

No Gibberellic Acid Found in Royal Jelly¹

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We were not able to detect the plant hormone, gibberellic acid (GA₃), in the brood food, otherwise known as royal jelly, fed to larval honey bees, *Apis mellifera* L., although Nation and Robinson (1966) suggested that it might be essential to development. These investigators found that nurse bees feeding on a semidefined diet could initiate laying by their queen, but could rear young larvae from hatching through only the 3rd or 4th day. If the larvae were to complete development, it was necessary to provide the nurse bees with additional nutrients, a minimum of 7.5% pollen or 0.85 mg of GA₃ per gram of diet.

If GA₃ was essential to larval growth, we should be able to detect measurable quantities in royal jelly by bioassay with seedlings of the pinto bean, *Phaseolus vulgaris* L., which elongate markedly when even a few micrograms of GA₃ are applied to a leaf (Adler et al. 1961). Seedlings of certain other plants respond similarly to GA₃, but pinto beans best withstood the heat during our tests.

MATERIALS AND METHODS

We collected royal jelly from queen cells 3 days after the cells were started and stored it frozen in glass vials. Then extracts were prepared (MacMillan et al. 1960) to recover and concentrate any GA₃. A 20- μ liter portion of the extract was considered the equivalent of about 5 g of royal jelly.

The pinto bean seedlings were grown in soil in separate 4-in. pots from seed sold commercially as edible dry beans. The pots were held in the greenhouse in shallow pans partly filled with water. Temperatures from April to June during the tests ranged from 27°C at night to 32–38°C during the day; however, on an occasional day in June, it rose to 41°C. Humidity was high. The plants were illuminated by sunlight passing through the glass.

The treatments listed in Table 1 were applied to the upper surfaces of the most recently unfolded leaves of the bean seedlings, the solutions with gas-tight microsyringes and hydrated royal jelly smeared on with a stick. The 20- μ liter applications were applied as two 10- μ liter applications to 2 leaves or leaflets; all other treatments were applied to 1 leaf or leaflet. The internode above the treated leaf or leaves was measured when elongation of that part of the stem was completed. In Test I, 13-day-old seedlings were treated on 1 or 2 leaflets of the leaves at Node 4, counting upward from the cotyledons at Node 1; then 7 days later Internode 4 was measured, and 15 days later Internode 5 was measured. In Tests II and III, 7-day-old seedlings were treated on leaves at Node 2, and Internode 2 was measured 6 days later. Each test was made with 15 plants/treatment, but in Test III, some plants died, so the data was reduced to 13 plants/treatment.

RESULTS AND DISCUSSION

The stems of the pinto bean seedlings treated with extracts or smears of royal jelly failed to elongate more than the stems of untreated plants. In contrast, seedlings

Table 1.—Effects of GA₃, extracts of royal jelly, and royal jelly on the elongation of pinto bean seedlings.

Treatment/plant	Mean elongation (mm) of selected internodes in 3 tests ^a		
	I	II	III
None	22.5 a	22.4 a	22.2 a
80% acetone (1 μ liter)	22.1 a		
GA ₃ (3.3 μ g) in 80% acetone (1 μ liter)	147.3 b	107.8 b	125.3 b
GA ₃ (3.3 μ g) in ethyl acetate (20 μ liter)			109.8 b
Extract of hydrated royal jelly in			
80% acetone ^b (20 μ liter)	12.8 a		
ethyl acetate (20 μ liter)		13.3 a	
Extract of lyophilized royal jelly in ethyl acetate (20 μ liter)		17.9 a	
Royal jelly (0.5 ml)			22.8 a
Common SE	8.7	4.3	4.5

^a Within each test, means with letters in common are not significantly different by Tukey's test.

^b Extract in ethyl acetate dried and redissolved in 80% acetone.

treated with 3.3 μ g of GA₃ per plant elongated greatly. The difference was apparent from observation and was verified by measurement, Table 1. However, the response of older seedlings to GA₃ was delayed and started farther from the point of treatment. In the younger plants treated with GA₃ at 7 days (Tests II and III), increased elongation began within a few days after treatment in the internode immediately above the treated leaves, and was completed within 6 days. In the older plants treated at 13 days (Test I), increased elongation began more than 7 days after treatment in the 2nd internode beyond the treated leaves, and was completed between the 7th and 15th days. Therefore, the measurements for Test I in Table 1 are for growth that occurred during the 2nd posttreatment week; those for the 1st posttreatment week showed no significant differences. Also, the measurements of older seedlings were more variable; the common standard error for Test I was about twice that in Tests II or III.

The slight, nonsignificant depression of growth that occurred after treatment with extracts of royal jelly probably resulted from a phytotoxic response to the solvents rather than from any toxicity of materials in the royal jelly. Although every 20- μ liter application made immediate soaked spots on the leaves, and the tissues in those spots subsequently died, the phytotoxic effect of 20 μ liters of ethyl acetate did not negate the response to GA₃. Also some leaf tissue died under the smears of royal jelly a few days after treatment, but the effect did not appear as rapidly as when acetone or ethyl acetate was used, and subsequent growth seemed unaffected.

The failure to find gibberellin-like activity in royal jelly led us to make corollary experiments with results that supplemented our earlier findings. We were able to detect the presence of 10 μ g of GA₃ added to 25 g of lyophilized royal jelly by using the pinto bean assay. GA₃ therefore cannot be present in royal jelly in more than trace quantities. Then, since diets containing as much as 430 μ g of GA₃ per g (Nation and Robinson 1966) did not permit nurse bees to rear young larvae

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beyond the 4th day, these possible trace quantities cannot support larval growth.

When we repeated the nutritional tests (Nation and Robinson 1966) with pure GA_3 , the synthetic diet would not allow nurse bees to sustain larval growth. The GA_3 used by Nation and Robinson was only 85% pure. We therefore suspect that the large percentage of impurities (nature not known) present in commercial GA_3 may have been responsible for the apparent ability of this compound to support larval growth. Possibly, these impurities are fermentation products that act as feeding stimulants for bee larvae when they are present in royal jelly. The failure of synthetic diets to support the growth of honey bees may thus result from gustatory rather than nutritional inadequacy.

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