Biological Control of Weeds in Compacted Soil Cultures

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APPROXIMATELY $500,000 worth of irrigation water is lost annually from canals west of the Mississippi River because of the transpiration of weeds, notwithstanding the asphalt linings of the canals. The weeds, which are able to pierce such linings as well as thin linings of concrete, are a major problem in all irrigated agricultures, especially where water is conveyed in open canals over long distances.

Thick linings of concrete would prevent the weeds from growing through. Their cost, however, would be prohibitive, and their maintenance difficult. Application of arsenates, copper salts, and other herbicidal inorganic chemicals to soil supporting the flexible bituminous canal linings is an unpromising measure. Tolerance of weeds for such poisons requires rather costly applications; the herbicidal effects, if any, are apparently temporary; finally, there is a strong opposition to such pretreatment of the soil among the users of water, however unreasonable this opposition may appear to be. Certain organic compounds, notably derivatives of benzene, are capable of destroying weeds economically and rapidly.[2] Unfortunately, they do not attack the seeds and must be used frequently and repeatedly. Technological research may still result in the discovery of a cheap weed-resistant material for the canal linings, or of effective, enduring, and unobjectionable herbicidals for the sterilization of the soil. Developments are slow, however, and disappointments are too numerous to be stimulating.

Such is a rough outline of the problem as I learned it from R. S. Rosenfels and John S. Shaw, in 1947, in Denver. Its biological aspects reminded me of some earlier findings in California,[3] confirmed subsequently by later investigators.[4]

These earlier studies dealt with susceptibility of feeder roots to certain pathogenic fungi developing in consequence of stresses induced in the respiratory system of the plants through exposure to weak poisons or to mechanical-physical shock. Plants so conditioned would yield to parasites or pathogens, which they could resist successfully as long as stress or shock was avoided. The state of susceptibility induced in the plants was temporary. A large number of plant species responded to the conditioning in about the same manner (citrus, avocado, walnut, corn, wheat, tomato, radish, lettuce, cauliflower, etc.), whereas the nature of the pathogen seemed relatively unimportant. An inference from these earlier findings was reasonable:

If such a state of susceptibility could be induced and maintained economically in the germinating seeds of noxious weeds, the problem of weed control in the field would be on its way to a satisfactory solution. The testing of this hypothesis was carried out as follows:

Test plants. Smooth brome grass, yellow sweet clover, buffalo grass, crested wheat, and Great Northern beans were chosen as test plants for the experiments. The first four are common weeds in Colorado and elsewhere; the beans were used because of their vigorous growth and their previously observed capacity to penetrate almost any bituminous lining and to split thin linings of concrete. My earlier and later observations lead me to believe that practically any plant, wild or cultivated, would respond in about the same way to the succession of regimes described below. The seeds were a part of Mr. Shaw's collection and were of excellent quality, yielding practically 100 per cent germination in test lots.

Soil. The humous A horizon of an alluvial soil from the banks of an irrigation canal, Denver Federal Center, was the test material. The soil was a dark-gray, heavy, silty clay loam to clay loam with a chernozem-like structure and a pH of 7.4 in 1:1 aqueous suspensions. This is one of the fertile soils of the area, and it supports an abundant growth of weeds and also of volunteer oats, alfalfa, wheat, etc. Its compaction characteristics are unfavorable because of the relatively high silt content. The choice of this particular soil for the experiments was determined by its unfavorable properties from the point of view of weed control. If positive experimental results were obtained with this control-resistant material, one

[1] I am indebted to the administrative officers of the U. S. Bureau of Reclamation at the Denver Federal Center for their hospitality, to R. S. Rosenfels (now of Richland, Wash.), and to John S. Shaw for their interest and active participation in the studies, and to many others of the bureau's staff for aid and advice. I am indebted also to G. F. Carter, chairman of the Isaiah Bowman School of Geography, The Johns Hopkins University, for his interest in and encouragement of these studies; to C. F. Miller, of the School of Engineering, for his enlightened comment; and to H. Bentley Glass, of the Department of Biology, for his interest in and criticism of the paper.

[2] John S. Shaw, U. S. Bureau of Reclamation, has met with some success in this method.

[3] Sokoloff, V. P., and Koltz, L. J. *Calif. Citrograph*, **28**, 290, 308 (Nov. 1943); Klotz, L. J., and Sokoloff, V. P. *Citrus Leaves*, **23**, 1, 22 (1943).

[4] Curtis, D. S., Chapman, H. D., and Zentmyer, G. A. *1949 Yearbook.* Calif. Avocado Soc., 155.

would be encouraged with respect to less difficult soils.

In earlier, unpublished work, I observed nearly the same response of plants to the treatment shortly to be described on soils ranging in texture from sand to clay, as well as on peat, muck, gravel, and in water cultures—in short, throughout a wide range of test materials and conditions, as long as certain basic features of the treatment remained the same.

Containers. Cylindrical tin cans were preferred to other containers in our tests, chiefly because of their suitability for the mechanical compaction treatment. The inside diameter of the cans was 5.1 inches; their inside depth, 5.9 inches; their volume, 155 cubic inches; and their capacity, about 8 pounds of compacted soil at 100 pounds per cubic foot.

Chemicals. Chemically pure nitrates of calcium and potassium, glucose, and sodium phosphates were used as aqueous solutions in distilled water. In the second series of tests, cane molasses was substituted for the glucose and tap water for distilled water. Only refined, table-quality cane molasses (Brer Rabbit) was available at the time, which was somewhat regrettable, although the results here reported are not affected by quality of the molasses. My preference for the crudest and cheapest cane molasses was determined not only by the successful results previously obtained, but by its low cost. As a matter of fact, any carbohydrate waste such as sweet-potato peelings or sugar-beet refuse would yield about the same results. The common impurities in low-grade molasses, both mineral and organic, are desirable in the control of weeds. The usual variations in their content are unimportant. Their presence serves to obviate the necessity of adding essentially the same substances to the enrichment cultures.

The following sequence of environments in soil cultures is generally conducive to the attainment of our purpose—namely, the prevention of weed growth:

Reduction of pore space to the practical minimum. This is accomplished by compaction in the presence of moisture optimal for the purpose. The function of moisture is to serve as a lubricant enabling soil particles of various sizes to slide into spaces or interstices between other particles. There is a maximum compaction for any given soil, corresponding to a certain minimum pore space, beyond which it is not possible to go. In this particular case the optimum compaction moisture was determined to be 21.7 per cent, and the maximum attainable density, 90–100 pounds per cubic foot, corresponding to about 40–46 per cent total pore space on a volume basis. Some sandy soils may be compacted to 125 pounds per cubic foot, corresponding to only 24 per cent total pore space, assuming the true density of the soil substance to be 2.65.

The effect of compaction is to reduce the total pore space to about two thirds of its original proportion and accordingly to influence adversely the aeration of seeds during their germination. The physical effect of the compaction is to increase the mechanical stability of the ground and to render it better suited for the linings or pavement to be imposed.

Compaction alone is insufficient to prevent the germination of seeds, however, with or without the asphalt capping of the cultures.

Further interference with respiration of germinating seeds. An economic way rapidly to exhaust oxygen in the soil air is to encourage bacterial growth in the soil by enrichment with certain added materials. The choice of the type of growth to be encouraged and, accordingly, the choice of materials to be added are determined by the following considerations:

1) The bacteria should be a common ubiquitous group, facultatively anaerobic, with simple nutritive requirements, mesothermal, and nonpathogenic to man and animal.

2) The group in question must *not* be effective fermenters of carbohydrates or producers of acid. In other words, the anaerobic metabolism of the bacterial group to be enriched should be such as not to allow rapid and significant changes in the volume of the gaseous phase of the confined soil, in order to protect the bituminous cap.

3) The anaerobic growth of bacteria to be enriched should be conducive not only to the deprivation of the germinating seeds of oxygen but also to a production of poisons, however mild, further to facilitate the destruction of the germinating seeds.

4) The organic substratum of the desired bacterial group should be cheap, harmless to man and animal, and noncorrosive to the bituminous linings.

It is evident that only one group of soil microorganisms satisfies the enumerated conditions, namely, the heterotrophic, facultatively anaerobic denitrifiers—that is, the reducers of nitrate capable of using organic substances as donors of hydrogen.[5]

The reduction of nitrate has been shown[6] to take place in the following well-defined steps:

$NO_3^- + 2H = NO_2^- + H_2O$; i. e., nitrate to nitrite
$NO_2^- + 2H = NO^- + H_2O$; i. e., nitrite to hyponitrite
$2NO^- + 2H = N_2 + 2OH^-$; i. e., nitrate to nitrogen gas[7]

Denitrification may be arrested at either the nitrite stage or the hyponitrite stage by controlling the proportion of the hydrogen donor (i.e., the carbohydrate substance) to the hydrogen acceptor (i.e., the oxidized forms of N), preventing thereby the evolution of gaseous nitrogen. As to the carbon dioxide and its derivatives produced during the denitrification, the bulk of the gas may be immobilized in the soil, in the slightly alkaline optimum range of the reaction and in the presence of calcium.

The gaseous economy of the denitrification may be

[5] The taxonomy of this large group is unknown. The *Bacillus denitrificans* of standard manuals, monographs, etc., covers a multitude of things. My collection of denitrifiers in Riverside, Calif., in 1940–43, contained more than 60 species, mostly unidentified, with many more remaining to be isolated.

[6] "Report on Bacterial Denitrification." University of California Citrus Experiment Station files (1941). See also Elema, B. *De Bepaling van de Oxydatie—Reductiepotentiaal in Bakterienculture en hare Beteekensis vor de Stoffwisseling.* Delft: (1931).

[7] The third stage, hyponitrite to nitrogen gas, is established by measurements of NO^- and N_2 as well as of OH^-. The pH optimum for this last stage of the denitrification is on the alkaline side of 7.

TABLE 1

Effects of Compaction, Capping, Carbohydrate, and Nitrate on Germination of Seeds in Soil Cultures and on Damage to Cap by Plants or Gas

Treatment				Response								
				Germination					Damage to cap			
Compaction	Cap	Carbohydrate	Nitrate	Brome	Buffalo	Clover	Wheat	Beans	By plants	By gas	No.	C:N
		(% of soil)		(Number of seeds per 10)								
+*	+	10.0	1.2	—	0†	—	0	0	0	+	17	34
+	+	7.7	1.8	—	0	—	0	0	0	+	18	17
+	+	5.5	2.4	—	0	—	0	0	0	+	19	9
+	+	3.1	2.8	—	0	—	0	0	0	0	20	5
+	+	2.2	3.1	—	0	—	0	0	0	0	21	3
+	+	1.2	3.3	—	0	—	0	0	0	0	22	2
+	+	0.6	3.4	—	0	—	0	0	0	0	23	1
+	+	0	0	—	7	—	5	8	+	0	24	—
+	0	0	0	—	9	—	8	10	+	0	25	—
0	0	0	0	—	8	—	6	10	+	0	30	—
0	+	0	0	—	9	—	8	10	+	0	31	—
0	0	0	0	10	8	6	5	—	+	0	1	—
0	0	0	0	10	7	5	5	—	+	0	2	—
0	0	0.6	0.5	3	3	0	0	—	—	—	5	4
0	0	0.6	0.5	9	0	0	0	—	—	—	6	4
0	0	0.6	0	8	3	6	0	—	—	—	9	—
0	0	0.6	0	8	6	3	4	—	—	—	10	—
0	0	0	0.5	5	0	0	2	—	—	—	13	—
0	0	0	0.5	6	0	0	0	—	—	—	14	—
0	+	0	0	2	2	1	1	—	+	0	3	—
0	+	0	0	3	3	1	2	—	+	0	4	—
0	+	0.6	0.5	0	0	0	0	—	0	0	7	4
0	+	0.6	0.5	0	0	0	0	—	0	0	8	4
0	+	0.6	0	0	0	0	0	—	0	+	11	—
0	+	0.6	0	0	0	0	0	—	0	+	12	—
0	+	0	0.5	6	5	0	1	—	+	0	15	—
0	+	0	0.5	5	1	0	1	—	+	0	16	—

* + means "treatment applied."
† 0 means "treatment not applied" or "absent."

controlled, therefore, by the proportion of the carbohydrate to the nitrate and by the pH of the system. For all practical purposes the carbohydrate-nitrate ratio is not critical, provided it remains below a certain threshold. This threshold is not affected by the nonnitrate nitrogen of the system. In our particular case the threshold corresponds to the weight ratio of molasses to $Ca(NO_3)_2$ as, roughly, 2:3 (Table 1).

An important consequence of the anaerobic metabolism of the denitrifiers is an impairment of the respiratory mechanisms of small roots in contact with the culture. It is this additional effect, accompanying the withdrawal of oxygen from the soil air by the facultative anaerobes, that seems to contribute to the collapse of the germinated seeds.

Secondary invaders. It has been observed consistently that young rootlets and germinating seeds become overgrown by fungi in soil cultures previously enriched with respect to the denitrifying bacteria. The fungi appear to be parasites or saphrophytes as well as mild pathogens, such as the brown root rot species.

The importance of this last stage of visible destruction of germinated seeds remains to be ascertained.

The experiments were carried out as follows:

a) The stock of soil, *ca.* 200 pounds, was screened to remove gravel and plant roots, mixed, and stored in a wooden bin. The air-dry moisture content of the stock was 3.5 per cent.

b) Eight-pound aliquots of the stock were brought to the optimum compaction moisture of 21.7 per cent, placed in the tin cans, and compacted, in layers, to maximum density.[8]

c) Seeds were planted as described in Table 1 and covered by soil five times as thick as the seed's largest diameter. The soil covering the seed was recompacted, to restore the original surface.

d) The asphalt caps were poured over the compacted soil, with care to keep the temperature at the minimum.[8]

e) Condition of compacted cultures, plant growth, etc., were observed daily.

f) After 23–83 days the experiments were discontinued; the asphalt caps were removed and examined for damage; condition of seeds was ascertained, together with the pH of the incubated soils and other changes in the cultures.

A summary of observations is given in Table 1. The extent of compaction was between 90 and 100

[8] I am indebted to the Soils Laboratory of the U. S. Bureau of Reclamation for these operations. See *Earth Materials Test Procedures* (rev.), Denver: USBR (1948).

pounds per cubic foot. Thickness of the asphalt cap, 30–40 pen., was ¼ inch in all cases. The carbohydrate was supplied as cane molasses in Nos. 17–31, and as glucose in the others; nitrate, as $Ca(NO_3)_2$ in Nos. 17–31, and as KNO_3 in the others. The number of seeds planted in staggered rows was 10 of each kind, but 8 for the beans. The injury of the asphalt cap was by a direct penetration of the germinated seed, by pockets of gas trapped in the bituminous substance, and, in Nos. 17, 18, and 26, by a disturbance of the soil surface under the cap. "0" damage means a complete absence of visible damage of any kind.

The C : N ratio is the atomic ratio of carbon in the carbohydrate to nitrogen in the nitrate, assuming 1 gram-atom C per 41 g of molasses or per 30 g of glucose and 1 gram-atom N per 82 g $Ca(NO_3)_2$ or 101 g KNO_3.

Nos. 17–31 were unbuffered, except by the added materials. The others were buffered at pH 7.4 at 0-time, with the aid of 5 millimols of a phosphate buffer per can. All materials added were introduced with the moisture used at the time of the compaction.

Nos. 17–31 were examined after 83 days of incubation; the others, after 23 days. The loss of moisture was not appreciable from either the capped or the uncapped cultures. There was some evidence of alcoholic fermentation in Nos. 17, 18, and 26—the only cultures where pH of the soil fell to 6.3–6.8 after the incubation. The pH of the others ranged from 7.0 to 8.4. There was no evidence of precipitation of $CaCO_3$.

The problem of weed control along canal embankments lined with bituminous materials is posed rather than solved in the present report, despite the rather spectacular positive results obtained in the laboratory. A complete solution of the problem in the field could not be carried out because of the lack of time, funds, and interest. A detailed laboratory investigation of the possibilities here demonstrated could not be undertaken for the same reasons, plus the lack of facilities for the microbiological research. It is my hope, however, that the present beginning may stimulate further studies by interested agencies or individuals.

Field tests should not be difficult, once the optimum treatment schedules could be established in the laboratory. Assuming an establishment of such schedules, the field practice will require attention chiefly with respect to the following probable difficulties:

1) Reproducibility of results obtained in closed systems in the laboratory, in systems only partially confined as in the field. There should be no difficulty in applying the carbohydrate-nitrate mixtures to soils undergoing compaction or in controlling rather nicely the amounts and the distribution of the solutes in the soil. The existing machinery and skills are adequate for the purpose. The real difficulty is likely to arise with the stability of the oxygen-impoverished carbohydrate and nitrate-enriched environment in the compacted root zone under the linings. This problem can be solved only empirically. Regardless of the kind of leads or solutions obtainable in the laboratory, only a series of field trials could justify the economic application of the method.

2) Stability of the herbicidal effects in the field environment likewise needs to be ascertained. In my view, there are reasons in favor of the presumption of this stability, provided the compaction and the stabilization are performed with care sufficient to minimize seepage, capillary exchanges of moisture, and the gaseous exchanges between the enriched compacted zone and its periphery. Here, again, only a series of field trials can ascertain this possibility.

3) Costs of materials and treatments were not considered in this study. This aspect of the problem would be critical in the field. Granted the locally low cost of crude cane molasses, for example, there is no reason to limit oneself to this particular source of carbohydrate. There may be cheaper sources. In fact, practically any carbohydrate can be employed in the method proposed here.

The effects and the relationships observed have significance in many fields of biological knowledge. The underlying mechanisms are suggested plausibly but not proved conclusively. Their proof lies in the biochemical fields now only partially or wrongly developed. For example, the conventional line of research involving isolations of organisms, "pure-culture" studies, single-substance effects, isolation of "pure substances," etc., leads more often than not into blind alleys, wherein contact with nature is sacrificed to conformity with unrealistic standards of the epigoni.

The relative sterility of modern soil microbiology is due to the neglect of mixed culture studies and to the adherence to the postulates of Koch, so useful in their day, but already transcended, in part, by modern medicine.

In our problem we are dealing with more or less controlled cultures, mixed and impure, and with susceptibility-resistance phenomena in germinating seeds, which are understood but imperfectly; with shifting balances between components of soil populations; and with delayed effects and aftereffects of factors and substances remaining to be ascertained. The technological problem posed is of a field engineering type, where the method must fit the need. In this study it has been observed that (1) seeds of brome grass, buffalo grass, yellow sweet clover, crested wheat, and beans can be largely prevented from germination and the growth of germinated seeds can be effectively arrested in compacted asphalt-capped soil cultures receiving a mixture of carbohydrate-nitrate, under certain conditions; and that (2) the evolution of gas in the carbohydrate-nitrate enriched cultures can be minimized by a control of the C : N ratio in the soil solution whereby the asphalt capping remains unimpaired.

These laboratory findings may be useful in the biological control of weeds in irrigation canals, and field tests to determine their utility appear to be in order.