sensitive response to acid concentration and pH variations in the external medium. A similar observation was reported by McCalla and Duley (3), Takijima, (7, 8, 9, 10); Kimber, (2); Patrick et al., (5). Root elongation has been used as an indicator of the effects of the factors studied in this experiment and are discussed later.

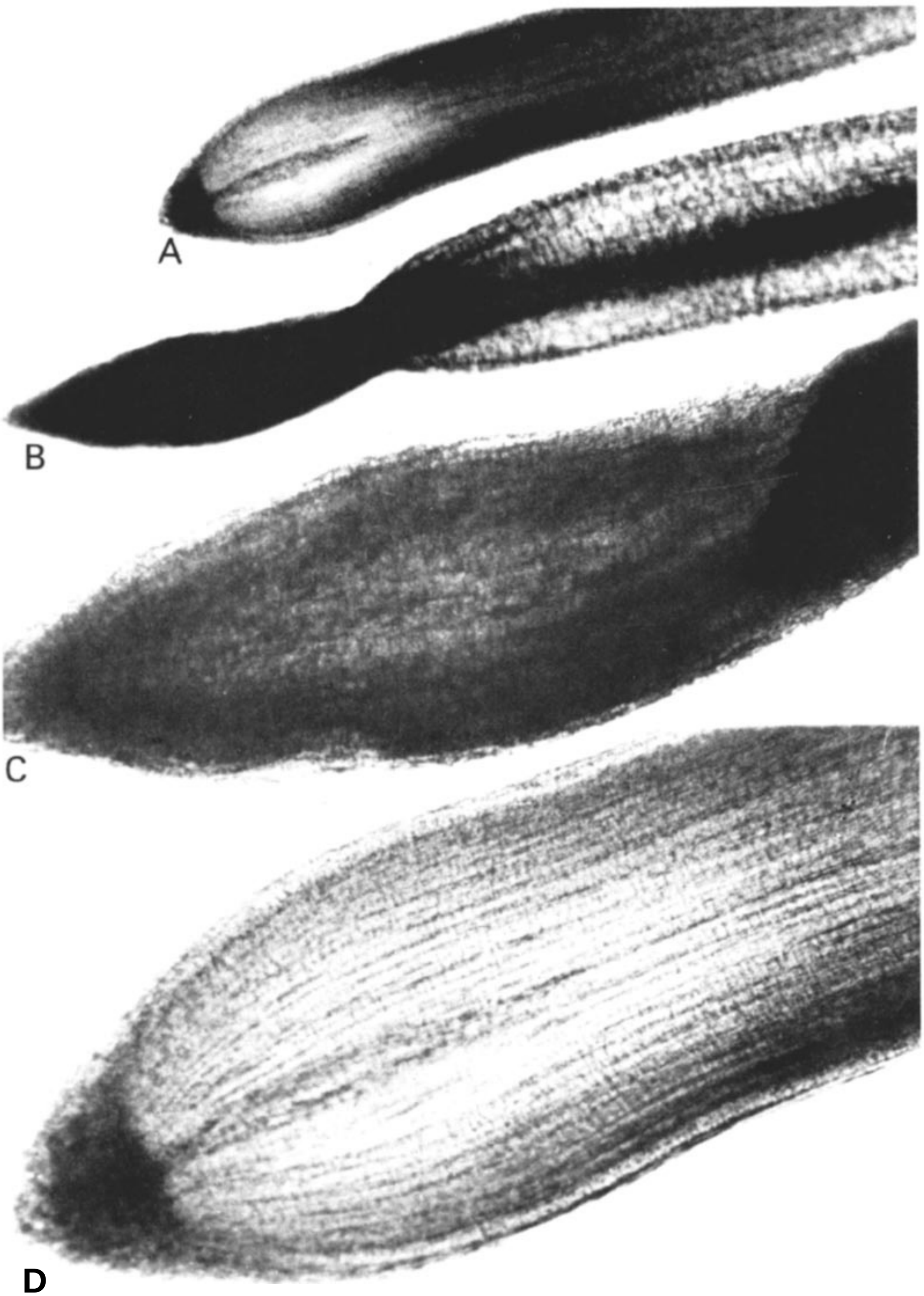

Fig. 1. Microscopic section of root meristem showing injury from 5 mN organic acid concentrations. A. Healthy, normal root tip. B. Root tip injured at 5 mN organic acid concentration. C. Close-up of the injured root showing the dead meristematic region. D. Close-up of the healthy root tip showing active meristematic elongation.

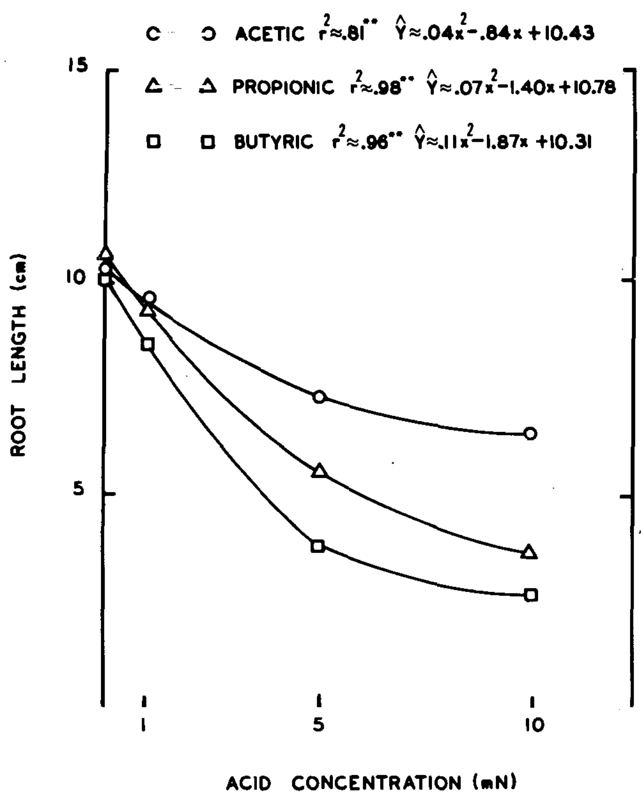

Fig. 2. Effect of acetic, propionic, and butyric acids on the root elongation of rice seedlings.

Plant height was also influenced by both acid concentration and pH. The average plant height for the control treatment (without acid) was 9.78 cm. Acetic acid reduced plant height to 6.67 cm at 1 mN, 5.98 cm at 5 mN, and 5.71 cm at 10 mN. Concentrations of propionic acid additions at 1, 5 and 10 mN gave, respectively, reduced plant heights of 6.64, 6.06, and 4.77 cm. Butyric acid at 1, 5, and 10 mN reduced plant heights to 6.08, 5.67, and 4.77 cm. All three organic acids at all concentrations reduced plant height.

The character least affected by kind of organic acid and concentration was shoot dry weight. Plant height was reduced less by organic acids than was root elongation, contributing to the small decrease in dry weight of shoots. The short exposure time used in the experiment was in part the reason for the small reduction in shoot dry weight and height. A 7-day exposure period was used: a) to insure that the seedlings were still dependent on the seeds as an energy source; and b) to maintain the composition of the nutrient solution and the organic acid concentrations as constant as possible. With seedlings primarily dependent upon seed reserve, the shoots were least affected while the roots, exposed to the treatments, were most afffected.

Even though root elongation varied appreciably with increased acid concentrations and pH, the differences in root dry weights were not always significant (Tables 1 and 2). It was observed that when root elongation was inhibited, an increased number of new roots were initiated from the crown. This occurred at all organic acid concentrations except for 10 mN of propionic and butyric acids, in which cases root initiation itself was inhibited. The increase in number of roots initiated with a decrease in root elongation suggests that apical dominance was operative where root elongation was not inhibited. This mechanism appears to reduce the differences between treatments in terms of root dry weight.

After the shoots and roots were separated the residual seed was weighed. The residual seed weights increased significantly with increasing acid concentrations (Table 1) but did not differ significantly between the three acids and pH's (Table 1 and 2). The residual seed weights at 1, 5, and 10 m*N* of acetic acid were respectively 14.20, 16.43, and 17.68 mg per seed. The trend was similar with propionic and butyric acids. These data suggests that the translocation of energy materials from the seed to the growing parts (roots and shoots) was inhibited with increased acid concentrations. This observation is further supported by the observed reduction of shoot and root dry weights at high acid concentrations (Table 1). In the initial stages the seedlings depend completely on the seeds as their metabolic energy source. The main reason for reduced seedling growth was due perhaps to inhibited translocation of energy products. Another factor associated with reduced seedling growth may be the reduced respiration of plants growing in high acid concentrations, as has been suggested by Robinson and Taylor (6) and Tanaka and Navasero (12).

Root length was the most sensitive indicator of the effects of organic acid concentrations and pH's. This is quantified and discussed below.

Effect of Organic Acids

Figure 2 shows the inhibition of root elongation by acetic, propionic, and butyric acids at 1, 5, and 10 m*N*. A quadratic regression equation of the type $Y = \pm ax^2 \pm bx \pm c$ was used to express the response curves. With the variable (independent) x being the concentration, and the coefficient c being the intercept. Only rate coefficients a (curvilinear) and b (linear) are used to illustrate the nature of the curves. For all three acids the coefficient a was positive whereas b was negative.

The inhibition of root elongation with acetic acid shows a proportional decrease of root elongation with an increase in acetic acid concentration. With propionic acid the linearity of reduced root elongation was similarly maintained. Propionic acid decreased the root elongation at an increased rate over acetic acid. Butyric acid was more inhibitive of root elongation than were propionic and acetic acids at all concentrations. These results agree with the data of Takijima (7), Takijima et al. (11), and Chandrasekaran and Yoshida (1) wherein they demonstrated that the higher the molecular weight of the organic acids (monobasic aliphatic), the higher was its inhibitory effect on rice seedlings.

Effect of Concentrations

Figure 3 shows the effects of 1, 5, and 10 m*N* concentrations of acids used in this experiment on root elongation at various solution pH values. The na-

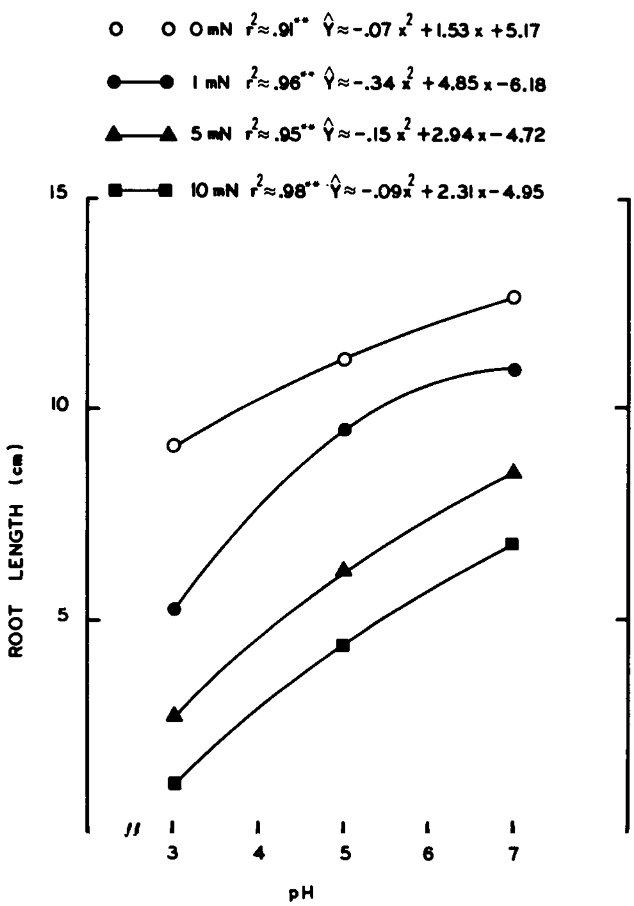

Fig. 3. Interactions of organic acid concentration and solution pH on the root elongation of rice seedlings.

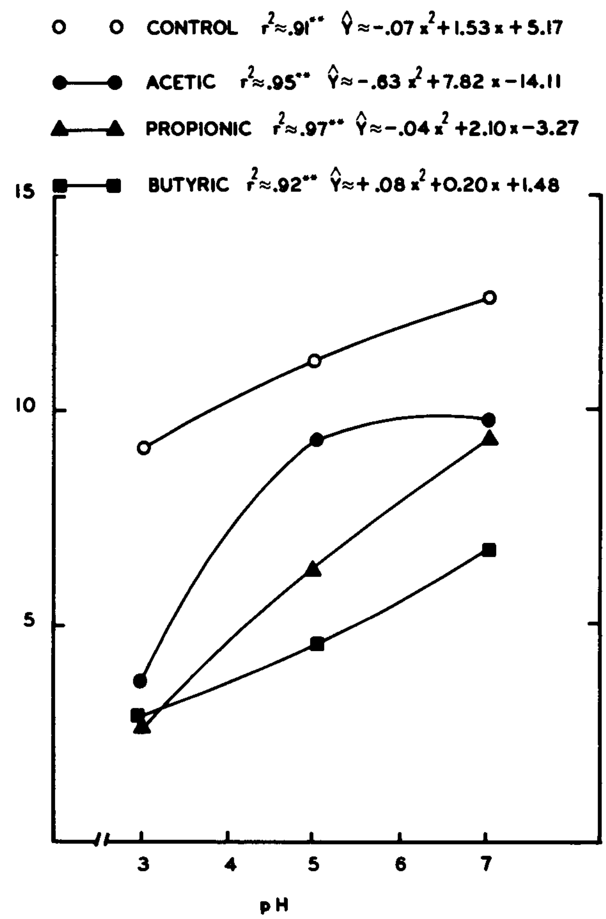

Fig. 4. Effect of acetic, propionic, and butyric acids and solution pH on the root elongation of rice seedlings.

ture of the responses is presented in a quadratic regression equation. With a decrease in pH from 7.0 to 3.0, root elongation was decreased even in the absence of organic acids. The reduction of root length was largely linear. At 1 m*N* the rate of decrease was curvilinear and the response to increased pH was also larger than at other concentrations. This was due to a lower root inhibition at this concentration, where adjustment of the pH could assist the recovery of seedlings. At 5 m*N* the curvilinear coefficient was lower (—0.15), as was the linear coefficient b (2.94). At 10 m*N* the curve is linear, with an a value of —0.09 and a b value of 2.31. In general, root elongation was inhibited at 1 m*N* and severely inhibited at 5 m*N*. At 10 m*N*, especially with propionic and butyric acids, root initiation itself was inhibited.

At the intermediate concentration, 5 m*N*, the root tip showed evidence of toxicity. Under a miscroscope the affected roots had dark spots rather than a distinguishable meristematic region that would be observed in a healthy root tip (refer Fig. 1). The dark spot suggests death of the tissue probably from the accumulation of some toxic products. This feature was observed only on roots which grew for a short time and then died, and it was present at the 5 m*N* concentration of each acid.

Effect of pH

One of the variables affecting root elongation most strongly was solution pH. Figure 3 presents the interactions between the pH and organic acid concentration on root elongation. The behavior of each acid is explained by the same quadratic regression equation. The organic acid and pH interactions are presented in Fig. 4.

Acetic acid was least effective in inhibiting root elongation, propionic acid was intermediate, and butyric acid was most effective. The increase in root elongation with an increase in pH with acetic acid was curvilinear, indicated by a high a value of —0.63, while with propionic and butyric acids the curves were more linear, with respective low a values of —0.04 and 0.08.

The inhibition of root elongation with a decrease in pH at the same acid concentration shows that the increased concentration of undissociated acids at lower pH caused the greater inhibition, rather than the concentration of organic acid per se.

CONCLUSIONS

The order of greater inhibition of root growth was: increasing acid concentration > decreasing pH's > organic acids (butyric > propionic > acetic). All organic acids reduced root length, at 1 m*N* concentration and pH 3, to 40 to 50% of that of the control. The main effect at 1 m*N* concentration was on root elongation. At 5 m*N* concentration, roots elongated for some time but some of the root tips then died, showing an injured dark meristematic region. Initiation of new roots still continued. However, at 10 m*N*, both root elongation and initiation were severely inhibited, especially at lower pH's.

Although root growth was greatly influenced, shoot height and weight were affected less, as shown by all acids at 10 m*N* concentration and pH 3.0. This treatment inhibited root length by 70 to 90%, and affected shoot height much less. Thus, translocation was affected only partially, but was not totally impaired.

Increase in pH from 3.0 to 7.0 decreased the inhibiting effect of all organic acids at all concentrations, more dramatically so at higher concentrations. This decreasing inhibition of root elongation with increasing pH was due mainly to a decrease in undissociated organic acids at a given concentration.

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