Technical Papers

The Effects of Indole-3-acetic Acid on the Plant Disease-Inhibiting Properties of Antibiotics[1]

R. N. Goodman and D. D. Hemphill

Department of Horticulture,
University of Missouri, Columbia

It has been reported recently that streptomycin (*1–8*) and combinations of streptomycin and Terramycin (*3–5*) effectively control fireblight (causal agent, *Erwinia amylovora*), a heretofore uncontrollable bacterial disease of apples and pears. Disease control was excellent under both orchard and greenhouse conditions.

In greenhouse experiments a single spray combining streptomycin and Terramycin each at 250 ppm was applied to the foliage of one-year-old potted apple trees (var. Jonathan). The inoculum was administered subepidermally to these plants 24 hr after the antibiotic sprays were applied. Under these conditions, control of the disease varied from 90 to 100 percent, indicating that the antibiotics were systemic in their action (*3, 4*).

Efforts were made to repeat this work with apple trees of the same age and variety that had been kept in cold storage (32° F) from Nov., 1952 through Sept., 1953. These trees failed to respond to the antibiotic treatment previously described. Control of disease in these experiments varied from 10 to 50 percent (*9*).

There were several observable differences in the trees that were kept in an extended period of dormancy: (1) a major portion of the lateral buds on the single main stem had broken in storage; (2) all lateral buds that had broken commenced growing upon exposure to favorable conditions in the greenhouse; (3) the activity of the numerous growing points appeared to reduce the vigor of the individual shoots; (4) there was a distinct loss of apical dominance displayed by the individual tree; (5) the characteristic foliar chlorosis caused by streptomycin (*4, 5*) was not observed, although the spray formulation and streptomycin concentration remained the same.

The performance of these trees indicated that the substance(s) responsible for the apical dominance phenomenon had been destroyed or changed during the prolonged storage period. Apical dominance has been demonstrated to be an auxin-regulated growth response (*10*). Recently natural disease resistance has been demonstrated to be increased by plant growth regulators (*2*). Moreover, small quantities of antibiotics have been shown to have a synergistic effect on plant response to auxin (*11*). These experimental results and the aforementioned differences observed on the apple trees held under conditions of prolonged dormancy suggested that indole-3-acetic acid might possibly increase the effectiveness of antibiotics in the control of *Erwinia amylovora*.

The antibiotic spray formulation was a combination of streptomycin and Terramycin, each at 250 ppm and 1 percent each of methyl cellosolve and Carbowax 4000. The latter two materials were used to facilitate penetration of the plant tissue by the antibiotic (*4*). The spray was applied 24 hr prior to inoculation.

An aqueous suspension of a 48-hr culture of *Erwinia amylovora* was injected subepidermally at a point midway between the apex and the first node of a vigorously growing shoot. Eight trees, two shoots per tree, were used in each treatment as follows: (1) control—methyl cellosolve, Carbowax 4000, and 100 ppm indole-3-acetic acid; (2) antibiotics plus methyl cellosolve and Carbowax 4000; (3) antibiotics, methyl cellosolve, Carbowax 4000, and 100 ppm indole-3-acetic acid. The data are shown graphically in Fig. 1. This

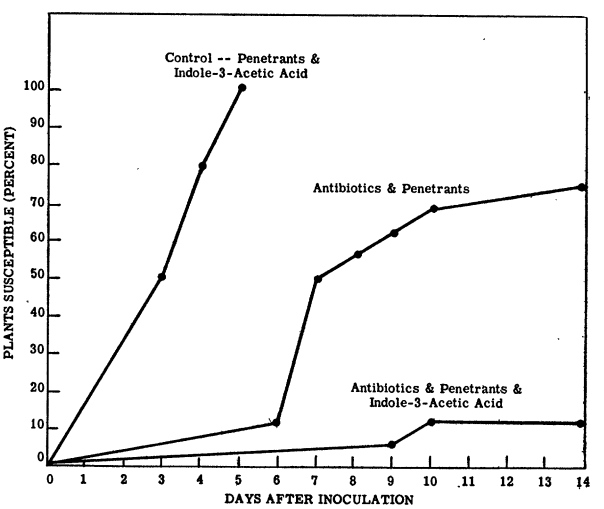

FIG. 1. Increased effectiveness of antibiotics by indole-3-acetic acid.

experiment was repeated five times with similar results.

The trees that were subjected to the prolonged dormancy (Nov., 1952 to Sept., 1953) did not respond as favorably to antibiotic treatment as those kept in cold storage for a shorter time (Nov., 1952 to Jan., 1953). The addition of 100 ppm of indole-3-acetic acid to the antibiotic formulation increased the protection from 25 to 87 percent.

The data suggest that indole-3-acetic acid plays a role in the disease-inhibiting action of antibiotics and possibly the disease inhibiting mechanism of the plant.

Experiments are in progress to determine if the various groups of synthetic growth regulators have the same effect as the native substance (heteroauxin). Precursors of indole-3-acetic acid are also being evalu-

[1] Journal Series Paper No. 1406 approved by the director of the Missouri Agricultural Experiment Station.

ated. This synergistic effect of plant growth regulators on antibiotic inhibition of disease may have an important practical application in the economic use of antibiotics in plant disease control.

References

1. ARK, P. A. *Plant Disease Reptr.* **37**, 404 (1953).
2. DAVIS, D., and DIMOND, A. E. *Phytopath.* **43**, 137 (1953).
3. GOODMAN, R. N. *Am. Fruit Grower* **73**, 7 (1953).
4. ———. *Missouri Agr. Exptl. Sta. Research Bull.* In press.
5. ———. *Proc. Am. Soc. Hort. Sci.* In press.
6. HUEBERGER, J. W., and POULOS, P. L. *Plant Disease Reptr.* **37**, 81 (1953).
7. MURNEEK, A. E. *Phytopath.* **42**, 57 (1952).
8. WINTER, H. F., and YOUNG, H. C. *Plant Disease Reptr.* **37**, 463 (1953).
9. MORGAN, B. S., and GOODMAN, R. N. Unpublished data.
10. WENT, F. W., and THIMANN, K. V. *Phytohormones.* New York: Macmillan, 1937.
11. IYENGAR, M. R. S., and STARKEY, R. L. *Science* **118**, 357 (1953).

Received December 21, 1953.

Effect of Piromen on Survival Following Severe Thermal Injury in Rats[1]

M. D. McCarthy and Virginia Blackburn

Harrison Department of Surgical Research, Schools of Medicine, University of Pennsylvania, Philadelphia and *Department of Zoology, Pomona College, Claremont, California*

A series of experiments conducted by McCarthy *et al.* (*1–4*) leads us to conclude that rather large volumes of various parenteral solutions were necessary to promote survival of rats following severe thermal injuries of 32 percent or more of the total body surface. Hence, we were very much interested in the experiments reported by Greene, Stuart, and Joralemon (*5*) in which they showed that significantly increased survival rates over control survival rates of thermally injured rats were obtained following treatment with the drug Piromen.[2] We were most anxious to learn whether rats receiving severe thermal injuries of 50 ± 2 percent of the body surface would survive if treated by single intraperitoneal injections of Piromen postburn. Recent work (*3*) with this extent of injury demonstrated that survival of more than 60 percent of rats burned in this way necessitated continuous intravenous infusions, begun immediately postburn, and continued for 10 hr, during which time a total of 18 percent of the body weight of sodium lactate, sodium chloride, whole blood, and plasma was infused. Consequently, we treated a number of rats receiving 50 ± 2 percent burns with single, postburn intraperitoneal injections of 1 μg of Piromen per rat. All these animals died. During a discussion of this experiment with Greene and Stuart, one of us was advised that our dosage was excessive. Hence, we treated a second group of rats receiving 50 ± 2 percent burns with 0.1 μg of Piromen per rat. These animals also died. We have subsequently reduced the percentage area burned and varied the dosage of Piromen to conform to that which Stuart (*6*) considered optimum, that is, 0.65 μg/kg. We have been unable to confirm the findings of Greene *et al.* (*5*).

Material and Methods. Unshaved male Wistar rats under ether anesthesia were subjected to back burns in water at 90° C for 35 sec according to the procedure reported by McCarthy (*7*). In those experimental groups that included simultaneous controls, the animals were segregated for test or control treatment by lot prior to burning. Therapy in all cases was administered intraperitoneally immediately following burning according to the method reported by Greene *et al.* (*5*). The Piromen,[3] lot number N-P-68, was diluted with pyrogen-free distilled water just prior to use. A new stock bottle of Piromen was used for each experiment. All syringes and needles were autoclaved and rinsed thoroughly with pyrogen-free distilled water. The control treatment consisted of intraperitoneal injections of 0.9 percent NaCl solution given in volumes equivalent to those which the Piromen-treated animals received. Following the burning and treatment, all animals were returned to individual wire-mesh cages where they had free access to water and Purina Laboratory Chow. The animals were checked every 3 hr for the first 12 hr following the burn and twice daily for the remainder of a 10-day postburn interval, at which time any living animals were sacrificed. Each animal listed in these experiments was skinned upon its death or sacrifice and a planimeter measurement made of the burned area. Any animals found to have injured areas outside of the range designated for a particular group were not included in such a group but were discarded. The weight variations of the animals in the various experimental groups in no case exceeded 20 g. In experiments A and B, all rats received 50 ± 2 percent burns. In A, each animal received 1 μg of Piromen (P), and in B, each animal received 0.1 μg of P. In experiment C, each animal received a 37 ± 2 percent burn and 1 μg of P. In experiment D, all rats received a 36 ± 2 percent burn; half of these animals each received 1.0 μg of P and the other half received no therapy. In experiment E, all rats received a 32 ± 2 percent burn; half of these received 0.1 μg of P, a fourth of them each received a volume of 0.9 percent NaCl solution equivalent to that which the Piromen-treated group received, and a fourth of them received no treatment. All Piromen-treated animals in experiments A to E inclusive received their respective P concentrations in 0.1 cc of diluent. In the experiments F, G, and H, 10 rats in each, selected by lot, received P and 10 rats in each received placebo therapy. The dosage of P was calculated individually so that each rat received 0.65 μg/kg; the placebo therapy of 0.9 percent NaCl solution was given in volumes calculated individually so that each rat received a volume equivalent to that which an animal of similar weight would have received of the P solution.

[1] Supported in part by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army, Contract DA-49-007-MD-88.

[2] A bacterial polysaccharide complex.

[3] The Piromen was made available to us through the courtesy of Dr. William F. Windle of the Travenol Laboratories, Inc., Morton Grove, Ill.