EFFECT OF INSECTICIDES AND HERBICIDES APPLIED TO SOIL ON THE DEVELOPMENT OF PLANT DISEASES. I. THE SEEDLING DISEASE OF BARLEY CAUSED BY *HELMINTHOSPORIUM SATIVUM* P. K. & B.[1]

LLOYD T. RICHARDSON

Canada Department of Agriculture, London, Ontario

[Received for publication September 21, 1956]

ABSTRACT

From the results obtained when nine insecticides and ten herbicides were applied at a series of dosages to soil infested to various degrees with *Helminthosporium sativum* P.K. & B. it was found possible to separate these chemicals into four groups on the basis of their effects on the growth of barley seedlings and on the development of rootrot infection. Group A (schradan, isodrin, DCU, IPX, and TCA) affected neither host nor disease development. Group B (lindane, dieldrin, and DDT) stimulated the growth of barley seedlings without affecting disease development. Group C (maleic hydrazide and heptachlor) stimulated seedling growth but increased the severity of infection. Group D (aldrin, endrin, chlordane, NPA, 2,4-D, monuron, DNBP, and dalapon) did not affect the growth of seedlings but reduced rootrot infection. Only DNBP was toxic to *H. sativum* in culture.

INTRODUCTION

Many agricultural chemicals used for insect and weed control find their way into the soil either through direct application or through accumulation of debris from sprayed plants. A knowledge of the effects of these materials on soil micro-organisms, both parasites and saprophytes, is obviously desirable but difficult to achieve because of the complexity of the problem and the limitations of available techniques. Since the pertinent literature is scattered in various journals, many of which are not devoted to plant pathology, it seems advisable to present a brief review at this point.

Changes in the population of soil micro-organisms following applications of insecticides and herbicides have been observed by many workers. The effects of these chemicals on bacterial populations have been studied principally in relation to soil fertility and therefore will not be discussed here. Their effect on the fungal populations may have some significance in relation to disease development. A decrease in the numbers of soil fungi has been reported following the application of BHC (2,23), chlordane (23), DDT (2) and aldrin (2). Increased numbers of fungi were found after treatment with toxaphene (2,23). 2,4-D applied at normal rates for killing plants was reported to have no appreciable effect on the soil fungi (14,22).

The effects of insecticides and herbicides on the germination and growth of fungi in culture have also been studied. Parathion and related organophosphorus compounds were found to inhibit the germination of

[1] Contribution No. 84 from Science Service Laboratory, Canada Department of Agriculture, London, Ont.

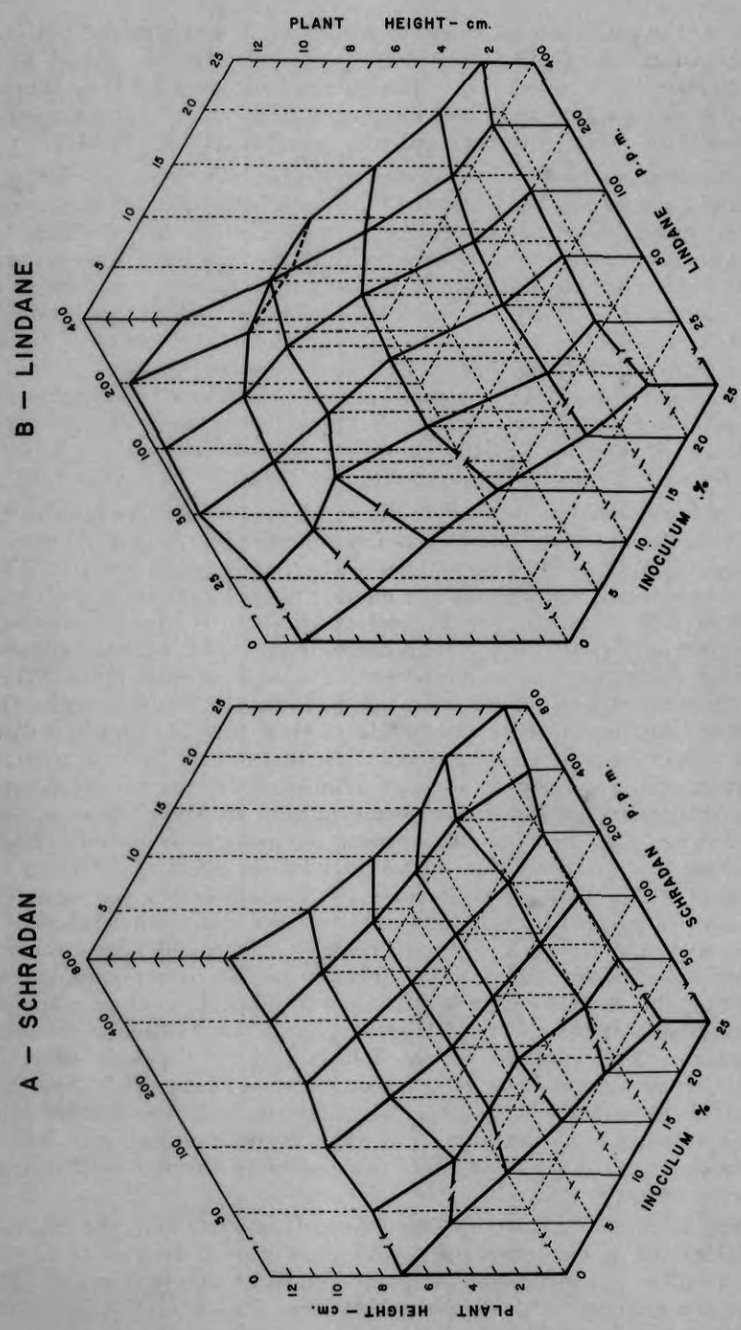
[2] Senior Plant Pathologist.

spores of *Venturia inaequalis* and *V. pyrina* while schradan did not have this fungistatic effect (13). The delta isomer of BHC was found to be toxic to *Rhizoctonia solani* (27). The reported effects of 2,4-D on fungi in culture vary from fungitoxic and fungistatic to stimulatory, depending on the formulation, concentration and test organism (1,7,8,9,19,24).

Several cases have been reported where the development of fungus diseases of plants has been affected by insecticides. Khapli wheat seedlings, for example, became susceptible to races of stem rust to which they were normally resistant when sprayed with DDT a few days prior to inoculation. This response was specific to the variety, and the pathogenicity of the rust races involved was not changed (12, 10). High concentrations of DDT in soil apparently rendered seedlings of many plants more susceptible to damping-off fungi whereas BHC and chlordane appeared to inhibit these pathogens (4). DDT and BHC preparations showed some fungicidal value against *Puccinia antirrhini* when applied with spores of this rust to snapdragon leaves (6). Systox applied to sugar-beets as a prophylactic spray reduced the incidence of infection by *Cercosporella beticola* (26).

The herbicides found to affect disease development have been of the growth-regulating type. 2,4-D was found to inhibit the germination of urediospores and growth of germ tubes of *Puccinia graminis avenae*. When applied to leaves it also reduced the number of uredia on a susceptible oat variety but the characteristic varietal reactions to stem-rust races were not altered significantly (11). High concentrations of 2,4-D were required to inhibit germination of urediospores of *Puccinia coronata* (18). Wheat seedlings sprayed with maleic hydrazide had larger leaf rust pustules (15) and were consistently more susceptible to stem rust (3) than untreated plants. Immune reactions were not altered, however, and a resistant type reaction did not change to susceptible except in the variety Khapli. The pathogenicity of *Helminthosporium sativum* to Mida wheat was increased when the inoculum was grown on medium containing 2,4-D. This result was evidently due to the predisposing effect of 2,4-D on the host plant rather than to an increase in virulence of the pathogen (9). 2,4-D and other growth-regulators did not alter the normal reaction of Red Kidney and Kentucky Wonder beans to alpha and beta strains of *Colletotrichum lindemuthianum* (17). There was some reduction of severity of infection, however, which was attributed to suppression of development of susceptible host tissues by 2,4-D rather than a modification of the plant metabolism. 2,4-D applied to flax foliage had no apparent effect on its reaction to pasmo, rust, authracnose, or stem break (20). Spraying tomato foliage with 2,4-D and other plant growth regulators was found to increase resistance to Fusarium wilt (5). Plants sprayed with maleic hydrazide, on the other hand, were more severely affected by *Fusarium lycopersici* (25).

With a view to gaining further information concerning the effect of insecticides and herbicides on the development of plant diseases of various types, a series of investigations was undertaken at this laboratory. The present communication deals with the effect of such chemicals on the development of the seedling disease of barley caused by *Helminthosporium sativum* P.K. & B.


PLANT HEIGHT – cm.
B — LINDANE
LINDANE P.P.M.
INOCULUM %
A — SCHRADAN
SCHRADAN P.P.M.
INOCULUM %
PLANT HEIGHT – cm.

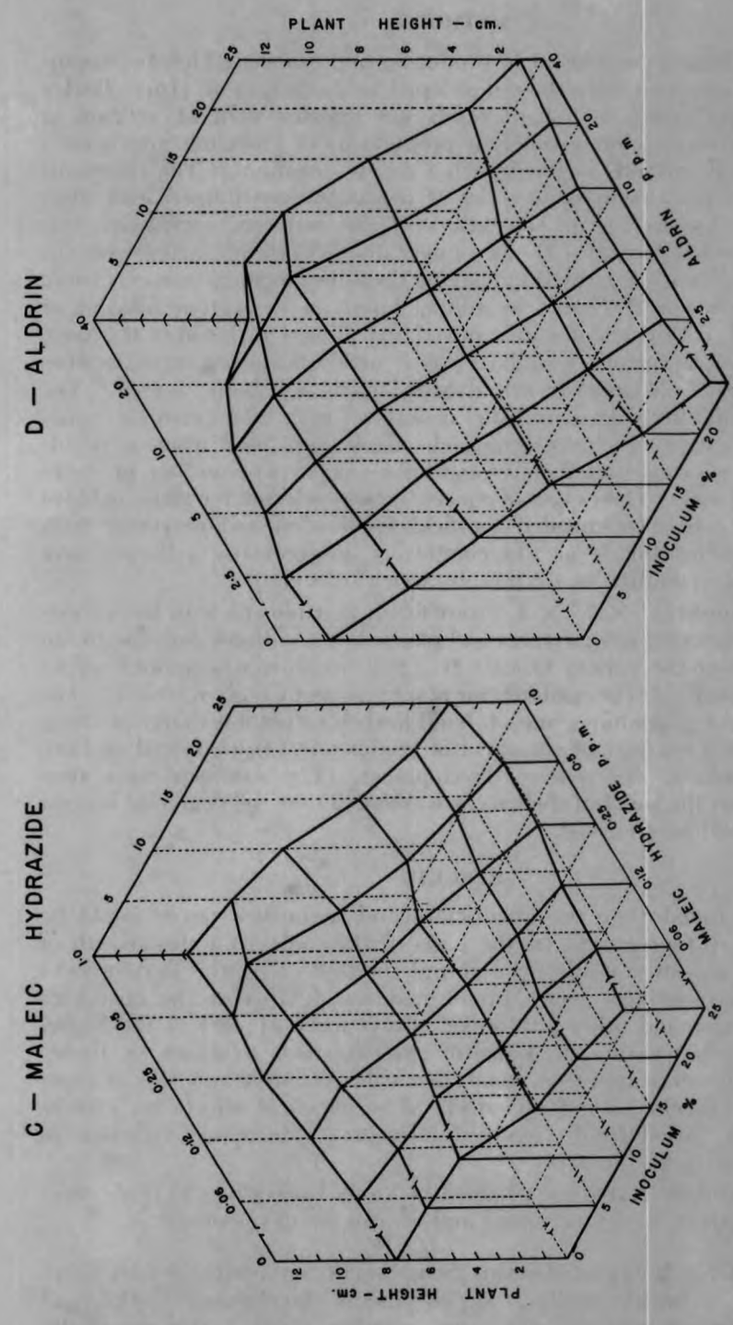


FIGURE 1. The effect of insecticides and herbicides applied to soils infested with *Helminthosporium sativum* on the height of barley seedlings. The examples are representative of four groups of chemicals separated on the basis of their effect on host and disease development (see Table I and text).

METHODS

The techniques employed in producing and assessing Helminthosporium rootrot infection were those developed by Ludwig *et al.* (16). Barley seedlings were grown in a light sandy soil infested with *H. sativum* to various degrees by mixing different proportions of inoculum grown on a sand-cornmeal mixture fortified with Czapek's medium. The chemicals were applied in a logarithmic series of concentrations immediately after infestation. Depending on the nature of the material, application was made in one of three ways: (1) as a finely divided powder mixed with the soil and diluted with untreated soil having the same inoculum concentration; (2) as a suspension (prepared by adding 1 part of an acetone solution of the chemical to 99 parts of water) mixed and diluted with soil in the same way; (3) as an aqueous solution applied at the required concentration directly to each container of soil after the seeds had been planted. The insecticides and herbicides are listed in Table I with their chemical names and dosage rates. Technical grade chemicals were used when available and dosages were calculated on a weight basis as parts per million of active ingredient in soil. The ranges of concentrations selected for tests included the highest rate recommended for field application and extended both above and below this level. In computing dosage rates, 1 lb. per acre was taken as equivalent to 1 p.p.m. by weight of soil.

Plant bands (2″ × 2″ × 3″) were filled, in triplicate, with the various treated and infested soil mixtures and planted at a uniform depth with ten barley seeds of the variety O.A.C. 21. Soil moisture was maintained as nearly as possible at the optimum for plant growth by daily watering. The average height of seedlings in each band a week to ten days after planting was taken as a measure of the effect of the chemical being tested on both host development and disease development. The seedlings were then removed from the soil and their roots washed so that the degree of rootrot infection could be observed.

RESULTS

It was found that the insecticides and herbicides tested could be separated into four groups on the basis of their effect on the growth of barley seedlings in non-infested soil and their effect on the development of rootrot in seedlings grown in infested soil. Most of the chemicals caused stunting and other phytotoxic effects when applied at the higher dosage rates, but within the range of concentrations tolerated by barley seedlings differences appeared, some chemicals causing stimulation of plant growth and others having no effect. The chemical effects on disease development varied from decreased infection to increased infection in comparison with the controls.

The chemicals tested are grouped in Table I according to their effect on the growth of barley seedlings and on disease development:

Group A. Chemicals placed in this group had no apparent effect on either the growth of barley seedlings or on disease development. Thus, all seedlings grown in soil with the same inoculum content were essentially of equal height and showed the same degree of infection, regardless of the

TABLE 1.—INSECTICIDES AND HERBICIDES EXAMINED FOR THEIR EFFECT ON THE DISEASE OF BARLEY SEEDLINGS CAUSED BY *Helminthosporium sativum*

Group[1]	Common name	Chemical name	Dosage range (p.p.m.)
A	schradan[2]	bis (dimethylamino) phosphonus anhydride	50–800
	isodrin[2]	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1:4, 5:8-*endo-endo*-dimethano-naphthalene	25–40
	DCU[3]	bis(1-hydroxy-2:2:2-trichloroethyl)urea	0.62–10
	IPX (sodium salt)	sodium isopropyl xanthic acid	0.62–10
	IPC[3]	isopropyl N-phenylcarbamate	0.25–4
	TCA (sodium salt)[3]	trichloroacetic acid	0.06–10
B	lindane[2]	gamma-1:2:3:4:5:6 hexachloro*cyclo*hexane	25–400
	dieldrin[2]	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a octohydro-1,4,5,8 dimethano-naphthalene	5–80
	DDT[2]	1:1:1-trichloro-2:2-di(*p*-chlorophenyl)ethane	6.2–100
C	maleic hydrazide[3]	1:2-dihydropyridazine-3:6-dione	0.06–1
	heptachlor[2]	1,4,5,6,7,8,8a-heptachloro-3a,4,7,7a-tetrahydro-4,7-*endo*-methanoindene	5–80
D	aldrin[2]	1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1:4,5:8-dimethano-naphthalene	2.5–80
	endrin[2]	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5,8-*endo-endo*-dimethano-naphthalene	5–80
	chlordane[2]	2,3,4,5,6,7,8,8-octochlor-3a,4,7,7a-tetrahydro-4:7-methanoindan	2.5–40
	NPA[3]	N-1-naphthyl phthalamic acid	0.6–10
	2,4-D(amine)[3]	2,4-dichlorophenoxyacetic acid	2.5–40
	monuron[3]	3-(*p*-chlorophenyl)-1:1-dimethyl urea	0.3–5.0
	DNBP (alkanol-amine salts)[3]	4-6-dinitro-ortho-*sec*.-butylphenol	0.6–10
	dalapon (sodium salt)[3]	2-2-dichloro-propionic acid	0.6–10

1 Grouping based on the effect of chemical on host plant and disease development (see text).
2 Insecticide.
3 Herbicide.

dosage of chemical applied, up to the concentration causing stunting. The plant height data for schradan, a typical Group A chemical, are presented as a three-dimensional graph in Figure 1A so that the effect of both variable factors, soil inoculum content and chemical dosage, may be compared directly.

An unusual effect was observed when the soil was treated with TCA. In non-infested soil, 0.06 to 1.0 p.p.m. TCA had no effect on the host; 1.25 p.p.m. caused slight stunting; while higher concentrations produced aborted, short-lived, blue-green seedlings. In infested soils, on the other hand, 2.5 p.p.m. TCA had no effect where 5 to 15 per cent inoculum was present, and 5 p.p.m. had no effect at the higher inoculum levels. Thus the inoculum in some way counteracted the phytotoxicity of this chemical.

Group B. Chemicals in this group stimulated the growth of barley seedlings without altering the degree of rootrot infection. The effect of lindane on plant heights is represented in Figure 1B. In non-infested soil and

at each inoculum level the heights increased as the chemical dosage increased up to 20 p.p.m. and then decreased; yet all plants from soil infested to the same degree showed equal rootrot infection. Thus treating soil with Group B chemicals resulted in more vigorous seedlings, due to chemical stimulation, not disease control.

Group C. Chemicals in this group also stimulated the growth of seedlings, as indicated by the heights of seedlings grown in non-infested soil. In infested soils, however, this beneficial effect was more than offset by an increase in severity of rootrot so that shorter plants resulted from chemical treatment. The effect of maleic hydrazide may be seen in Figure 1C. With 0 and 5 per cent inoculum in the soil the plant heights increased as the chemical dosage increased; at higher inoculum levels the heights decreased as the chemical increased. Washed roots of the latter plants showed a progressive increase in severity of rootrot with increasing chemical dosage.

Group D. The chemicals listed in the final group had no stimulatory effect on barley seedlings but improved those grown in infested soils by reducing the severity of rootrot infection. The plant height data for aldrin treatments are presented in Figure 1D. Aldrin treatments up to 10 p.p.m. had virtually no effect on the height of seedlings grown in non-infested soil, while higher concentrations reduced it. In infested soils, however, the plant heights increased with increasing concentrations, even to 40 p.p.m. That this effect was correlated with a reduction of rootrot may be seen from the photograph (Figure 2) of one replicate of these plants.

Figure 3 illustrates a set of barley seedlings growing in soils treated with NPA, another chemical of Group D. Not only has the chemical improved the seedlings by reducing rootrot, but the inoculum has in some way overcome the phytoxic effects of the highest concentration of the chemical (stunting, rolling and distortion of leaves).

EFFECT OF INSECTICIDES AND HERBICIDES ON *H. SAVITUM* IN CULTURE

In order to determine to what extent the effect of an insecticide or herbicide on disease development could be attributed to a direct effect on the causal organism, each of the chemicals was tested for toxicity to *H. sativum* in culture. For this purpose tubes of Czapek's agar were made up with a series of concentrations of each chemical in solution or suspension, the required amount of chemical being added to each tube as either a water or acetone solution after autoclaving. The final acetone content of the medium, where present, was 2 per cent. Insecticides were tested at 12.5, 25, 50 and 100 p.p.m., and herbicides at 1.24, 2.5, 5 and 10 p.p.m., to cover the dosage ranges tested in the disease trials. The tubes were cooled to 50°F., inoculated with spore suspension, shaken, and sloped for final cooling and solidification. After incubation at room temperature the relative amounts of mycelial growth were observed. DNBP was the only chemical to affect the growth of *H. sativum*, retarding it at 1.25 p.p.m. and inhibiting it completely at higher concentrations.

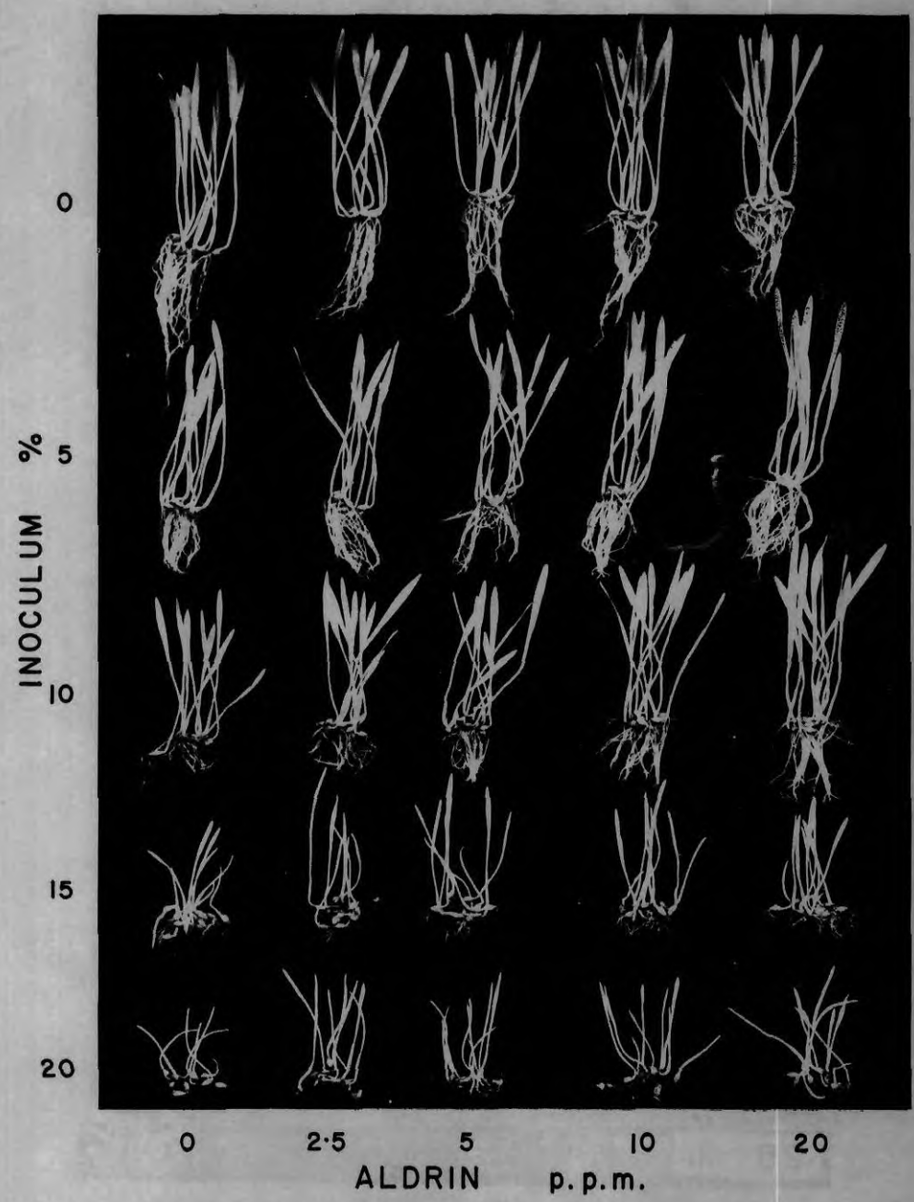


FIGURE 2. The effect of aldrin applied to soils infested with *Helminthosporium sativum* on rootrot and height of barley seedlings.

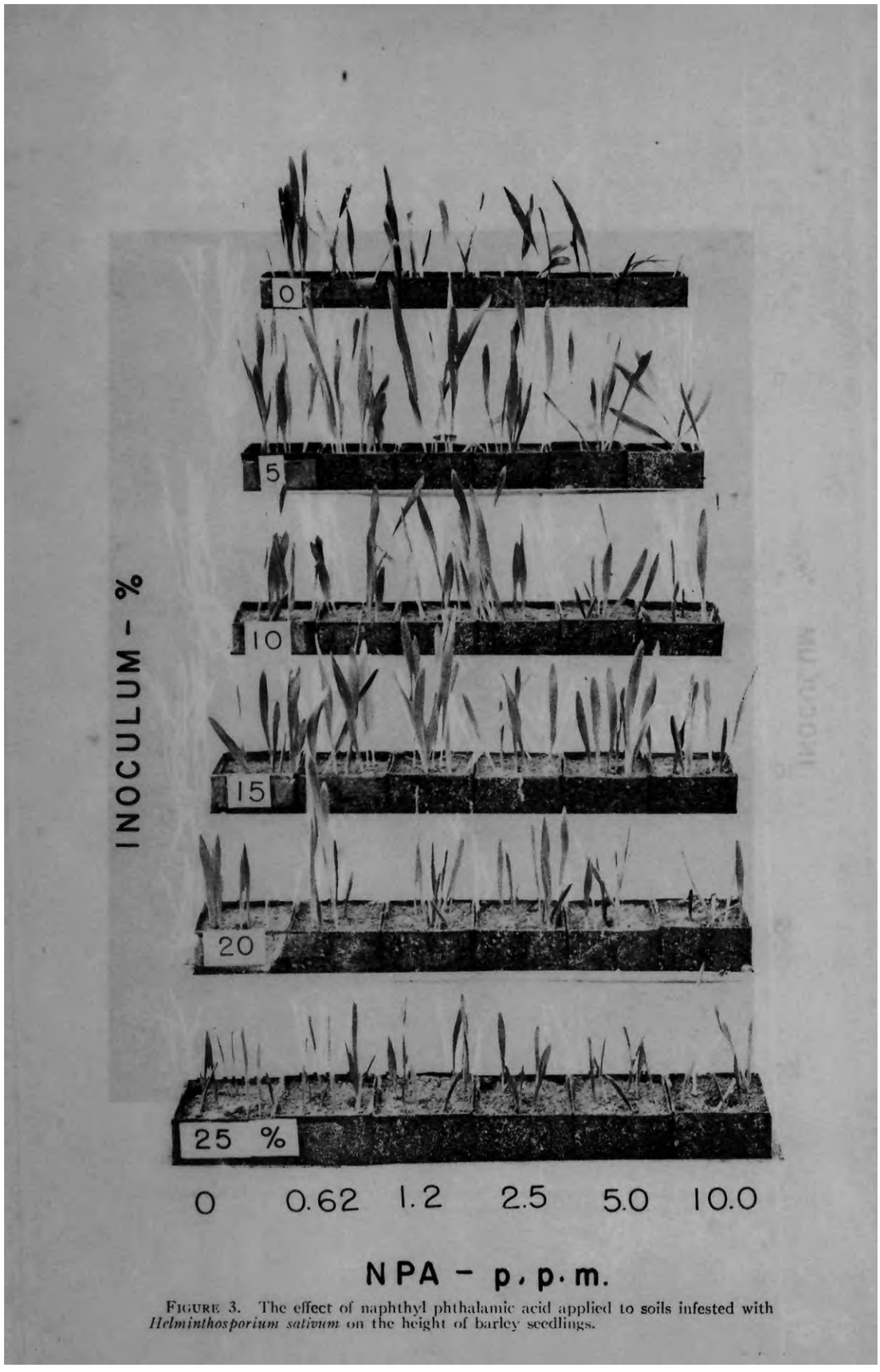


FIGURE 3. The effect of naphthyl phthalamic acid applied to soils infested with *Helminthosporium sativum* on the height of barley seedlings.

Since the hydrogen-ion concentration of the medium is known to affect the toxicity of many compounds (21), the experiment was repeated using a buffered synthetic medium adjusted to pH levels 2.5, 5.5 and 7.5. Again only DNBP was fungitoxic and its effect did vary with the acidity of the medium. At pH 3.5 growth was inhibited by 1.25 p.p.m. DNBP; at pH 5.5 growth was retarded by 2.5 p.p.m. and inhibited by 5.0 p.p.m., at pH 7.5 growth was only retarded by 10.0 p.p.m.

DISCUSSION

From the results of these studies it appears unlikely that the application of these insecticides and herbicides to the soil will aggravate the disease of barley seedlings caused by *Helminthosporium sativum*. Only two of the nineteen chemicals tested gave any indication of increasing the degree of infection, whereas eight were beneficial in that they reduced infection and the remainder had no effect on disease development.

The mechanism of action of these chemicals which did affect disease development is a matter of speculation. Since only one chemical, DNBP, was toxic to *H. sativum* in culture, the effect cannot be explained in all cases by a direct action of the chemicals on the causal organism. It is possible that the chemicals alter the metabolism of the host in a way which increases or decreases its resistance to the disease. There was no apparent relationship between effect on disease development and chemical structure. Aldrin and dieldrin, for example, were not grouped with their isomers isodrin and endrin.

ACKNOWLEDGEMENTS

The author is indebted to R. A. Ludwig for his interest and guidance in this study, and to B. N. Smallman and W. H. Minshall for advice concerning insecticides and herbicides respectively.

REFERENCES

1. Bever, W. M., and F. W. Slife. Effect of 2,4-D in culture medium on the growth of three pathogenic fungi. Phytopathology 38 : 1038. 1948.
2. Bollen, W. B., H. E. Morrison, and H. H. Crowell. Effect of field treatments of insecticides on numbers of bacteria, *Streptomyces* and molds in the soil. J. Econ. Entomol. 47 : 302-306. 1954.
3. Bromfield, K. R., and C. E. Peet. Chemical modification of rust reaction of seedling wheat plants infected with *Puccinia graminis tritici*. (Abstract). Phytopathology 44 : 483. 1954.
4. Cullinan, F. P. Phytotoxicity of chlorinated hydrocarbon insecticides. J. Econ. Entomol. 42 : 387-391. 1949.
5. Davis, D., and A. E. Dimond. Inducing disease resistance with plant growth regulators. Phytopathology 43 : 137-140. 1953.
6. Dimock, A. W. The dispersal of viable fungus spores by insecticides. Phytopathology 41 : 152-156. 1951.
7. Fenner, L. M., and L. R. Fate. *Ceratostomella ulmi* on elm bark treated with 2,4-dichlorophenoxyacetic acid. Phytopathology 37 : 925-928. 1947.
8. Guiscafre-Arrillaga, J. Inhibitory action of 2,4-dichlorophenoxyacetic acid on *Penicillum digitatum* and *Phomopsis citri*. (Abstract). Phytopathology 39 : 8. 1949.
9. Hsia, Y., and J. J. Christensen. Effect of 2,4-D on seedling blight of wheat caused by *Helminthosporium sativum*. Phytopathology 41 : 1011-1020. 1951.
10. Hotson, H. H. Some chemotherapeutic agents for wheat stem rust. Phytopathology 43 : 659-662. 1953.

11. Ibrahim, I. A. Effect of 2,4-D on stem rust development of oats. Phytopathology 41 : 951-953. 1951.

12. Johnson, T. The effect of DDT on the stem rust reaction of Khapli wheat. Can. J. Research, C, 24 : 23-25. 1946.

13. Kirby, A. H. M., and E. L. Frick. The fungistatic activity of certain organophosphorus insecticides. Ann. Rept. East Malling Research Sta., 1950 : 172-176. 1951.

14. Lewis, R. W., and C. L. Hamner. The effect of 2,4-D on some micro-organisms. Mich. Agr. Expt. Sta. Quart. Bull. 29 : 112-114. 1946.

15. Livingston, J. E. The control of leaf and stem rust of wheat with chemotherapeutants. Phytopathology 43 : 497-499. 1953.

16. Ludwig, R. A., R. V. Clark, J. B. Julien, and D. B. Robinson. Studies on the seedling disease of barley caused by *Helminthosporium sativum* P.K. and B. Can. J. Botany 34 : 653-673. 1956.

17. Natti, J. J., and M. Szkolnik. Influence of growth regulators on resistance of beans to *Colletotrichum lindemuthianum*. (Abstract). Phytopathology 44 : 111-112. 1954.

18. Peturson, B. Effect of growth-promoting substances on the germination of uredio-spores. Phytopathology 41 : 1039-1040. 1951.

19. Richards, R. R. Responses of representative fungi to certain growth regulating substances. Botan. Gaz. 110 : 523-550. 1948.

20. Sackston, W. E. The effect of 2,4-D on some diseases of flax. U.S.D.A. Plant Disease Reptr. 32 : 386-387. 1948.

21. Simon, E. W., and G. E. Blackman. The significance of hydrogen-ion concentration in the study of toxicity. Soc. Exptl. Biol. Symposia 3 : 253-265. 1949.

22. Smith, N. R., V. T. Dowson, and M. E. Wenzel. The effect of certain herbicides on soil microorganisms. Soil Sci. Soc. Amer. Proc. 10 : 197-201. 1945.

23. Smith, N. R., and M. E. Wenzel. Soil microorganisms are affected by some of the new insecticides. Soil Sci. Soc. Amer. Proc. 12 : 227-233. 1947.

24. Stevenson, E. C., and J. W. Mitchell. Bacteriostatic and bactericidal properties of 2,4-dichlorophenoxyacetic acid. Science 101 : 642-644. 1945.

25. Waggoner, P. E., and A. E. Dimond. Effect of stunting agents, *Fusarium lycopersici*, and maleic hydrazide upon phosphorus distribution in tomato. (Abstract). Phytopathology 42 : 22. 1952.

26. Wenzl, H., A. Graf, R. Meinx, and R. Krexner. Uber die Wirkung des innertherapeutischen Insektizides Systox gegen *Cercosporella beticola* an Rube. Pflanzenschutz Berichte 14 : 65-75. 1955.

27. Wilson, J. K., and R. S. Choudri. BHC and soil microflora. J. Agr. Research 77 : 25-32. 1948.