10. M. J. THIRUMALACHAR. — Romanoa, a new genus of soil fungus with antibacterial activity.

Summary. — A new soil inhabiting fungus of the family Clavicipitaceae has been isolated and described. The fungus is characterised by a white mycelium which produces superficial, hyaline, lageniform perithecia with blunt apices. The asci are aparaphysate and narrowly clavate containing eight filiform, septate ascospores, and are crowned with a hyaline refractive gelatinous cap. A new genus, Romanoa, has been erected for this fungus, the type species being named *Romanoa terricola*.

R. Terricola was found to produce sprout cells when grown in submerged culture. Broth filtrates had no antibacterial activity against the organisms tested, whereas agar cultures showed antibacterial activity against *Bacillus subtilis* and *Micrococcus pyogenes*.

Riassunto. — Si è isolato e descritto un nuovo fungo abitatore del suolo della famiglia Clavicipitacee. Il fungo è caratterizzato da un micelio bianco che produce periteci superficiali, ialini, lageniformi con apici ottusi. Gli asci sono aparafisati e strettamente clavati contenenti otto ascospore filiformi, munite di setti, e sono coronate con cappello ialino rifrangente gelatinoso. Un nuovo genere, Romanoa, è stato formato per questo fungo, e la specie tipica è chiamata *Romanoa terricola*.

Si è trovato che *R. terricola* produce celluli di germogli quando è cresciuta in coltura sommersa. I filtrati di brodo non presentarono attività antibatterica contro i microorganismi esaminati, mentre colture di agar mostrarono una attività antibatterica contro il *Bacillus subtilis* e il *Micrococcus pyogenes*.

Résumé. — On a isolé et décrit un nouveau champignon du sol de la famille des Clavicipitacées. Ce champignon est caractérisé par un mycélium blanc qui produit des perithécium superficiels, hyalins et lagéniformes avec des sommets obtus. L'ascus aparaphysé et étroitement clavé contient huit ascospres filiformes séparés et se couronne d'un revêtement gélatineux hyalin et réfringeant. On a créé pour ce champignon, un genre nouveau, Romanoa, l'espèce étant nommée *Romanoa terricola*.

R. terricola produit des cellules germinantes en culture submergée. Les bouillons filtrés n'ont pas montré d'activité contre les organismes à

examinés, alors que les cultures sur agar avaient une activité anti-biotique contre *Bacillus subtilis* et *Micrococcus pyogenes*.

Zusammenfassung. — Eine neue Art von Bodenpilz der Familie Clavicipitaceae wurde isoliert und beschrieben. Kennzeichen des Pilzes ist ein weisses Mycelium, das durchsichtige flaschenförmige Oberflächen-Perithecia mit stumpfer Spitze hervorruft. Die Asci sind aparaphysisch und klumpenförmig und enthalten acht fadenförmige mit einem Saeptum versehene Askosporen und sind mit einer durchsichtigen refraktiven, gelatineartigen Kapsel versehen. Für diesen Fungus wurde ein neus Genus die ROMANOA, geschaffen, wobei die Species *Romanoa terricola* benannt wurde.

Es wurde gefunden, dass die *Romanoa terricola* in flüssiger Kultur Samenzellen hervorbringt. Bouillonfiltrate zeigten bei den Versuchsorganismen keine bakterientötende Wirkung, während Agarkulturen sich Wirksamkeit gegen *Bacillus subtilis* und *Micrococcus pyogenes* aufwiesen.

Introduction.

During the course of studies on soil fungi isolated from soil samples collected in Rome an ascomycetous fungus was observed, which on further studies proved to belong to an undescribed genus. The fungus was found to grow quite easily in artificial culture, producing abundant perithecia. In addition to the interesting morphological characters, the fungus was found to produce on agar cultures an antibiotic substance which inhibited the growth of some Gram positive bacteria. While there are very few soil ascomycetes known so far outside the groups of Plectascaceae, Sordariaceae, Xylariaceae and Chaetomiaceae, several of the fungi included in the above-mentioned families have been studied intensively for the production of numerous antibiotics.

Morphology of the fungus.

The fungus grows vigorously in surface cultures on several of the semi-solid media tested, such as Moyer's agar, glucose-peptone agar, Czapek-Dox agar and others. The colonies are white, arachnoid, spreading, with granular appearance after the formation of perithecia.

Since the perithecia are hyaline, they do not impart any dark shades of colour to the colony after their formation.

The mycelium is thin, long, septate and branching. The septa are indistinct in young hyphae, but may be easily observed in old hyphae. It is of interest to note that the fungus does not produce a conidial stage in the surface cultures on agar media. When the cultures are incubated for long periods and allowed to desiccate, the young hyphae along the margin of the colonies become closely septate and break into *Oidia*-like fragments.

The perithecia are formed in large masses even in very young colonies which are 8 to 10 days old (Fig. 1). During the formation of perithecia, the differentiation of fertile and sterile hyphae become evident. While the sterile hyphae of the colony are floccose and loosely grouped, the fertile hyphae are closely interwoven forming a pseudoparenchymatous layer. The perithecia are differentiated from this layer in large aggregations.

Mature perithecia are hyaline, covered with loose strands of hyphae, obclavate, lageniform to vermiform and never subglobose or spherical. They are rounded at the base and taper into a blunt papillate apex. The ostiole is imperfectly differentiated, and becomes evident after the discharge of asci and ascospores. The walls of the perithecia are composed of a pseudoparenchymatous layer of closely grouped cells and an inner layer of loosely grouped hyphal cells. The apeces of the perithecia are covered with numerous free hyphal tips appearing as papillate projections (Fig. 2). The asci are formed from the base of perithecia and never from the side walls. They are clustered on short stalk cells, aparaphysate, long and narrowly clavate, and thin-walled. The ascospores are 8 in number, long and needle-like, hyaline, very thin and appear to be faintly septate when examined in deeply stained microscopic preparations. The asci are crowned at the top with hyaline refractive gelatinous caps, which get separated off during the emergence of the ascospores from the asci.

When a mature perithecium is mounted on a slide and gently pressed with the coverglass under the microscope, the long asci are ejected out enmasse leaving the empty shells of the perithecia. The ascospores are not discharged out of the asci immediately. Observations have shown that in most of the cases the asci were extruded with the ascospores enclosed and the ascospores emerged later by throwing off the gelatinous cap. When plated out on nutrient semi-solid media, the ascospores in many cases germinate while still enclosed within the asci.

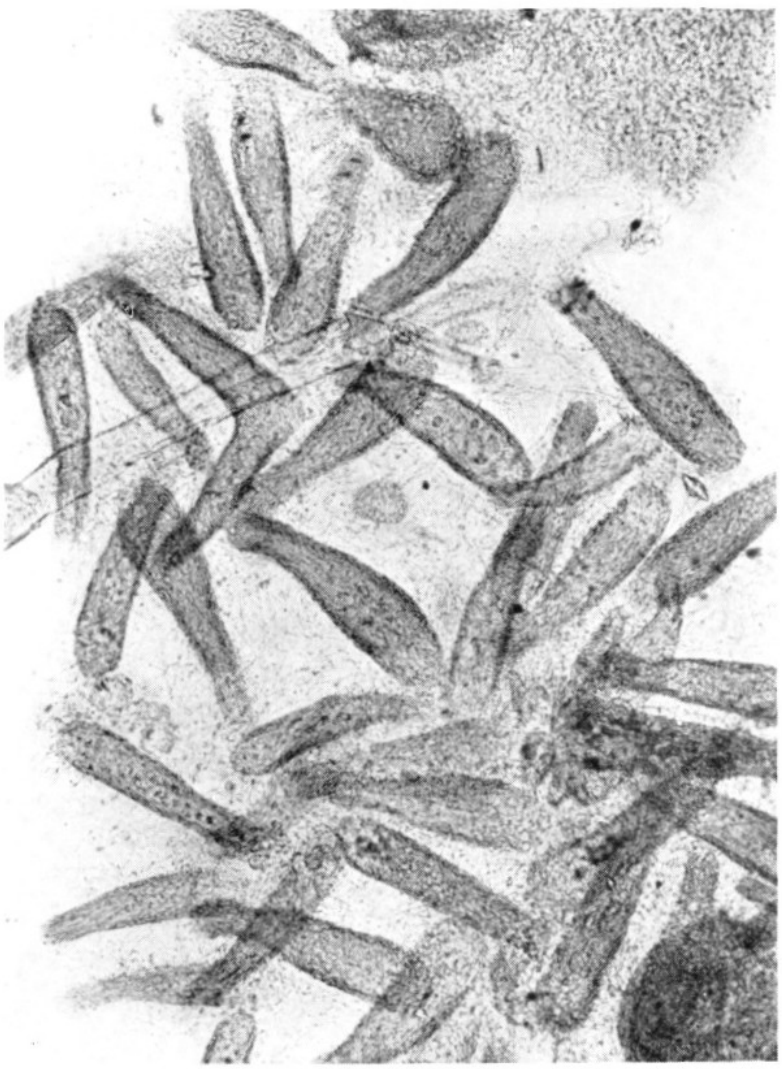

Fig. 1. - Showing the perithecia in artificial culture. × 250.

Fig. 2. - Enlarged perithecium showing the poorly developed ostiole and ascicular asci. × 500.

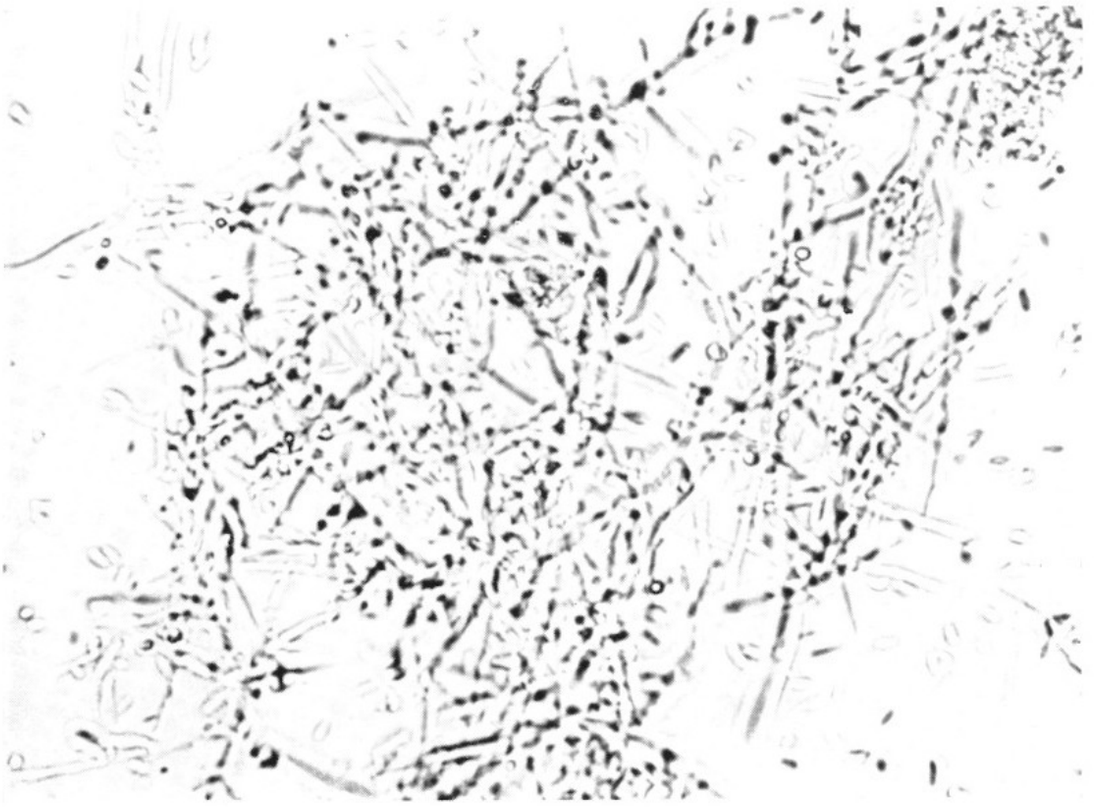

Fig. 3. - Mycelium from submerged culture producing sprout cells × 900.

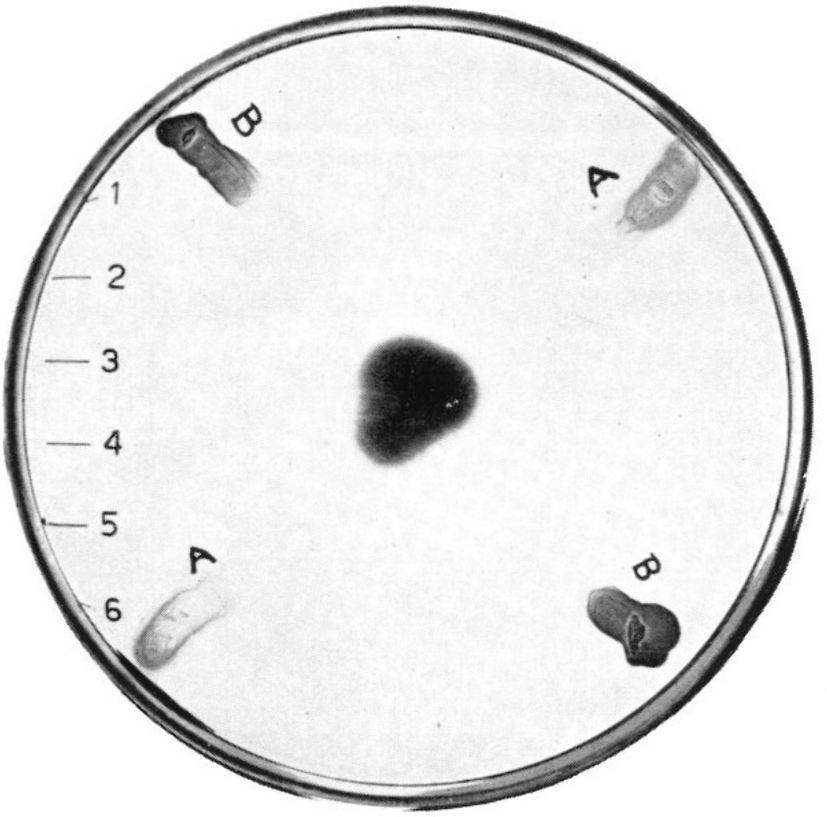

Fig. 4. - Showing the antibiotic activity on agar culture against. A. *Bacillus subtilis* and B. *Micrococcus pyogenes* var. *aureus*.

IDENTITY OF THE FUNGUS.

The characters of the fungus enumerated above indicate that it is a member of the Clavicipitaceae under the Hypocreales. The family Clavicipitaceae according to Miller (1949) with the type *Claviceps* Tul. constitutes a well defined group. There is a very marked cap at the apex of the long cylindrical ascus with filiform spores, which are ejected out by pushing off the gelatinous cap. Paraphyses and periphyses are present in some genera, but they deliquesce in early stages.

In comparing the fungus under study with closely related genera, *Ophionoctria* Sacc. and *Globulina* Speg. may be considered. In the genus *Ophionectria* which includes species parasitic on insects, the asci have needle-like ascospores, but the perithecia are globose unlike the lageniform to vermiform perithecia in the fungus under study. The genus *Globulina* described by Spegazzini (1891) with the type *G. erysiphoides* Speg. for a leaf parasite on a Compositae in Brazil, also possesses perithecia with acicular ascospores, but the perithecia are globose and astomate unlike the fungus under study. *Globulina trichocarpa* described by Sydow (1930) from Venezuela on *Sida carpinifolia* may not belong to that genus, since the perithecia have well developed ostioles, with copious periphyses. The characteristic gelatinous cap for the asci is a distinctive feature of the present fungus, and these characters warrant the allocation of a separate genus for its accomodation.

ROMANOA Thirumalachar gen. nov.

Mycelium white, floccose-arachnoid, loosely grouped. Perithecia minute, superficial, hyaline obclavate; lageniform to vermiform, tapering into a blunt apex. Asci narrowly clavate, aparaphysate, with gelatinous cap at the tip; ascospores filiform, hyaline, septate. Type species: *Romanoa terricola* Thirumalachar.

Mycelium albidum; floccose-arachnoideum, effusum. Perithecia minuta, superficialia, obclavata, lageniformia vel vermiformia, hyalina, attenuata, ad apicem obtusa. Asci anguste obclavati, aparaphysata, cum corona gelatinosa in apice; sporidia filiformia, hyalina, septata. Species typica: *Romanoa terricola* Thirumalachar.

ROMANOA TERRICOLA Thirumalachar sp. nov. (*)

Colonies white, mycelium white, floccose; arachnoid, effuse, septate, branched. Perithecia gregarious, hyaline; obclavate, lageniform to vermiform, 147-190 × 27-45, 34-45 μ; at base, 18-34 μ; at the top, papillate at the apex, ostiole indistinct, wall membranous, pseudoparenchymatic, hyaline. Asci numerous, stipitate, aparaphysate, narrowly obclavate, with gelatinous cap at top, 8-spored, 72-123 × 4-5 μ; Spores hyaline, acicular, 70-100 × 1.2-2 μ, septate.

Hab. in the soil, Rome, Italy, Sept. 1953.

Plagulae albidae, mycelium albidum, floccoso-arachnoideum, effusum, septatum, ramosaum. Perithecia aggregata, hyalina, obclavata, lageniformia vel vermiformia, 147-190 × 27-45, 34-45 μ, ad basim, 18-34 μ; ad apicem; superne papillata, ostiolo indistincto, pariete membranaceo, pseudoparenchymatico, hyalino. Asci numerosi, stipitati, anguste obclavati, postice corona gelatinosa, 8-spori, 72-123 × 4-5 μ. Spora hyalina, acicularia, septata, magnitudinis 70-100 × 1.2-2 μ.

Growth in culture.

The fungus was grown both in surface culture on semi-solid media and liquid cultures as well as in agitated submerged cultures. On the different agar media such as Moyer's agar, glucose-peptone agar and Czapek-Dox agar, the growth of the fungus is as fleecy-white colonies, spreading, producing abundant perithecia. There is no evidence of any conidial stage in this surface culture, the perithecia being the only fruiting structure. While no detailed studies on the temperature relationship of the fungus influencing growth have been worked out, it was found that the fungus on Moyer's agar grows vigorously at 24 °C; (75 to 80 mm; diameter of colonies in 10 days), and very slowly or not at all at 30°C (3 to 5 mm. diameter in 10 days).

In the case of surface cultures on liquid media, the mycelium forms a dense felt on the liquid surface, producing numerous perithecia. In submerged cultures (in 500 cc. flasks with 125 cc. culture solution) agitated on rotary shakers with speed of 250 revolutions per minute and incubated at 24°C, the following media were used; 1. glucose-corn steep solution (3% glucose, 3% corn steep solids, 0.7% $CaCO_3$, with pH at 6.0),

(*) Cultures have been deposited with the Centraalbureau voor Schimmelcultures Baarn, and the Istituto Superiore di Sanità, Rome.

2. Moyer's media (without agar) and 3; Peptone-glucose-yeast extract solution (peptone 1%, glucose 2%, yeast extract 0.3%, NaCl 0.5%; pH 6.8). The flasks were inoculated with fragments of mycelium taken from agar surface culture. Observations after 48 hours in submerged culture indicated that the mycelium after making some growth had fragmented into numerous small bits. On each of these mycelial fragments, several obconical branchlets resembling conidiophores had been formed, and these abstricted off numerous sprout cells. These resembled similar sprout cells found in the artificial cultures of smut fungi and *Taphrina* sp. giving rise to numerous yeast-like cells by budding. The culture solution turned turbid instead of showing mycelial growth, and contained numerous sprout cells with fragments of mycelia. When these sprout cells were aseptically streaked on Moyer's agar and other semi-solid media, the characteristic mycelial colonies of *Romanoa terricola* developed again. In the case of surface cultures on liquid media, the submerged portion of the mycelium was found to abstrict off a few sprout cells, but these were of rare occurrence.

While the fungus in the mycelial stage failed to grow on agar or liquid media at temperatures of 30°C, the sprout cells were found to be capable of showing some further development at 30°C and up to 37°C tested in the present study. When the inoculum consisting of sprout cells was transferred to corn-steep-glucose solution and placed on a rotary shaker at 30°C the sprout cells were found to bud numerous secondary ones thus increasing in number.

ANTIBACTERIAL ACTIVITY.

The fungus when grown on peptone-glucose agar and incubated at 24°C was found to produce an antibiotic substance which inhibited the growth of some gram positive bacteria. Five days old growth cultures on peptone-glucose meat-extract agar were streaked with the test organisms and incubated at 37°C. The zones of inhibition after 12 hours incubation were as as follows:

Bacillus subtilis 20 mms.
Micrococcus pyogenes var *aureus* 22 mms.
Escherichia coli Nil.
Klebsiella pneumoniae Nil.

4 mm. discs of agar taken from the margins of 10 days old colonies of *Romanoa terricola* grown on Moyers agar, when placed on nutrient agar seeded with the above mentioned bacterial organisms individually, have

shown the same result of inhibition, that the substance produced by the fungus is active only against *B. subtilis* and *M. pyogenes* var *aureus*.

The broth filtrate from submerged cultures had no activity against the bacterial organisms tested. This may be due to the fact that there was very poor or no mycelial growth at all and only sprout cells were formed. In surface cultures on liquid media, the filtrate after 30 days growth at 24°C, showed little activity (10 mm; zone of inhibition) when tested against B. subtilis (I.C.I. strain). The factors influencing maximum production of the antibiotic substance in liquid media need further study.

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Roma — Istituto Superiore di Sanità - International Research Centre for Chemical Microbiology.

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