Pathology of Strawberry Root Rot Caused by Ceratobasidium Species

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ABSTRACT

Ceratobasidium sp. (*Rhizoctonia fragariae*) invades strawberry roots by direct penetration, and causes sloughing of the cortex and death of rootlets. Naturally infected plants of an everbearing cultivar compared under field conditions with plants free of *Ceratobasidium* showed reduced vigor as measured by leaf size, slow degeneration, and premature collapse. Encasement of the pathogen in sloughed, melanized cortical cells probably protects it from antagonists and insures the presence of inoculum in the root zone throughout the life of a strawberry plant.

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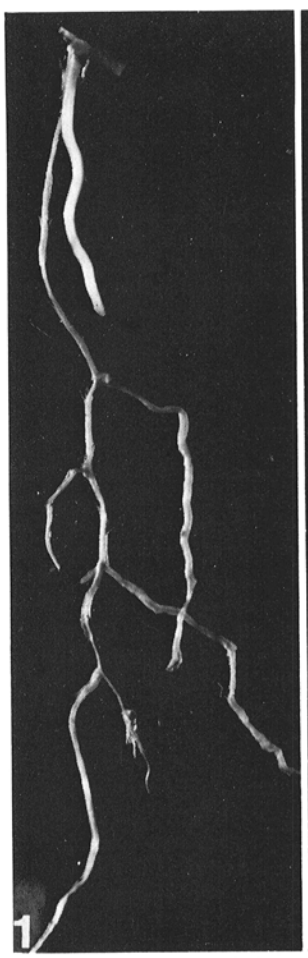
The blackroot complex disease of strawberry is characterized by deterioration of the rootlet cortex. Previous investigations have contributed to knowledge of pathogens among previously unknown or little-known fungi (11, 19), rhizosphere-dependent bacteria capable of pathogenesis but not parasitism (4, 8), ubiquitous root parasites of the genus *Endogone* and their role as endotrophic mycorrhizae (10, 12), and a concept of health of root systems of perennial plants as exemplified by strawberry, where the rootlets age, become apparently functionless as absorbing structures, die, and are replaced in situ (18). The unknown factors alluded to by Lipman (9) as being responsible for the decline in strawberry productivity on fertile soils of the California Pajaro Valley when they were planted to strawberries a second time, in our opinion, primarily are rootlet-attacking fungus pathogens (17). The present paper presents evidence that a *Rhizoctonia* species reported previously as an "orchid type" (7, 20) is pathogenic to strawberry roots and capable of reducing plant growth. The fungus in question is considered to be identical to *Rhizoctonia fragariae* (2, 5, 16). Because the vegetative cells are binucleate, we consider the fungus to be an unnamed species of the perfect genus *Ceratobasidium* in accordance with the views of Parmeter et al. (13).

Preplant soil fumigation with methyl bromide-chloropicrin mixtures of fields known to be free of Verticillium wilt and *Pythium ultimum* has resulted in growth and yield increases of as much as 100% over the checks. In one instance, plots fumigated at the rate of 255 kg/hectare (225 lb./acre) of methyl bromide-chloropicrin (2:1, w/w) yielded slightly over 50,000 kg/hectare (44,000 lb./acre) of strawberries during the April to June harvest; nonfumigated plots yielded only 27,272 kg/hectare (24,000 lb./acre). *Endogone* sp., *Olpidium* sp., and "white monilioid types of *Rhizoctonia solani*" were reported as root parasites from the nonfumigated plots; they were infrequent in the fumigated plots (7). Ultimately, rootlets in the fumigated plots became brown, and the cortex fell apart in flakes when placed in water (Fig. 1). Cleared rootlet cortex showed the characteristic monilioid structures and cell masses of the invading fungus which was subsequently identified as *Ceratobasidium* (Fig. 5). As the season progressed, *Ceratobasidium* sp. was isolated with increasing frequency from the fumigated plots. Reddish-brown lesions on otherwise white or light brown roots indicated its presence. The fungus was also isolated on numerous occasions from strawberry plants growing in the major strawberry districts of California and from recently set plants which had initiated new, vigorous growth and then collapsed.

The purpose of this study was to gain knowledge on infection, pathological anatomy, and effect of the disease on growth.

MATERIALS AND METHODS.—*Anatomical studies.*—Field soil from a fumigation test site was placed in 20-cm clay pots, brought to the laboratory, and treated with 3 ml of chloropicrin per pot. After aeration, isolates of *Ceratobasidium* sp. which had

Fig. 1-6. 1) Rootlet from a field-grown strawberry root system infected with *Ceratobasidium* sp. showing fraying of the cortex. Note the production of a new rootlet at the point of attachment to the larger root. **2)** Check plant (left) and inoculated plant (right) grown in soil infested with a strawberry isolate of *Ceratobasidium* sp. Note the darkening and deterioration of the adventitious roots at the point of attachment to the crown on the inoculated plant. **3)** Longitudinal section of an infected strawberry root showing a mycelial mass of *Ceratobasidium* sp. in the outermost root cell (× 625). **4)** Large dark mycelial strand of *Ceratobasidium* sp. on the surface of a strawberry root with a short lateral branch penetrating (arrow) an epidermal cell directly (× 600). **5)** Intracellular mycelial mass of *Ceratobasidium* sp. formed in the outermost cells of a strawberry root (× 600). **6)** Portion of a transverse section of a constricted strawberry root showing intracellular mycelial masses of *Ceratobasidium* sp. in thick walled cortical cells (× 700).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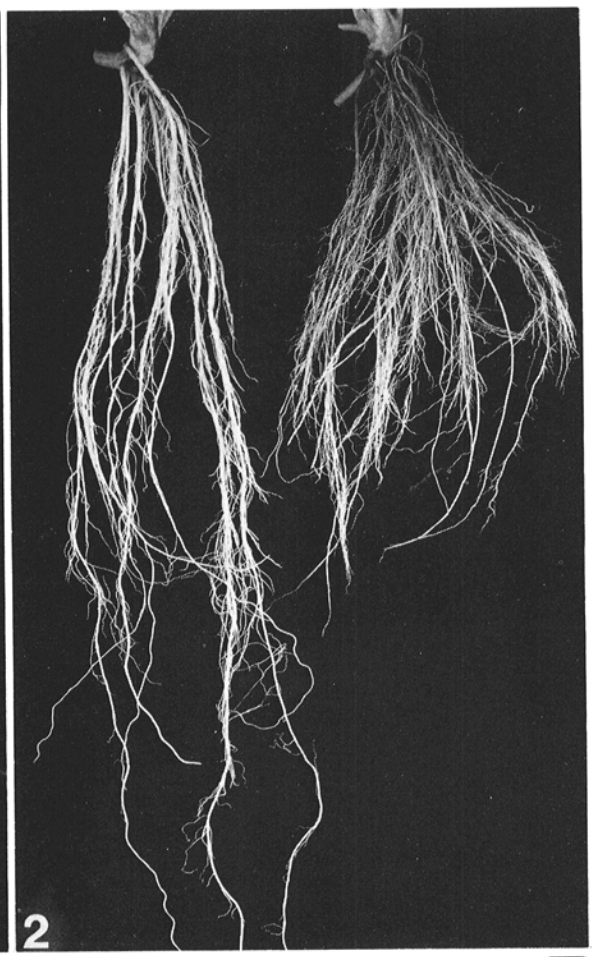
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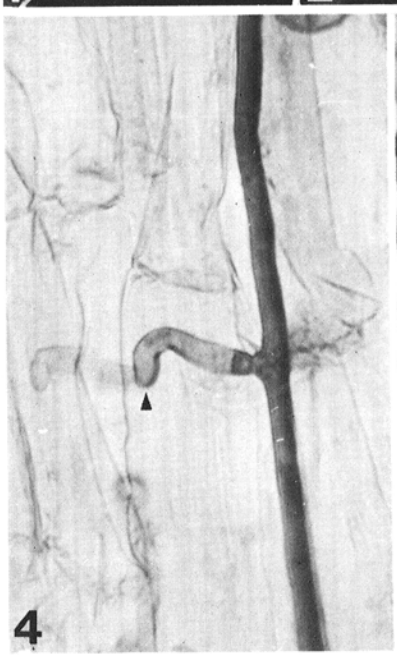
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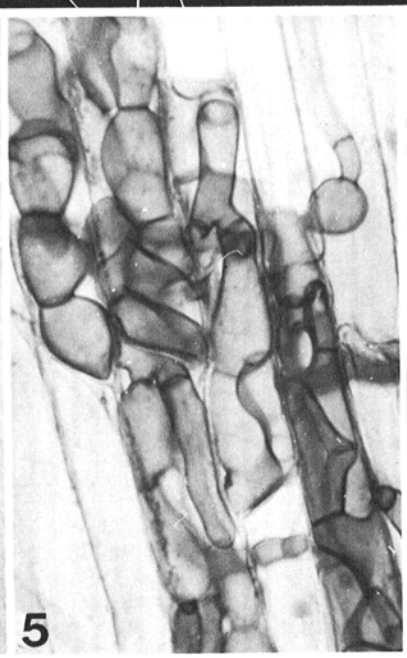
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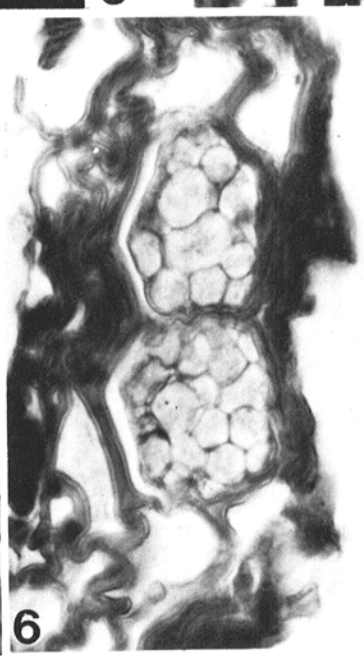
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been allowed to colonize sterilized tomato stem pieces were mixed throughout the soil. Seven isolates from strawberry roots were used. Tomatoes were seeded in the pots and grown for 1 month to serve as a "nurse crop" for *Ceratobasidium*. Tomato roots were removed from the soil, washed, and cultured on straw water agar medium (3), and only those pots were saved from which recovery was positive for the specific clonal type of *Ceratobasidium* sp. used to infest the soil. Dormant Shasta strawberry plants (*Fragaria ananassa* Duch.) were set in 32 of the original 40 pots infested; checks consisted of plants grown in the infested soil after it was fumigated. The plants were grown outdoors in a lath house. Strawberry plants were sacrificed during a period of 1 year to examine individual root systems. We gathered correlative data by studying root systems of plants taken from commercial fields; these were carefully dug with a large ball of earth, soaked in water, and washed with a fine spray to free the roots from soil.

Selected rootlets were cleared by a modification of Popp's technique (14). Specific portions of the root systems were cultured for *Ceratobasidium* sp., and adjacent portions were fixed in either alcohol-Formalin-acetic acid fixing solution (15) or alcohol-Formalin-propionic acid fixing solution (6) for 48 hr or longer. The fixed material was dehydrated in a tertiary butyl alcohol schedule (6) and embedded in Tissuemat. When necessary, we softened root material prior to sectioning in a solution consisting of 90 ml of a 1% aqueous solution of Dreft (sodium lauryl sulfate) and 10 ml of glycerol (1). Material was sectioned on a rotary microtome at 10 μ. Sections were mounted on slides with Haupt's adhesive and stained with Johansen's Quadruple stain (6).

Field studies.—Field studies were carried out under conditions of commercial cultivation of strawberry. Plants of the strawberry cultivar E-5, developed by Driscoll Strawberry Associates, Inc., were freed from *Ceratobasidium* infection, increased in a field nursery, then planted in a commercial field along with E-5 stock known to be at least 60% infected. We established the *Ceratobasidium*-free clones of E-5 by training daughter runner plants into pots of sterilized soil placed on inverted flower pots to raise them above the greenhouse bench. The daughter plants were checked periodically for infection by *Ceratobasidium* sp. by the culturing and microscopic examination of cleared roots. Despite the precautions taken, we detected occasional infections. During the course of 1 year, ca. 50 *Ceratobasidium*-free plants were secured and transplanted for increase in a commercial nursery which had been fumigated previously at the rate of 370 kg/hectare (325 lb./acre) with methyl bromide-chloropicrin (2:1, w/w). The nursery soil was of volcanic ash origin and proved to be an excellent medium for strawberry root development. During the period of plant increase and prior to digging in the winter, cultures were made of roots and petiole bases of ca. 10 daughter plants of each clone. The clone was labeled as infected when a culture yielded either *Ceratobasidium* or any other fungus considered to be a Basidiomycete. On several occasions, we isolated Basidiomycete-type fungi which were similar to *Ceratobasidium* in morphology, but which grew faster in culture and were generally hyaline or only faintly pigmented.

RESULTS.—*Anatomical studies.*—Root systems of inoculated plants were smaller than those from check plants, and showed reddish-brown discoloration and deterioration of the adventitious roots at the point of attachment to the crown (Fig. 2). Cleared roots from the pot tests showed large, dark hyphae of *Ceratobasidium* growing in the lengthwise direction of the roots, closely appressed to the root surface (Fig. 4). Behind the hyphal apices, short lateral branches penetrated the epidermal cells directly (Fig. 4) and gave rise to intercellular mycelial masses which tended to conform to the shape of the invaded cells (Fig. 3, 5, 6). Invaded cortical tissues darkened and collapsed, and ultimately were sloughed (Fig. 9). Usually the endodermis served as an effective barrier to the invading hyphae, but occasionally the fungus reached the stele, killing the rootlet. The main structural roots, which have the capacity for secondary growth, appeared little damaged by the cortical lesions caused by *Ceratobasidium*. Cell masses of *Ceratobasidium* became encased in the sloughed cortical cells (Fig. 6, 9); from these, new lateral roots were attacked at their points of attachment to the main roots. The deep lesions which resulted ultimately caused "pinching off" of the lateral root and loss of all its branches (Fig. 8). The seven isolates were essentially identical as pathogens.

Field studies.—In February 1966, 16 clones consisting of up to 90 plants, each rated as noninfected with *Ceratobasidium*, and 12 clones rated as carrying Basidiomycete-type fungi were planted in a commercial field together with the E-5 cultivar obtained from three different nurseries in the same general area. Starting growth, and growth throughout the 1st year, of plants derived from clones which were rated as free from *Ceratobasidium* sp. were approximately equal to that of clones carrying the Basidiomycete-type fungi, and vastly surpassed that of the commercial stocks. Strawberry

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Fig. 7-10. 7) Root systems of strawberry plants grown in field soil naturally infested with *Ceratobasidium* sp. showing deterioration of the main adventitious roots and scarcity of feeder rootlets. **8)** Main adventitious strawberry root showing cortical rot and deterioration of branch roots as well as constriction of branch roots (arrows) at the point of origin from the main adventitious root. **9)** Cross section of a constricted strawberry branch root showing cortical deterioration and mycelial masses (arrows) of *Ceratobasidium* sp. in cortical cells (× 334). **10)** Main adventitious root grown in soil infested with *Ceratobasidium* sp. showing deterioration of the cortex and attached rootlets.

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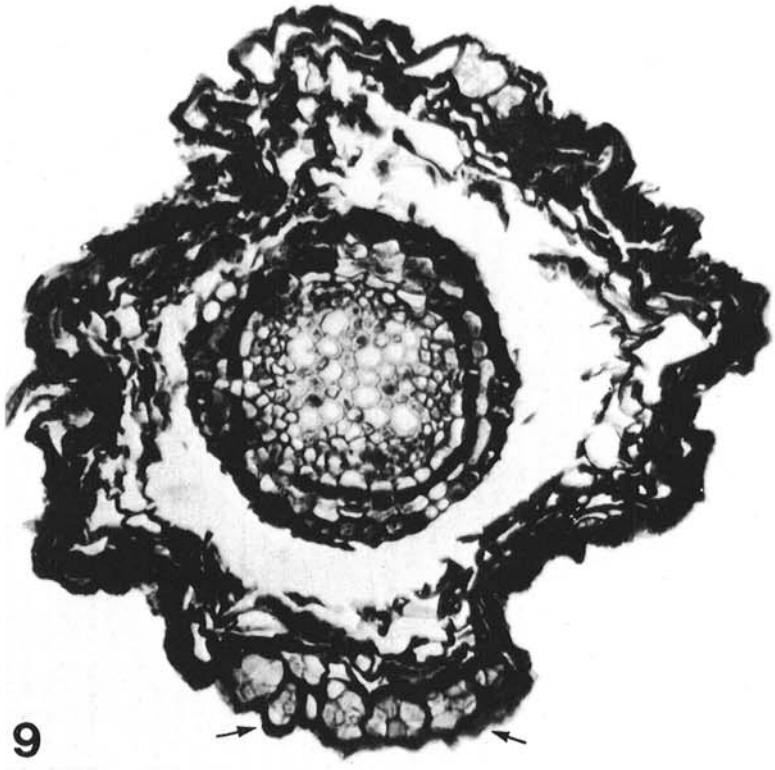

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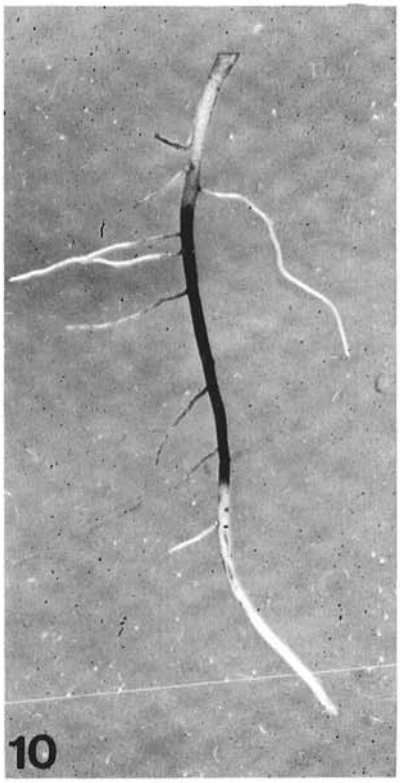

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beds planted with indexed plants appeared at least 50% larger than beds planted to commercial stocks, and leaf measurements confirmed these observations. In June, mean petiole length and blade width (distance across three leaflets) of the largest mature leaf of two plants of each clone were 11.2 ± 3.1 and 11.0 ± 2.7 cm, and blade widths of 14.8 ± 2.3 and 14.3 ± 1.7 cm, for the *Ceratobasidium*-free and Basidiomycete infected clones, respectively. From these data, it was concluded that the Basidiomycete-type were nonpathogenic tenants of strawberry root surfaces. Measurements made of the commercial stock gave a mean petiole length of 6.6 ± 1.8 cm and a mean blade width of 11.0 ± 1.8 cm. Data on yield were not obtained. In August, during a hot spell, beds of the commercial E-5 stock began to wilt and ultimately collapsed, suggesting the Rhizoctonia wilt described by Zeller (21). The root systems were deteriorated and black (Fig. 7), and cultures of all plants yielded *Ceratobasidium*. Subsequently, plants of the indexed beds began to wilt. Reddish-brown lesions were present on the major roots (Fig. 10), and *Ceratobasidium* was isolated from all of these.

DISCUSSION.—Evidently one of the most ubiquitous of the strawberry root-infecting fungi and thus probably considered to be mycorrhizal (2, 16), *Ceratobasidium* is shown nonetheless to be a pathogen capable of infecting root tissues and causing the cortex to slough. Destruction of cortex is not necessarily injurious to main structural roots, but it destroys the function and is ultimately lethal to the feeder rootlets. Feeder rootlets also die naturally, but infection with *Ceratobasidium* certainly hastens their death. Encasement of *Ceratobasidium* in sloughed, melanized cortical cells no doubt protects it from microbiological factors of control and favors survival of the pathogen within the specific soil zone in which new strawberry rootlets develop. Thus, the fibrous strawberry root system which grows and regenerates itself within a circumscribed volume of soil cannot escape from the pathogen by growing away from it. Dissemination by nursery stock of the cultivar studied, contiguity of pathogen and susceptible root tissue, and the possibility of the occurrence of a virulent strawberry strain of the pathogen insure disease outbreaks in field-grown plants.

Although our studies dealt primarily with the reaction of one everbearing strawberry cultivar to *Ceratobasidium*, our observations (*unpublished data*) indicate that differences in susceptibility to *Ceratobasidium* injury are related to inherent root vigor and fruitfulness of the cultivar. High-yielding cultivars, particularly those of the everbearing class, exemplified by E-5, are more severely affected by loss of feeder rootlets than vigorously growing cultivars which produce only a single crop of fruit per year. In fact, it is only since development of soil fumigation with methyl bromide-chloropicrin mixtures that everbearing strawberry cultivars have been profitable to grow in California.

Pathogenesis by soil-borne saprophytes may be initiated through necrotic lesions caused by *Ceratobasidium* sp. on strawberry roots. Xylem of main supporting roots, for instance, is commonly occupied by a number of different kinds of soil-borne fungi, bacteria, and Actinomycetes (18). These could gain entrance to the plant through a channel of dead interconnected tissues which the xylem of dead rootlets provides.

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