Short Communication

Florigenic Acid From Fungal Culture

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Summary. The extraction procedures which have been successfully employed in the preparation of a florigenic principle from the tissues of Xanthium, are applicable to the derivation of an entity of similar activity from Calonectria culture. The Xanthium principle is acetic, with $pK_a$ values characteristic of a carboxylic acid (6). Although definitive chemical comparisons have not been completed, the extraction and solvent partition procedures that have been applicable to the extraction of the active entity from higher plant tissues have yielded florigenic preparations from fungal culture. The chemical principle from higher plant tissue may be the same or similar to the florigenic agent of Calonectria.

The many responses of higher plants to growth regulators produced by microorganisms are well known. The presence of a flower-producing principle from Calonectria (Fusarium) rigidiuscula parallels closely the pattern exhibited by those fungal species capable of the production of auxin and gibberellins.

A substance, acidic in nature, has been extracted from the tissues of several flowering plants which will cause the initiation of floral primordia when applied to vegetative Xanthium strumarium test plants, Chicago strain (1, 2, 4, 5, 6). This florigenic acid is apparently present in the tissues of flowering higher plants in very small amounts. The extraction of this material in quantities appropriate for purposes of chemical identification has posed familiar and formidable problems.

In the search for a more abundant source of the florigenic factor, we have recently extracted cultures of the parasitic gall fungus of Cacao, Calonectria (Fusarium) rigidiuscula, (Berk. and Br.) Sn. and H.

This fungus is known to produce a hypertrophic condition of the host which develops as a proliferation of vegetative buds known as green point. The fungus can also be consistently isolated from flower galls of the same plant. A culture of the Calonectria fungus, clone No. 176, which had been isolated from flowering galls of Cacao in Central America, was obtained from the collection of W. C. Snyder, University of California, Berkeley. Clonal lines of the fungus were maintained in stock culture by single spore transfer. Obvious morphological variants from the original single spore isolate were discarded. The fungus was mass cultured on potato dextrose agar by seeding large flasks of media with a dilute conidial suspension from the stock material. The cultures were allowed to develop for 2 weeks at which time the contents of the flasks were homogenized in a blender with the addition of 20 ml of water for every 100 ml of media. The resulting slurry was centrifuged at 12000 $\times g$ for 30 minutes. The supernatant was decanted and retained; the agar pellet was discarded. The liquid was neutralized with 1 N NaOH and the neutral fractions administered to vegetative Xanthium test plants by means of a stem-flap method (1). Each of the 20 test plants accepted approximately 8 ml of the solution. Twenty untreated plants of the same age were retained as controls. All plants were maintained in the greenhouse on continuous light for 60 days at which time they were dissected and the morphological stage of development of the terminal, staminate inflorescence recorded according to the system of Lincoln et al. (4). The results are presented in table I.

A second preparation of the active extract in the manner described yielded a fraction which caused the initiation of floral primordia in 25% of the treated plants. In each instance, all of the control plants remained in the vegetative condition.

Previous work with extracts from the tissues of higher plants has yielded a florigenic principle. Based on the assumption that the active entity of the fungal extract may be attributed to a substance

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Table I. Flowering Response of Xanthium strumarium Following Applications of an Extract of Calonectria rigidula

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<th>No. of flowering stage flowers</th>
<th>% of numerical avg plants</th>
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<tbody>
<tr>
<td>Untreated control plants</td>
<td>20</td>
<td>0.0</td>
</tr>
<tr>
<td>Treated plants</td>
<td>20</td>
<td>1.0</td>
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of similar chemical properties, a procedure involving solvent partition was attempted. The fungus was shake-cultured in potato dextrose broth for 2 weeks. The mycelial mass was then separated from the liquid by use of a basket centrifuge. The liquid was acidified to pH 3 with 1 N HCl and then shaken with twice its volume of ethyl acetate. The water portion was discarded and the ethyl acetate portion treated with an equal volume of 2% (w/v) KHCO₃. The aqueous material was again brought to pH 3 with 1 N HCl and the resultant acetic solution treated with twice its volume of ethyl acetate. The aqueous phase was discarded and the organic portion treated with half its volume of 1 N NH₄OH. The basic, aqueous phase was evaporated to dryness under vacuum. The crystalline residue was reconstituted to a 1% (w/v) aqueous solution and after neutralization with HCl, was introduced into the test plants in the manner described earlier. Four plants were treated with the extract and 10 untreated plants kept as controls. All plants were dissected 30 days after treatment. All control plants were vegetative whereas 2 of the treated plants flowered, each at the stage 3 level. This procedure has at this time been successfully repeated several times. The taxonomic proximity of Calonectria (Fusarium) rigidulosa to Gibberella (Fusarium) moniliformae suggests that substances related to gibberellin may be active in the induction of flowering by this extract. The active extract has been tested for the presence of gibberellins by the dwarf corn and barley endosperm assay methods. There has been no evidence of any of the gibberellins for which these tests are sensitive.

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Literature Cited