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13. Area of cacao productivity (12) is a function of the modern (1950 to present) maximum annual productivity of cocoa. The correlation coefficient  $r = .8598$ ,  $P < .001$ , leaving only 26 percent of the variance in cultivation area of cacao unexplained by the modern maximum of annual cocoa productivity. This justifies the indirect species-area curve of Fig. 2, which includes more points than Fig. 1. The equation  $Y = 0.735X + 2.093$  describes the regression of "area in cacao" upon "annual cacao productivity."
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## Rhizoplane Fibrils in Wheat: Demonstration and Derivation

**Abstract.** *Aggregates of fine, curved fibrils extend from the rhizoplane of soil-grown wheat roots into the rhizosphere. The fibril diameter is usually between 3 and 10 nanometers. The fibrils arise as extracellular products of the root, and some aggregates are intimately associated with microbial cells and soil particles.*

The roots of plants play a vital role in the activities of the biosphere. Epstein (1) stated that "the root is the interface between terrestrial life and the mineral substrate supplying all other essential elements." The interface between root and soil can be a region of high metabolic activity as a result of plant-microflora associations (2-4). Many noninvading microorganisms are concentrated at the root surface or rhizoplane (5) and in the immediate soil zone or rhizosphere. This microflora influences the mineral nutrition of the root (2, 6) and is in turn subject to influences from the soil environment. Some attempts have been

made by agricultural scientists to describe the structure of the rhizoplane and rhizosphere in terms of the three-dimensional disposition of plant and microbial cells (7, 8). These attempts have been revealing but can and should be pursued further.

In recent years, considerable attention has been focused on extracellular plant materials (9). One class of these, fibrillar polygalacturonic acid (10, 11), can form extensive weblike structures of great surface area which project from cells into the external milieu. These extracellular structures (made up of distinctive curved fibrils which have diameters between 20 nm and the

resolution limit for sectioned material) are known to exist on the surface of some roots grown in laboratory culture (12). Their function has not been studied, but these fibril aggregates with their acidic character could take part in the uptake of minerals (including heavy metals) and act as anchor sites for both microbes and extracellular enzymes. Their small but significant protein component (13, 14) indicates that they may have some enzymatic activity of their own (10), a suggestion reinforced by some findings of Halperin (15). It is of interest to know if these or similar fibrils exist at the rhizoplane (or in the rhizosphere). This report documents the existence of electron-opaque fibrils on soil-grown roots of common wheat and demonstrates that these fibrils are plant-derived.

Wheat, *Triticum aestivum* L. emend Thell., was grown under controlled environmental conditions in a growth chamber in pots containing Lethbridge soil (Canada Agriculture Research Station, Lethbridge, Alberta) for 19 days, at which time soil samples containing well-developed roots were taken. Root tips plus adhering soil were placed for 90 minutes at room temperature in a large volume of chemical fixative (7 percent glutaraldehyde in 0.05M phosphate buffer at pH 6.8). Each sample was washed with buffer so as to minimize displacement of adhering soil. A cold postfixation in ruthenium-osmium (16) was given according to the method of Leppard *et al.* (13). Final washes were in distilled water at room temperature, and the sample was dehydrated slowly by using an ethanol series followed by propylene oxide. Embedding was in Spurr's low-viscosity medium (17). Sections were cut with glass knives on a Porter-Blum MT-2B ultramicrotome, as described previously for embedded material containing mineral

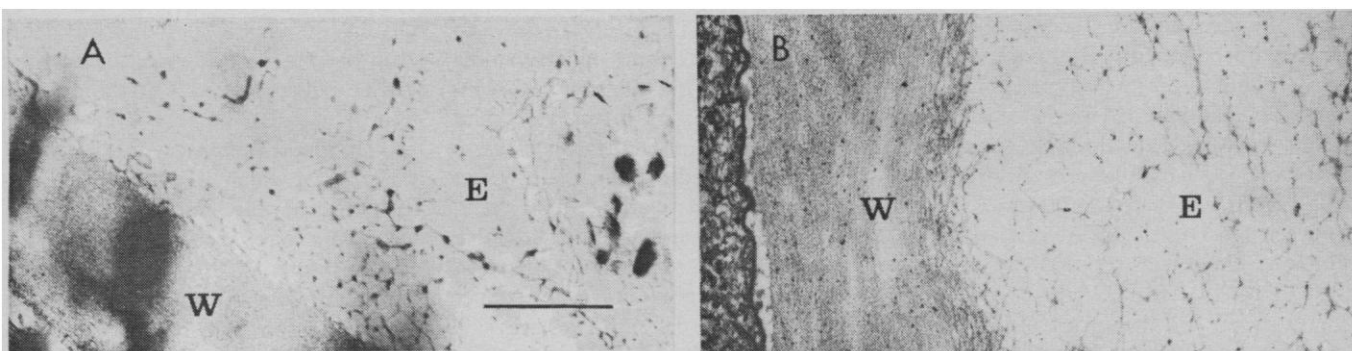


Fig. 1. Electron micrographs of sections through the surface of wheat roots. Abbreviations: W, cell wall; E, external milieu. The scale bar indicates 500 nm. (A) Electron-opaque fibrils extending from the cell wall into the rhizosphere of a soil-grown root. Many of these fibrils are coarse. (B) Electron-opaque fibrils extending from the cell wall of an axenically grown root. Many of these fibrils are delicate. It is not known whether the ratio of coarse to delicate fibrils is diagnostic of the growth conditions.

particles (18). The sections on grids were stained with uranyl acetate followed by lead citrate, and were then examined with an AEI transmission electron microscope at 50 kv. The experiment was repeated with surface-sterilized seeds grown on glass beads partially immersed in a sterile nutrient solution (19) and enclosed in sealed, aerated glass containers.

A diffuse network of distinctive, curved, electron-opaque fibrils (and loosely assembled tufts of them) was found at the rhizoplane of the tip of all roots examined. These rhizoplane fibrils at the root-soil interface, between the root hair zone and the root cap, are described here for the first time. They are curved ribbons whose diameter usually varies from 3 to 10 nm. Their form is similar to that of fibrils of a recently described, complex material composed mainly of polygalacturonic acid (10) and found at the surface of some plant cells grown axenically. Figure 1A shows the distinctive appearance of some fibrils which form a loose tuft projecting from the rhizoplane into the rhizosphere. Rhizosphere bacteria were often well preserved, and the fixation image of root cells internal to the epidermal layer was similar in quality to that of previous work (13) done with similar combinations of fixatives and "electron stains." Substances in the soil solution had no obvious deleterious effect on the primary fixation, but mineral particles interfered with the quality of sectioning. The distribution of fibrils in the rhizoplane of root tips was patchy, as was the distribution of fibril tufts projecting into the rhizosphere volume. Penetration of mineral particles by the fibrils could not be studied in sections approximately 50 nm thick. Sections of the root tips grown under sterile conditions (Fig. 1B) revealed rhizoplane fibrils (including a patchy distribution of tufts of fibrils) and a total absence of microbes. Scanning electron microscopy confirmed the absence of surface microbes. Thus, the fibrils were derived from plant cells.

The rhizoplane fibrils were revealed by a combination of electron stains previously used to reveal fibrillar polygalacturonic acid. The fibrils in each case have a similar form (13), polymers containing galacturonic acid do exist at some root surfaces (20), and the enzyme polygalacturonase is important in the invasion of some roots by microbes (21). These facts are consistent with the assumption that the rhizoplane fibrils may be composed in part of galacturonic acid polymers. Detailed

knowledge of their chemistry is required, particularly in view of recent suggestions by Ramamoorthy and Manning (22) concerning nutrient and heavy metal uptake by roots.

Structures considered to be composed partially of uronic acid polymers have been described previously for root surfaces (3, 8). These structures were not resolved as fibril aggregates, but perhaps reinvestigation would show them to be fibrillar. It is interesting to note that rhizoplane fibrils do not normally appear in electron micrographs in the literature on roots. A major reason for this anomaly appears to be inadequate enhancement of contrast by most electron-staining procedures. That the ruthenium-osmium stain enhances contrast of existing fibrils (as opposed to creating artifactual fibrils) has been shown previously (13).

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## Fenfluramine: Amphetamine Congener That Fails to Maintain Drug-Taking Behavior in the Rhesus Monkey

**Abstract.** *Fenfluramine, over a dose range from 0.003 to 3 milligrams per kilogram of body weight, failed to maintain self-injection behavior in rhesus monkeys that had initiated and maintained responding for cocaine or methohexital. This absence of a positive reinforcing effect could not be attributed to a slow onset of drug effect or to the use of behaviorally inactive doses. Fenfluramine, because of its distinctive properties, may produce fewer problems of human abuse than do amphetamine-type agents.*

Fenfluramine [N-ethyl-m-(trifluoromethyl)amphetamine] manifests some, but not all, of the pharmacological actions of amphetamine and related phenethylamines. Like the latter compounds, fenfluramine decreases food intake in animals (1) and is clinically efficacious in the initiation of treatment for obesity in man (2). In contrast to amphetamine, however, fenfluramine has much less pressor activity in animals and little, if any, pressor activity in man, it does not induce amphetamine-like response stereotypy, and it

lacks psychomotor stimulant actions in animals or humans (1, 3). Furthermore, the electroencephalographic effects of fenfluramine in man resemble those of amobarbital rather than those of amphetamine (4). The possibility that fenfluramine lacks an amphetamine-like subjective effect in man is suggested by the results of a study in which amphetamine users were asked to compare fenfluramine's effects to those of amphetamine. They judged fenfluramine to be no more similar to amphetamine than was the placebo (5).