A Nitrogen Deficiency Disease of Sugarcane Probably Caused by Repeated Pesticide Applications

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ABSTRACT

Yellowing and drying of leaves, stunted cane growth, and partial to complete death of sugarcane plants, occurring in more or less round patches in the field, surrounded by healthy canes, was observed at one of the Central Aguirre Farms in southern Puerto Rico in February 1968. No pathogenic organisms were found in cane roots or shoots. Soil from the affected area was significantly low in NO_3-N, nitrogen mineralization was low, and only *Penicillium citrinum* was found in this soil, as against a heterogeneous fungal population in soil from the unaffected area. The cane leaves from the affected area were low in N. Growth of test plants in the greenhouse in soils from the affected area was reduced both in sterilized and nonsterilized soils. The problem was remedied by the addition of mineral nitrogen.

Repeated applications of pesticides, especially the 19 applications of the fungicides maneb, zineb, and tribasic copper to the preceding tomato crop, probably changed the microbial population so much that mineralization of soil nitrogen was inhibited, resulting in N starvation of the following sugarcane crop. Phytopathology 60:485-487.

In February 1968, a severe case of sugarcane stunting and mortality was observed in a 30-acre field of the Central Aguirre Farm in southern Puerto Rico. The affected field was a 7-month-old plant crop of variety P.R. 1029. More or less round patches of stunted, partially to completely dead plants with yellow and dry leaves, surrounded by healthy, green, and normally growing canes were the characteristic features of the field. The cause was studied, and the results are presented in this paper.

MATERIALS AND METHODS.—Composite soil samples were collected from unaffected and affected sites of the sugarcane field, and were subjected to physical, chemical, and microbiological analyses in the laboratory. Plant samples were analyzed for N, P, and K. Physical and chemical analyses were done by commonly used methods in the soils laboratory.

In order to determine if soil microorganisms or their enzymes had directly affected the plant growth, a greenhouse experiment was conducted. Oats were planted in sterilized and nonsterilized soils from both unaffected and affected areas. Sterilization was done in an autoclave at 121 C for 1 hr in order to kill the microorganisms and destroy the enzymes. For this and other greenhouse studies, four oat seedlings were allowed to grow in 100-g soil samples maintained at field capacity in plastic cups without any holes at the bottom, to prevent leaching. The plants were harvested after 4 weeks and their wt recorded; they were then subjected to chemical analyses.

Soil incubation studies were conducted with 50-g (oven-dry basis) air-dry soil samples brought to near-neutral pH by adding the necessary amount of 1.0 N H_2SO_4. Half the number of soil samples received 100 ppm N as $(NH_4)_2SO_4$ in 1-ml water. After drying, the additives were thoroughly mixed and the samples transferred to 250-ml Erlenmeyer flasks. Distilled water was added to bring the soil to field capacity. The flasks were covered with Parafilm, which allows gaseous exchange but prevents escape of moisture. The samples were incubated in the laboratory at 24 C. The treatments were replicated three times. At intervals, sets of soil cultures were separated for determination of pH and mineral nitrogen. The 50-g soil cultures were extracted with 250 ml of 1.0 N KCl. Ammonium and NO_3-nitrogen were determined by first distilling off the ammonia with MgO, then using the same extract for determination of NO_3^- by reducing the NO_2^- and NO_3^- by Devarda's alloy. Value for NO_3^- was calculated by subtracting the value for NO_2^- from the value for $NO_2^- + NO_3^-$. Nitrite nitrogen was determined by 1-naphthylamine and sulfanilic acid method (5). The pH was determined in one of the replicates by glass electrodes in 1:1 soil:water suspension before extracting it with 1.0 N KCl solution.

Sodium albuminate agar medium was used to grow the soil bacteria and actinomycetes, and Martin's Rose Bengal medium with streptomycin for soil fungi.

RESULTS.—Examination of the roots, stems, and leaves of the stunted sugarcanes did not reveal symptoms of specific diseases. It was, therefore, assumed that the condition was definitely not due to soil-borne pathogens, but probably resulted from some chemical edaphic factor.

Physical and chemical analyses of the soil samples from the unaffected and affected areas revealed little or no difference between the two samples with regard to their texture, pH, organic matter, or exchangeable bases. Salt concentration up to 12 me/liter in the affected soil as compared to 8 me/liter in the unaffected soil could not be the factor, since sugarcane grew well in a similar soil with salt concentration of 42 me/liter in another field on the same farm.

Total N content in the two soils was almost the same (Table 1), and the NH_4- and NO_2-N contents differed little. But the soil from the unaffected area had 163 ppm NO_3-N, while that from the affected area had only 35 ppm. Also, the microbial population in the

TABLE 1. N content and microbial population in the unaffected and affected soils

Soil	Nitrogen				Microbial population/g soil		
	Total N	NH_4^+	NO_2^-	NO_3^-	Bacteria $\times 10^5$	Actinomycetes $\times 10^5$	Fungi $\times 10^3$
		ppm					
Unaffected	1634	4.1	0.08	163.7	97	133	246
Affected	1639	3.5	0.04	35.4	49	158	637

affected area was markedly changed. The bacterial population was reduced to half and the fungal population increased to three times that in the unaffected area.

The soil from the unaffected area had a heterogeneous fungal population, while that from the affected area had only one fungus, *Penicillium citrinum* Thom. There was no apparent difference in the actinomycete population.

Sugarcane leaves from affected areas were markedly low in N (0.81% vs. 1.21 from unaffected areas). There was hardly any difference in the P (0.13-0.1) and K (1.68-1.69) contents of sugarcane leaves from unaffected and affected areas.

Table 2 gives the shoot weight and N and P contents of 4-week-old oat seedlings. There was about 50% reduction in shoot weight of the plants in soil from affected area, irrespective of sterilization. Nitrogen content in these shoots was lower, but P content was higher than those growing in the unaffected soil. Root growth was also reduced in the affected soil, and they were low in N. There was no difference in P content of the roots. Except for reduced growth, there was no abnormality in the appearance of the roots or shoots. Soil microorganisms or the enzymes produced by them were apparently not responsible for plant growth reduction in the affected soil.

Because of the altered microbial population and its possible relationship to the low mineral N in the soil and low nitrogen uptake by the cane and oat plants, it was considered appropriate to investigate the nitrogen situation in the soil. A soil incubation experiment conducted in the laboratory indicated that the nitrification process was a little slow in the affected soil (Table 3). Although for some reason the nitrifying bacteria in the affected soil were being inhibited to some extent, this alone could not be held responsible for such a marked reduction in plant growth, since the effect on nitrification was small and transitory. Besides, sugarcane and oats can utilize NH_4-N as well as NO_3-N for normal growth (4).

Data on mineralization of soil N in the two soils are presented in Table 4. Over three times more NO_3-N was present in the unaffected soil than in the affected soil. This marked difference in the mineral N content between the two soils persisted even after 8 weeks of incubation under quite favorable conditions for microbial activity. There was no difference in the total N content of the two soils (Table 1). It was presumed, therefore, that deficiency of available mineral N was limiting the growth of the plants in the affected soil.

To confirm the above assumption, another experiment was set up in the greenhouse in which 100 ppm N as $(NH_4)_2SO_4$ was applied to one set of soil samples,

TABLE 2. Wt, N, and phosphorus contents of 4-week-old oats grown in sterilized and nonsterilized soils that had supported normal (unaffected) and abnormal (affected) sugarcane

Soil	Shoot			Root		
	Dry wt g/plant	N %	P %	Dry wt g/plant	N %	P %
			Sterilized soil			
Unaffected	0.28	2.69	0.28	0.125	1.24	0.16
Affected	0.16	1.61	0.44	0.112	0.52	0.13
			Nonsterilized soil			
Unaffected	0.29	3.96	0.34	0.087	2.06	0.18
Affected	0.22	2.71	0.43	0.043	1.14	0.19

TABLE 3. Nitrification of 100 ppm applied NH_4-N in unaffected and affected soils

Soil	Period of incubation						
	3 days			1 week			2 weeks[a]
	NH_4^+	NO_2^-	NO_3^-	NH_4^+	NO_2^-	NO_3^-	NO_3^-
				ppm			
Unaffected	46.6	21.0	21.7	3.6	0.0	89.4	101.0
Affected	61.0	15.4	14.1	7.4	3.3	90.2	90.0

[a] Only NO_3-N found at this time.

TABLE 4. Nitrogen mineralization in unaffected and affected soils

	Mineral nitrogen[a] formed after incubation, weeks								
	1			2			8		
	NH_4	NO_3	Total	NH_4^+	NO_3^-	Total	NH_4^+	NO_3^-	Total
					ppm				
Unaffected	3.5	163.7	167.2	0.6	167.8	168.4	0.0	171.3	171.3
Affected	2.6	39.2	41.8	2.9	52.5	55.4	0.0	46.9	46.9

[a] No NO_2-N was found.

both from unaffected and affected areas, while the other set did not receive any N. Nitrogen application caused a 16% increase in dry wt on affected soils as against only 3% increase on the unaffected soil. Plants in soil from affected areas were suffering from N deficiency, since the reduction in growth was largely overcome by application of N.

DISCUSSION.—It seems that for some reason the microbial population in the affected area of the field was drastically changed, affecting adversely the N mineralizing groups of microorganisms. The breakdown of nitrogenous organic compounds in the soil with a concomitant mineralization of N is brought about by a host of heterogeneous microorganisms.

An investigation into the history of pesticide applications to this field revealed how microbial populations may have been affected, causing the previously described results. The preceding tomato crop received 1 application of diphenamid, 4 of maneb, 10 of zineb, and 5 of tribasic copper. The cane, prior to appearance of the problem, received 1 application of aldrin, 2 of ametryne, and 3 of pentachlorophenol.

All these 26 applications of pesticides were made within 12 months before the sugarcane problem was noticed. The heavy applications of the above-noted pesticides, especially the 19 applications of fungicides, the drastic changes in the soil microbial population, coupled with a marked reduction in soil mineral nitrogen, and a sharp growth response to added mineral N, lead to the natural assumption that the repeated applications of pesticides so changed the soil microbial population that the N mineralization was inhibited, resulting in a severe N starvation of the sugarcane crop.

In this regard, some of the reports in the literature are worth mentioning. Kreutzer (3), after reviewing the literature on the toxicity of chemicals to soil microorganisms, concluded that, in general, most soil toxicants have no effect on the nonspecific bacterial population, or that they actually stimulate it. The specialized bacteria of the soil are more susceptible to toxicants than unspecialized types, and soil fungi appear to be more susceptible to a variety of toxicants than are either soil actinomycetes or bacteria. *Penicillium* spp., *Aspergillus* spp., and *Trichoderma viride* are the most resistant fungi to Formalin and carbon disulfide (2, 7, 8). Thiram at 6.2 ppm inhibited *Pythium ultimum,* but not *T. viride* and *Penicillium* spp., which survived exposures to 50 ppm thiram (6). Nabam was less toxic to bacteria and actinomycetes than to fungi (1). In this laboratory (*unpublished data*) maneb changed the microbial population quantitatively as well as qualitatively in a soil similar to the one under study. At 60 ppm and above, maneb drastically decreased the fungal population, only *Aspergillus* spp. and *Trichoderma* spp. surviving.

In the light of the above observations, the change in the bacterial population and the drastic change in the fungal population encountered in the affected area might well be the result of the repeated applications of the dithiocarbamates such as maneb and zineb. It is possible that other of the pesticides applied also might have contributed to this condition.

The affected areas appeared only in scattered round patches surrounded by normal cane along the irrigation channels. This may have resulted from a faster decomposition and possible leaching of the pesticides along, and in the vicinity of, the irrigation channels. On the other hand, in the central areas of the plots where moisture was not as plentiful, decomposition of the pesticides did not take place fast enough, owing to the slow microbial activities which are so instrumental in pesticide degradation. In such areas the problem was more pronounced.

LITERATURE CITED

1. CHANDRA, P., & W. B. BOLLEN. 1961. Effects of nabam and mylone on nitrification, soil respiration, and microbial numbers in four Oregon soils. Soil Sci. 92: 387-393.
2. EVANS, E. 1955. Survival and recolonization by fungi in soil treated with formalin or carbon disulfide. Brit. Mycol. Soc. Trans. 38:335-346.
3. KREUTZER, W. A. 1963. Selective toxicity of chemicals to soil microorganisms. Annu. Rev. Phytopathol. 1: 101-126.
4. NIGHTINGALE, G. T. 1948. The nitrogen nutrition of green plants. Bot. Rev. 14:185-221.
5. PRINCE, A. L. 1954. Determination of total nitrogen, ammonia, and nitrites in soils. Soil Sci. 59:47-52.
6. RICHARDSON, L. T. 1954. The persistence of thiram in soil and its relationship to the microbiological balance and damping-off control. Can. J. Bot. 32:335-346.
7. SAKSENA, S. B. 1960. Effect of carbon disulfide fumigation on *Trichoderma viride* and other soil fungi. Brit. Mycol. Soc. Trans. 43:111-116.
8. WARCUP, J. H. 1952. Effect of partial sterilization by steam or formalin on damping-off of Sitka Spruce. Brit. Mycol. Soc. Trans. 35:248-262.