Technical Papers

Studies on Natural Pollination of *Hevea brasiliensis* in Brazil[1]

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As the result of previous studies on pollination in the Pará rubber tree, *Hevea brasiliensis* (Willd. ex A. Juss.) Muell. Arg., evidence was obtained that natural pollination of this species in Puerto Rico was accomplished largely by midges of the family Heleidae (*1*). The presence of pollen grains on Hevea stigmas was found to be associated with the presence of small insect bristles, also found adhering to the stigmatic surfaces and presumably lost by the pollinating insects. The identity of the pollinating insects was finally established by tracing these bristles to three genera of heleid midges: *Atrichopogon, Dasyhelea,* and *Forcipomyia.*

Through the cooperation of the Division of Rubber Plant Investigations, it was possible to continue these studies in the Amazon region of Brazil. These additional studies seemed desirable in order to determine whether methods of natural pollination in Brazil, where Hevea is native, were similar to those in Puerto Rico, where Hevea is introduced.

The studies in Brazil were conducted at the Instituto Agronomico do Norte at Belém during the first two weeks of July and the first week in August 1951, and during the last two weeks of July at Belterra (the site of the old Ford plantation) some 600 miles up the Amazon from Belém.

In general, Hevea flowers were found to be well pollinated; an average of 68% of all stigmas examined at Belém and 48% at Belterra bore pollen grains. In general, also, the stigmas were adequately pollinated. Since these readings were taken early in the season and usually from flowers that had been open less than 24 hr, they represent minimum pollination percentages and probably not final percentages. This would indicate that the low fertility of Hevea in cultivated plantings in Brazil (as in Puerto Rico) probably is not attributable to a failure of natural pollination.

It soon became evident that heleid midges were also the chief agents of pollination in Brazil. Insect bristles were again found on Hevea stigmas, and from this clue the complete process of pollination was worked out, including several important steps that had not been observed in Puerto Rico.

The midges are barely visible to the naked eye (they are approx 1 mm in length) and are yellowish to dark brown in color. Their flight is zigzag and very difficult to follow. Once the eye is trained, however, the insects may be observed carrying on their pollination activities. They are so numerous that half a dozen may be observed at one time around a single inflorescence. They light on a petal and walk into the flower with their backs toward the anthers or stigma. They seem to be interested in the corolla, or the long hairs with which it is clothed on the inside, rather than in the reproductive structures of the flowers. Individuals have been observed to light on male flowers and to go in and out repeatedly, in an aimless fashion. Such individuals when caught and examined under a dissecting microscope were found to be carrying numerous pollen grains—especially on the antennae, the thorax, and the tops of the wings.

Likewise, the insects have been observed to light on the petals of female flowers and, with their wings toward the stigma, walk into the flower through the narrow opening between the stigma lobes and the corolla tube. They may pass down to the region on the ovary, stay there a short time, and then turn and walk out. One female flower that had been so visited by a midge was taken to the laboratory for examination. It was found to have pollen grains lodged on the edges of the stigma where, presumably, they were deposited as the insect walked past.

The midges were difficult to capture. They fly away rapidly at the least disturbance, but generally will return after a short time. Numerous specimens were finally caught by quickly pinching the corolla closed with thumb and forefinger, after an insect had been observed to enter, and then carefully dropping the entire flower in 70% alcohol. For some reason the method used so successfully in Puerto Rico, of placing an adhesive substance on the petal tips of open female flowers, gave indifferent results in Brazil.

The midges are most abundant and most active for 1–1½ hr after sunrise and for 1–1½ hr at sunset. During the remainder of the day only an occasional individual was observed. Wind or rain was found to discourage their appearance, even at sunrise or sunset. The small size, the similarity in color to petals, and the limited periods of activity probably explain the failure of earlier attempts to identify midges as pollinating agents of Hevea in Brazil.

The midges captured were identified as belonging to four genera of the family Heleidae: *Atrichopogon, Stilobezzia, Dasyhelea,* and *Culicoides.*[3] In the genus *Culicoides,* one specimen of *C. debilipalpis* var. *glabrior* Macfie and one of *C. guttatus* (Coq.) were identified. In the genus *Atrichopogon* four different types (probably species) were observed among 41 specimens. In this genus, however, as well as in *Stilobezzia* (3 specimens) and *Dasyhelea* (2 specimens), species identifications were not possible.

[1] Cooperative investigation with the Division of Rubber Plant Investigations, BPISAE, USDA, Beltsville, Md.

[2] Administered by the Office of Experiment Stations, Agricultural Research Administration, USDA.

[3] Identified by W. W. Wirth, Division of Insect Identification, Bureau of Entomology and Plant Quarantine, USDA, Washington, D. C.

There remains little doubt that members of this family, and of the genus *Atrichopogon* in particular, are responsible for most natural pollination of *H. brasiliensis* in Brazil. It is of interest that members of two of these genera (*Atrichopogon* and *Dasyhelea*) were also identified as among the pollinating agents in Puerto Rico. In Puerto Rico, however, members of the genus *Dasyhelea* were found to be most abundant, whereas in Brazil individuals of the genus *Atrichopogon* were most abundant. This difference in relative numbers of insects is believed to be related to the reduced number of stigmas bearing insect bristles in Brazil. Specimens of the predominant form in Brazil, which is yellowish in color, were found to shed few if any bristles when rubbed over the stigmas of fresh Hevea flowers. The dark form, most common in Puerto Rico, was found to shed bristles readily.

An insect belonging to the family Culicidae, tentatively identified as *Mansonia* sp., may also be responsible for some pollination in Brazil. This insect is somewhat larger than the midges and has wing veins and margins marked by a fringe of scales. These scales, or ones similar to them, have occasionally been found on Hevea stigmas and appear to be associated with the presence of pollen grains.

In Puerto Rico, thrips were found to be the most numerous insects around Hevea flowers and were shown to be agents of pollination, although not as important as the midges. In Brazil, thrips of three different genera (*Frankliniella, Scirtothrips,* and *Heterothrips*[4]) were collected from Hevea flowers. The actual numbers of flower thrips observed in Brazil at the time of these studies were so small, however, that these insects were probably of little or no importance as pollinating agents.

Reference

1. WARMKE, H. E. *Science,* **113**, 646 (1951).

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[4] Identified by J. D. Hood, of Cornell University.

The Rate of Endogenous Respiration as Affected by the Oxidation of Exogenous Substrates[1]

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Many manometric data in the literature are corrected on the premise that the endogenous respiration continues as its normal rate during the simultaneous oxidation of exogenous substrates. This assumption, unless valid, may lead to erroneous interpretations, especially in the case of such organisms as *Penicillium chrysogenum* Q-176, which possess a relatively high endogenous respiration as compared to their total respiration with a substrate. Inasmuch as it is impossible to distinguish directly between the oxygen needed for the metabolism of endogenous and exogenous materials, some information on the course of the endogenous respiration can be obtained indirectly by comparing the release of isotopically labeled CO_2 from labeled resting cells in the presence or absence of unlabeled substrates (*1–5*). This approach presupposes (*a*) that CO_2 production is a function of oxygen consumption and (*b*) that $C^{14}O_2$ release is representative of the total endogenous CO_2 production. This preliminary note illustrates some of the difficulties encountered in the use of this method.

Vegetative cells of *P. chrysogenum* Q-176 were grown from a spore inoculum at 25° C in 500 ml Erlenmeyer flasks containing 100 ml of a medium in which acetate was the main source of carbon (*6, 7*); the flasks were agitated on a reciprocating shaking machine, which made 90 4-in. strokes/min. After 42 hr of growth, the cells from 6 flasks were harvested, washed with *M*/15 phosphate buffer (pH 6), and added to a flask of fresh medium that also contained 2.0 mg each of 1-C^{14}- and 2-C^{14}-acetate with specific activities of 1 mc/mM. This flask was placed in a closed system similar to the one described by Martin and Wilson (*8*); air was circulated by a small diaphragm pump and freed of CO_2 by passage through alkali. The cells were allowed to grow for 6 hr, while the system was being shaken on a reciprocating shaker. The cells were then harvested, washed with buffer, minced for 15 sec in a Waring blendor, and diluted with buffer to give a cell concentration of about 2–4 mg (dry weight)/ml. Washing the cells after mincing rather than before had no effect on the results. Two ml aliquots of cells were placed in Warburg flasks containing 0.2 ml of 20% carbonate-free NaOH in the center well (without filter paper), 0.5 ml substrate or buffer in one side arm, and 0.5 ml 72% perchloric acid in the other. The experiment was started by the addition of substrate or buffer to the main compartment and stopped, at desired intervals of time, by the addition of perchloric acid. The temperature used was 30° C, and the gas phase was air. One hour or more after the completion of the experiment the alkali was removed quantitatively from the center wells. $BaCl_2$ was added to the alkali to precipitate the carbonate; the $BaCO_3$ was collected by centrifugation, washed twice with 95% ethanol, and then plated on a microporous disk. The radioactivity on the disk was measured by standard techniques, using a thin mica window Geiger-Müller counter. Counts were corrected, by a graphical method, to the activity at 0 self-absorption (*9*).

Fig. 1 shows the results of an experiment with 0.1 *M* glucose or sodium acetate as substrates; the values are averages from two or more determinations. Although the oxidation of glucose did not affect the rate at which $C^{14}O_2$ was released, the oxidation of acetate eventually suppressed the release of $C^{14}O_2$ almost com-

[1] Supported in part by the Atomic Energy Commission. Presented at the 51st General Meeting of the Society of American Bacteriologists in Chicago (*1*).

[2] Predoctoral fellow of the Atomic Energy Commission.