GROWTH SUBSTANCES IN SYMPTOM DEVELOPMENT IN DOWNY MILDEW OF SUNFLOWERS

By

M. A. Viswanathan and W. E. Sackston
Department of Plant Pathology
Macdonald College of McGill University
Ste. Anne de Bellevue, Quebec, Canada

The object of these studies is to determine the biochemical mechanisms involved in the symptom development in downy mildew of sunflowers caused by Plasmopara halstedii. Symptoms of the disease vary with the severity of infection. Infected plants are often very much stunted as a result of decrease in the length of their internodes. Leaves may often be distorted and show chlorotic areas on the upper surfaces. In older leaves, a symmetrical pattern of vein chlorosis may also be evident. Although the above mentioned symptoms are encountered in plants infected with fungi, bacteria or viruses, these symptoms appear to be characteristic for sunflowers affected by downy mildew. So far, no other pathogen is known to induce similar symptoms on this plant.

The reduction in growth and the various degrees of disorganization of sunflowers indicated that some imbalance in the metabolism of growth substances was involved. To investigate this, we started first with a comparative determination of indole-3-acetic acid (IAA) in healthy plants and those affected by downy mildew.

Preliminary work on the extraction of IAA from limited quantities of diseased material was not successful and it was found necessary to obtain relatively large quantities of diseased material. The availability of large quantities of diseased material was restricted to the growing season. Further, infection under natural conditions was often uncertain. Such samples, in addition, are likely to be heterogeneous with regard to the degree of infection and severity. It was therefore found necessary to devise means of obtaining consistently heavily infected plants of uniform age and growth. This problem was overcome by the following technique. Seeds of sunflowers were dehulled and surface sterilized in 1% sodium hypochlorite (commercial Javex solution diluted 1:1) for 5 minutes. They were washed several times in distilled water and germinated on moist filter papers. After 24 to 48 hours, when the radicle with numerous root hairs had appeared, they were kept in an active zoospore suspension of the disease inducing organism, P. halstedii. The zoospore suspension was prepared by placing a few leaves on which the fungus was sporulating (often stored in the freezer at -20°C), in distilled water at 10°C for a few hours. The germinated seeds were kept in this suspension for at least 4 to 5 hours. They were then planted

carefully in pots of pasteurized garden soil and transferred to growth chambers (1600 ft.cd., 20°C, 16 hr. day length). After 10 to 15 days when the cotyledonary leaves showed indications of infection, the plants were kept in a saturated atmosphere. After 24 to 48 hours very heavy sporulation was obtained. The plants were cut out, frozen in liquid air, and stored in a freezer at -20°C until they were needed.

Two methods were tried for the extraction of IAA, one involving cold 95% ethanol (Bentley, 1962), and the other boiling ethyl acetate (Sequeira, 1967). In the first method, excessive amounts of extracted lipids and pigments interfered with the satisfactory separation of IAA. The latter method, which was found to be more satisfactory, was therefore adopted.

Aliquots of ether extracts of both healthy and diseased plants obtained as above were spotted on Whatman No. 1 chromatography paper and substances were separated with isopropanol-acetone-water (10:1:1). The amount of IAA in the chromatograms was not sufficient to be estimated quantitatively by color reactions either with Ehrlich or Salkowski reagents. Hence, only bioassay was carried out.

The Avena Coleoptile Straight Growth Test was employed for the bioassay. Each strip of chromatogram from origin to front was divided into 20 parts, each approximately equal to 0.05 Rf unit. Each of these was then eluted in tubes with 2 ml of citrate buffer pH 5.0 containing 2% sucrose. Five millimeter long sections of coleoptiles of oat variety "Brighton" were cut and transferred to each tube and the tubes were placed in a rotator with a speed of 1 rpm in the dark at 20°C. After 20 hr. of growth, the coleoptiles were removed and measured with the aid of a projector. The growth of treated coleoptiles was calculated as a percentage of the growth obtained in the controls. Growth inhibitions and growth promotions are evident at various positions in the chromatogram (Fig. 1). The Rf value of IAA being 0.2 it is evident from Fig. 1 that in the diseased plants there is a rather considerable decrease of IAA.

Gibberellins are naturally occurring substances in plants which influence their growth, and it was interesting to study them. No extraction of these substances from the diseased sunflowers was attempted. However, we studied the effect of supplying gibberellic acid (GA) to diseased and healthy plants. One drop of 0.01% solution of GA was applied daily to the growing tips of both healthy and diseased plants and their height was recorded at regular intervals. In an earlier experiment, we found that GA actually had an adverse effect on the growth of diseased plants. This was contrary to what was reported in the literature with regard to the effect on diseased adult plants under field conditions (Novotelnova, 1966). It was thought that this apparent contradiction was due to the fact that our plants were already severely affected by the disease prior to the application of GA. Hence, in a later experi-

BIOASSAYS OF EXTRACTS

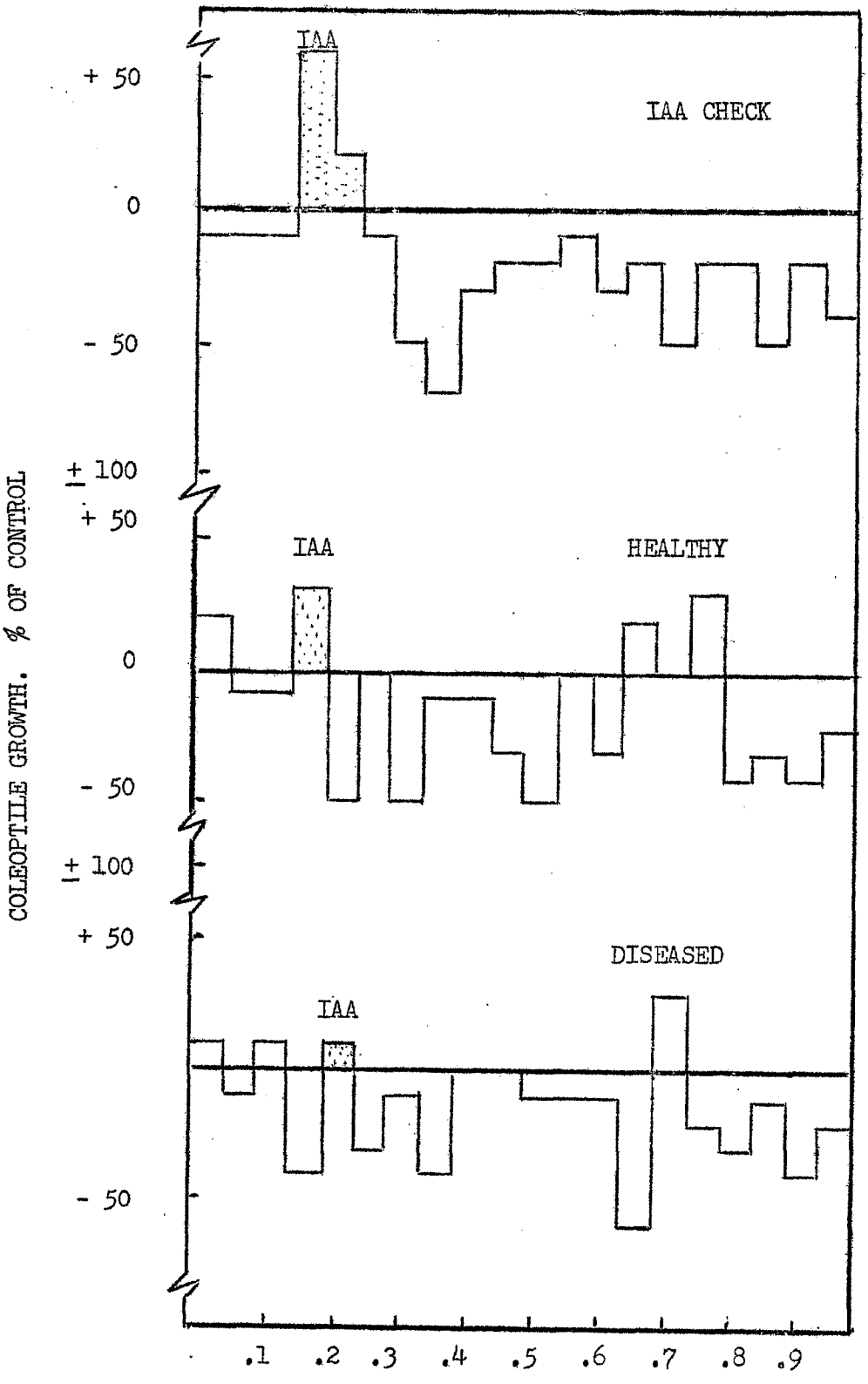

Fig. 1

ment, seedlings were inoculated with an active zoospore suspension when they were 5 days old, and the application of GA was started immediately thereafter. It was observed that in these plants which were inoculated a few days after their initial growth, the application of GA reduced the severity of symptoms. Similar results were obtained with the application of IAA alone or a mixture of both GA and IAA. In all cases a definite positive response was obtained. But the diseased plants never showed the same growth response as did the comparable healthy plants. It is proposed to continue work on this line with some refinement.

Our studies conducted so far on sunflowers affected by downy mildew showed the following indications: First, there was a reduction in the amount of IAA in the diseased plants. Whether this is also true for GAA remains to be verified. This we propose to do in further studies. Secondly, a positive response to the application of both IAA and GA was obtained. Thirdly, GA had a more pronounced effect than IAA on the diseased plants. Lastly, in no instance did the diseased plants respond to the same extent as comparable healthy plants, as a result of the application of either IAA or GA.

In a disease such as the downy mildew of sunflowers caused by an obligate parasite, any of the abnormal changes mentioned earlier cannot be positively attributed to the action of either the host or to the pathogen alone, since we are as yet not in a position to study the pathogen away from its host. The above studies have been made with seedlings of sunflowers that were between 10 and 15 days old, and it is quite conceivable that a different picture obtains in adult plants. In the seedling stage, the typical symptoms of downy mildew were often not observed. Techniques are being devised to obtain severely infected adult plants so that some of our earlier experiments may be conducted with them. Instead of IAA or GA alone, perhaps an interplay of both IAA and GA is involved in the disease syndrome. Any reduction in the level of growth substances in the diseased plants could be the result of inhibition of their synthesis or of increased breakdown of these substances by the host, the pathogen, or both. Perhaps the production of some specific inhibitory substances or substances elaborated in the diseased plants is responsible for interfering with the normal role of these growth substances. It is along these lines that we propose to conduct further studies on this disease.

References:

Bentley, Joyce A. 1962. Analysis of plant hormones. In David Glick (ed.) Methods of Biochemical Analysis Vol. IX: 75-125.

Novotelnova, N. S. 1966. Downy mildew of sunflowers. Taxonomy, biology of the pathogen, and pathogenesis of the disease (In Russian). Publishers "Science". Moscow-Leningrad. 150 pp. 49 figs.

Sequeira, L. 1967. Determination of auxin in culture filtrates of plant pathogens and in diseased plants. In Sourcebook of laboratory exercises in plant pathology. pp. 199-201.

* * *