

## A Plant Growth Promoting Substance Found in an Acorn Weevil of the Family Curculionidae

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Freshly fallen acorns of white oak, *Quercus alba* L., were collected in October 1959 by John C. Callahan of Purdue University in Orange County, (southern) Indiana as a part of an oak regeneration study. Approximately half of the acorns were found to be infested with the larva of an acorn weevil of the family Curculionidae. Callahan made the interesting observation that the endosperm of infested acorns was in a fresh green condition while the endosperm of acorns free of insects was brown. Whether the green condition of the endosperm is the result of premature abscission or of delayed maturation requires further investigation. Unfortunately no acorns have been produced during the past two seasons to carry on this work.

In the present study 170 larvae (having a combined fresh weight of 8.85 grams) were used in a preliminary test for the possible presence of a plant growth hormone in the insects that could account for the phenomenon observed. The larvae were stored at  $-18^{\circ}\text{C}$  until ready for use. They were then twice extracted with cold methanol and the extracts combined and taken to dryness in vacuo at  $45^{\circ}\text{C}$ . The resultant yellow oily substance was redissolved in a small amount of methanol and fractionated using ascending paper chromatography with 80% v/v isopropanol as the solvent.

The developed chromatogram was cut into 15 equal horizontal segments. These segments were each eluted in an aqueous solution of 0.1% polysorbate-80 wetting agent buffered at pH 5.5. Each of the eluates was tested for growth promoting (i.e., auxin) activity using an excised oat, *Avena sativa* L. Brighton, coleoptile section straight growth test modeled

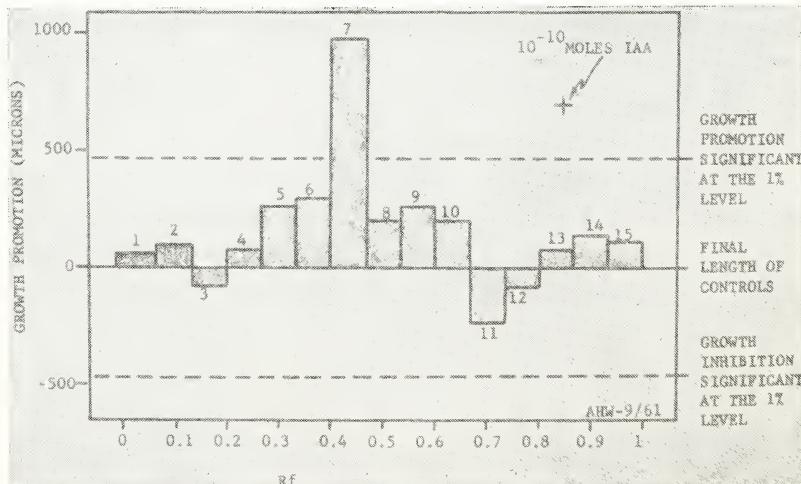


Figure 1. Plant growth substance bioassay of the fractionated extract of a curculionid acorn weevil. Fractionation was achieved by ascending paper chromatography using 80% v/v isopropanol. The oat coleoptile sections had an initial length of 4.01 mm; the final length attained by the untreated (control) sections was 5.27 mm.

after the one described by Nitsch et ux. (1) and tested by an analysis of variance followed by J. W. Tukey's procedure for multiple comparisons (3).

The eluate from segment 7 (i.e., at an Rf of 0.43) was demonstrated by the above assay to contain a growth-promoting factor; the other 14 eluates exhibited no significant activity (see Figure 1). The growth promoting substance in eluate 7 is unidentified as yet. It is not indoleacetic acid (which has an Rf of 0.86 in this system) and may not even be an indolic compound since it did not give a positive reaction with either Salkowski reagent (acidified FeCl<sub>3</sub>) or Ehrlich reagent (*p*-dimethylaminobenzaldehyde). A positive reaction (chartreuse color) with *p*-diazobenzenesulfonic acid could indicate the presence of a phenolic or aldehydic compound while a positive reaction (lavender color) with ninhydrin suggests the presence of amino plus carboxyl groups. Further tests and characterizations must await future collections.

An attempt was made to approximate the quantity of growth promoting substance extracted from each larva. Under the assay conditions used,  $1.8 \times 10^{-10}$  moles of indoleacetic acid resulted in the same amount of promotive activity as did the extracted and fractionated growth promoting substance of eluate 7. If one assumes similar molecular weights (175), similar biological activities, and 70% efficiency of extraction and recovery, each larva contributed roughly  $1.5 \times 10^{-12}$  moles of growth promoting substance. This is indeed a small amount but presumably the larva could be continuously producing the substance.

There have been several instances where it was conjectured that an insect was influencing plant development by producing auxins. The finding reported here is of interest because in perhaps the only other actual attempt to demonstrate this, the presence of such plant growth promoting substances in the insect could not be detected. This was the case when Plumb (2) tested to no avail a crude extract of the salivary gland of the fundatrix stage of the aphid *Adelges abietis* (L.), which produces a gall on Norway spruce, *Picea abies* (L.) Karst., using the split pea test.

Additional research on the plant growth substance extracted from the acorn weevil larvae should be directed towards its characterization and its biological activity with reference to maturation and abscission.

### Summary

The acorns of *Quercus alba* drop in a green rather than brown condition when infested with the larvae of an acorn weevil of the family Curculionidae. A methanolic extract of these larvae was found to contain a plant growth promoting substance, not indoleacetic acid.

### Literature Cited

1. NITSCH, J. P., and C. NITSCH. 1956. Studies on the growth of coleoptile and first internode sections. New, sensitive, straight-growth test for auxins. Plant Physiol. 31: 94-111.
2. PLUMB, G. H. 1953. Formation and development of the Norway spruce gall caused by *Adelges abietis* L. Conn. Agric. Exp. Sta. Bull. 566, 77 pp.
3. STEEL, R. G. D., and J. H. TORRIE. 1960. Principles and procedures of statistics with special reference to the biological sciences. N. Y.: McGraw-Hill, 481 pp.