A Fungus of Economic Importance on the Avocado

(Persea Americana).

By E. M. Doidge.

Comparatively little is known about the fungous diseases to which the Avocado is subject. In Florida it is said to suffer from certain leaf and fruit spot diseases caused by a *Colletotrichum* sp., and the young growth is attacked by a scab fungus said to be identical with *Cladosporium citri*, but none of these fungi is recorded as attacking the twigs and larger limbs.

During 1921 it was reported from Louis Trichardt that certain Avocado trees were in a very bad state, and that spraying with bordeaux mixture had proved quite ineffectual. The twigs which were sent for examination were covered with minute pustules, the fruiting bodies of an ascomycete, and as none of the diseased wood had been excised previous to spraying it was not surprising that the inroads of the fungus had not been checked. As a consequence the trees had become so badly cankered that it was impossible to remove all the diseased bark without seriously affecting the vitality of the trees, and at this stage it was impossible to arrest the progress of the malady.

Symptoms.

The effect of the fungus on the twigs and limbs of the Avocado is very similar to that produced by *Physalospora cydoniae* on the limbs and twigs of apple trees.

In the earlier stages of infection the bark becomes sunken and discoloured; the diseased area increases in size, and gradually girdle the twig or branch, so causing the death of the terminal portion. Branches, 2–3 inches in diameter, are killed in this way, and extensive cankers are produced on the larger limbs and the trunk. The bark of the cankered areas is dark-coloured and sunken, the boundary between the healthy and diseased tissue being marked by a raised, reddish-brown line. As the bark is destroyed it dries out and cracks horizontally, eventually falling away from the wood, which has become dead and discoloured. Numerous fruiting pustules of the pathogen may be observed scattered over the diseased areas of the bark.

The fruits on the diseased trees are affected, showing a corky growth at the lower end, which may possibly be due to the same fungus (but this fact has not been established, as it was not possible to obtain trees in bearing for inoculation purposes). The affected area is irregular in form and varies in size. On fruits examined it was roughly 10 × 5 cm. and 4 × 5 cm. The surface is very much roughened, verona brown (Ridgway) in colour, and deeply cracked, after the fashion of tortoise-shell.

This affection of the fruit is apparently identical with one which occurs in Florida and is mentioned in a paper by H. E. Stevens, published in the "Proceedings of the Florida State Horticultural Society" for 1918. His description is as follows: "Another common type of injury frequently noted on the fruits is referred to as anthracnose by some of the growers. This type of injury is very similar to melanose of citrus fruits in general appearance. It is superficial, and appears in the form of dark reddish-brown, caked masses on the surface of affected fruits. The markings are hard, compact, and the surface is cracked or broken. The injury may cover only a part or the whole surface of the fruit. It makes an unsightly fruit, but apparently does not affect the quality. The disease is apparently caused by a fungus, perhaps a *Gloeosporium* or a closely related species."

MORPHOLOGY.

Perithecia have been found on cankered areas of the twigs of the Avocado pear (*Persea Americana*). They are usually scattered, standing separate from one another. Sometimes, however, they occur in small groups of two to four, and occasionally they have been found so closely crowded as to form a pseudo-stroma, but no true stroma has been observed. They are buried in the cortical tissues, but at maturity the papillate ostiole breaks through to the surface and protrudes slightly.

The form of a typical perithecium is shown in fig. 1, which is a camera-lucida drawing from a medial longitudinal section. The form is subglobose or broadly ellipsoid, 130–190 μ in the horizontal diameter and 100–120 μ in the vertical diameter. The average dimensions are about 170 $\mu \times$ 100 μ.

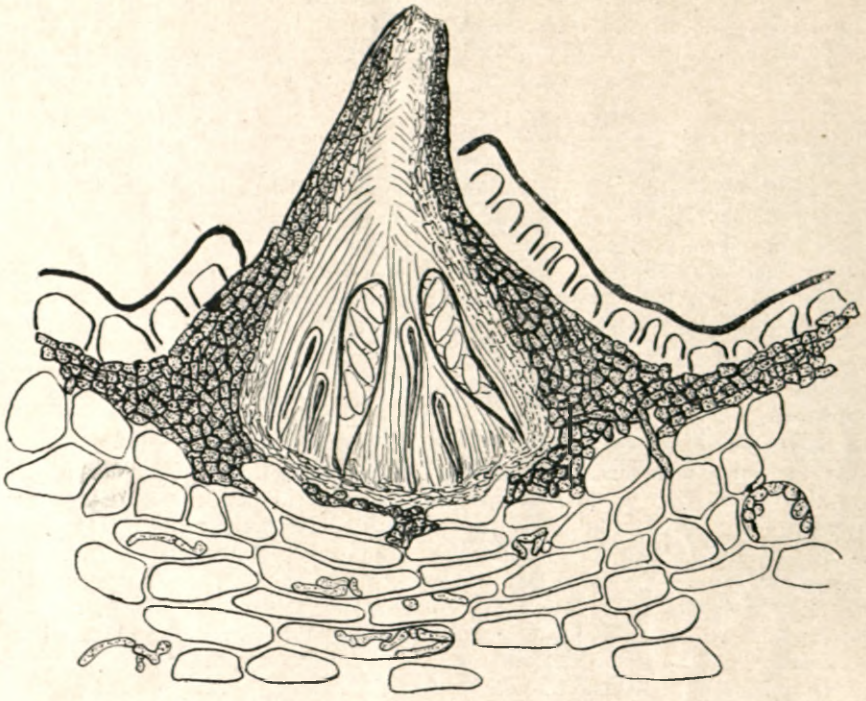

Fig. 1.
Section through a perithecium of *Physalospora perseae*.

The wall is differentiated into two layers: the thickness of the outer layer is variable being thinner or almost absent at the base, and thickest at the sides near the base, where it is 16–24 μ thick. The pseudo-cells are brown, polygonal, 4–5 μ in diameter, or somewhat flattened. Within this there is an inner layer of delicate, colourless, compressed hyphae of much more uniform thickness.

The papilla is usually central, but may be oblique: it is 80–100 μ long and 30–50 μ in diameter, and its walls show two distinct layers similar to those observed in the sides of the perithecium; the ostiole appears in section as a narrow passage between the walls of the papilla. The asci interspersed with paraphyses fill the cavity of the perithecium, and there are numerous thread-like periphyses lining the neck and the inside of the papilla.

The asci are abundant, usually clavate, sometimes tending to the cylindrical or curved, 80–100 $\mu \times$ 18–23 μ, eight-spored ; they are slightly thickened round the apex, but no canal or apical pore could be observed. The asci do not stain blue with iodine ; they disappear rapidly at maturity, so that one rarely finds an entire ascus containing mature spores ; the wall of the ascus seems to break down as soon as the spores are mature.

The ascospores are more or less distichous, hyaline, ellipsoid or subfusoid, tapering abruptly at each end to a blunt apex, 20–21 $\mu \times$ 8–10 μ. There are two to three large oil drops in each spore which stain pink with Guéguen's triple stain, the rest of the spore stains a deep blue.

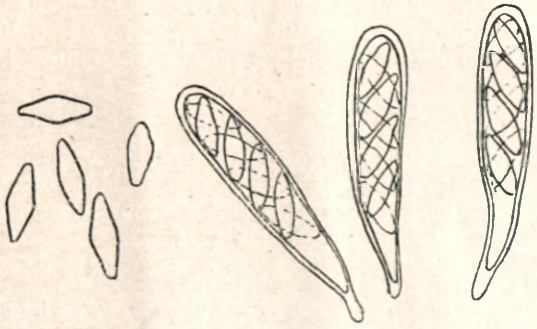

Fig. 2.
Asci and spores of *Physalospora persicae*.

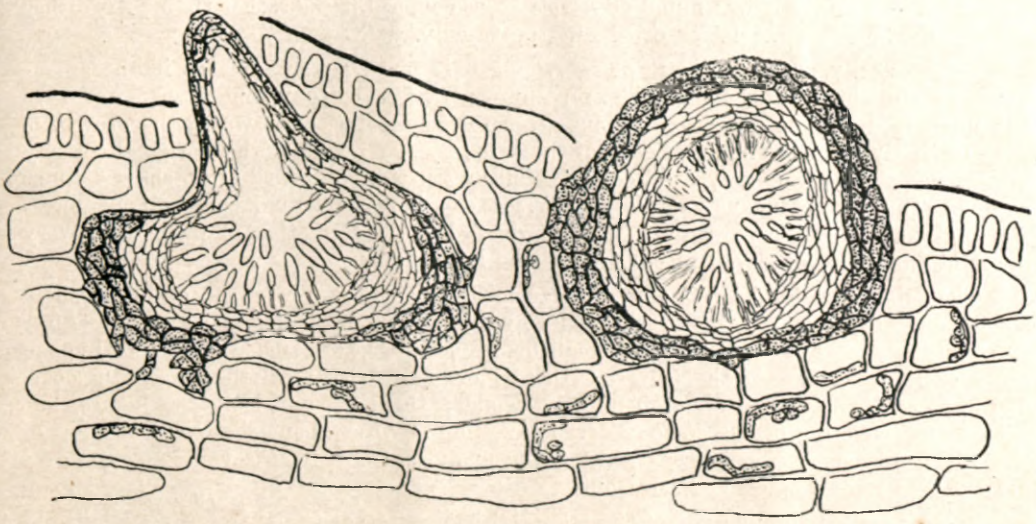

Fig. 3.
Section through two pycnidia.

Pycnidia are found on some of the stem cankers in the place of perithecia or are interspersed with them. The pycnidia are single or in small groups : some are spherical or sub-spherical with a distinct papilla, that is to say, they are similar to the perithecia ; others are conical, spherical or elongated, without a papilla (fig. 3). They are formed under the epidermis or in the cortical tissues, and are erumpent at maturity. The horizontal diameter is 120–130 μ, vertically they measure 70–187 μ, and there is a double wall, the two layers being similar to those described in the perithecia. From the inner wall arises

the spore-bearing layer, which occupies almost all the space not taken up by the ostiole. The cells lining the cavity arch inward, and elongate to form a stalk, from the tip of which the pycnospore is abstricted.

The pycnospores are ellipsoid and colourless, and do not turn brown at any time; they are 18–20 $\mu \times$ 5–6 μ in dimensions.

Physalospora Perseae, nov. sp.

Peritheciis sparsis v. gregariis, tectis, ostiolo papillato erumpente, atris, ellipsoideis v. subglobosis, 130–190 $\mu \times$ 100–120 μ; ascis clavatis, paraphysatis, 80–100 $\mu \times$ 18–23 μ, octosporis: paraphysibus filiformibus, numerosis, hyalinis: sporidiis distichis, continuis, hyalinis, ellipsoideis v. subfusiformis utrinque obtusis, 20–21 $\mu \times$ 8–10 μ. Pycnidiis subglobosis v. ellipsoides, 120–130 $\mu \times$ 70–187 μ; sporulis hyalinis ellipsoideis, 18–20 $\mu \times$ 5–6 μ.

Hab. in foliis *Perseae americanae*, Louis Trichardt, Transvaal, 25.4.21, leg. J. H. Cronwright.

When the spores have been discharged from the pycnidia or perithecia, one frequently finds the cavity occupied by another fungus, which is probably a saprophyte or secondary parasite. This organism produces spherical pycnidia about 90 μ in diameter, and filled with small, narrow, ellipsoid, fuliginous, two-celled spores.

The Fungus in Culture.

The fungus was isolated a number of times from diseased twigs by plating (1) ascospores and (2) pycnidia in agar, and (3) from the mycelium present in small pieces of diseased wood. In each case the same fungus was obtained, and the cultural characters were identical. It grows readily on most of the culture media in common use, but as the fruiting bodies develop slowly it was found advisable to use conical flasks rather than petri dishes for culture work, as the latter dried out too rapidly.

Cultures on prune agar, oatmeal agar, and maize-meal agar were kept in the greenhouse in a bright diffused light. The mycelium is at first cottony, but after a few days the submerged hyphae are green or blue-green for some time, after which the growth is dark or nearly black. The outer ends of the aerial hyphae maintain their original cottony appearance and become greyish in colour. Cultures in active growth often show distinct zoning. Thus far the fungus *Physalospora Perseae* closely resembles in its cultural characters *Ph. Cydoniae*, which is parasitic in apple twigs,* but the formation of pycnidia is very different from that observed in cultures of the latter fungus.

The submerged hyphae, as seen by transmitted light, are olive-green or brownish in colour, and are about 3 μ thick; the aerial hyphae are more slender and hyaline. After three or four weeks on oatmeal agar small bodies consisting of tangled masses of hyphae appear on the surface of the culture; these increase in size until they form tussocks, 3–6 mm. in diameter and about 3 mm. in height; these are dark-coloured bodies, but with a thin covering of the cottony aerial mycelium. After about six weeks the top of those bodies becomes covered with hard, black pustules, from which seven to eight weeks old cultures masses of conidia ooze out.

A cross-section through one of the sclerotial bodies first observed in a three weeks old culture shows that it consists of tangled masses of hyphae similar in form to the submerged hyphae of the ordinary mycelium. Later there is a differentiation with a sterile base, consisting of interlaced dark-coloured hyphae, brown or olive-green, by transmitted light, and an upper portion of more closely woven, lighter-coloured hyphae in which a number of cavities are developed. These are irregularly arranged in one or more layers, ellipsoid or flask-shaped—usually papillate, 250–320 $\mu \times$ 130–200 μ. The inside of the cavity is lined with a layer of compressed hyaline hyphae, which eventually arch inwards and form the conidiophores. The tip of the conidiophore swells up and the mature conidium is cut off.

* Hesler, L. R.: Black Rot, Leaf Spot, and Canker of Pomaceous Fruits. Cornell Univ. Agric. Exp. Sta. Bull. 379. Aug. 1916.

On maize-meal agar and prune agar slight differences are observed. On maize-meal agar the pycnidial cushions are up to 8 mm. in diameter; on prune agar the form is somewhat different, the pycnidial cushions are columnar, 5–6 mm. high and 3–5 mm. thick. They have a sterile base, which is usually rather smaller in diameter than the rounded head in which the pycnidial cavities are found.

Sugar agar appears to be an unsuitable medium; there is no aerial mycelium, and only a few small sterile sclerotia are formed. Potato agar is also an unfavourable medium. On solution N. agar only a few small sub-spherical cushions are formed, which are 2–3 mm. in diameter, and show some internal differentiation, but none were observed in which mature pycnidial chambers were formed.

The masses of conidia oozing out of the pycnidia are pale orange-yellow (Ridgway). Individual conidia are hyaline, thin-walled, with granular contents, and containing a few oil drops. The majority are ellipsoid in form, a few are sub-fusoid or sub-pyriform; they vary from 16·5–23·5 μ in length and from 6–7·5 μ in breadth, the greater number measuring 18–20 $\mu \times$ 6·5–6·7 μ.

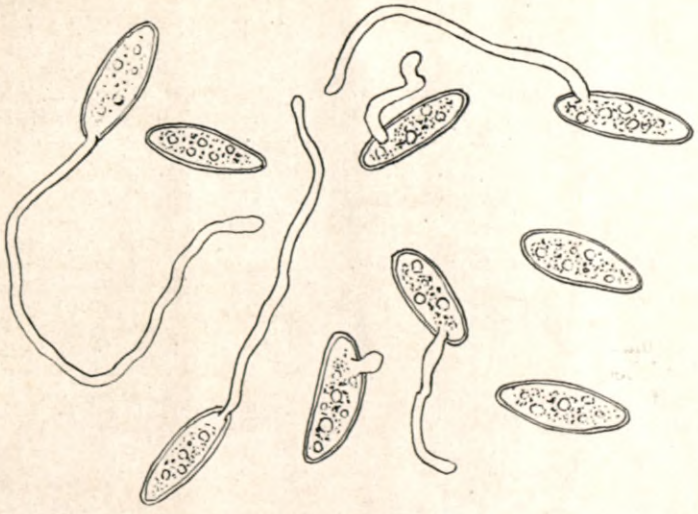

Fig. 4.
Germinating conidia after 6 hours at 25° C. in hanging drop.

A number of hanging drop cultures in Ward tubes, each containing a small number of conidia, were put into an incubator at 25° C. After six hours a considerable number had germinated (fig. 4). The form and character of the spore remained unchanged, and a single germ tube, 2·5–3 μ thick, colourless and non-septate, had been produced at or near one pole.

After twenty-four hours possibly 20 per cent. of the conidia remained unchanged and had not germinated. The remainder had lost their granular appearance and had become slightly broader and more broadly rounded at the ends, the majority being 6·7–7·5 μ broad. They were still quite hyaline, but had become septate: most commonly with 2-transverse septa, but a few were 1-septate, and there was an occasional one which was 3-septate. Those which were germinating had, with a few exceptions, produced two germ tubes, one from each terminal cell (fig. 5).

The conidia, which had germinated in six hours, had developed considerably, and had produced elongated hyphae which were septate and branched.

After forty-two hours there was no further change, except in the increased length of the germ tubes and the increased number of spores with two and three septations. At no time was any suggestion of a change of colour observable in the conidia.

The ascus stage was not found in culture, but, as stated elsewhere, its connection with the conidial stage has been traced.

Inoculation Experiments.

Several seedling Avocados in the greenhouse were inoculated with conidia from cultures on oatmeal agar and with pieces of mycelium. In each case a small incision was made in the bark, the infective material inserted, and the branch at the point of inoculation was kept moist for about forty-eight hours.

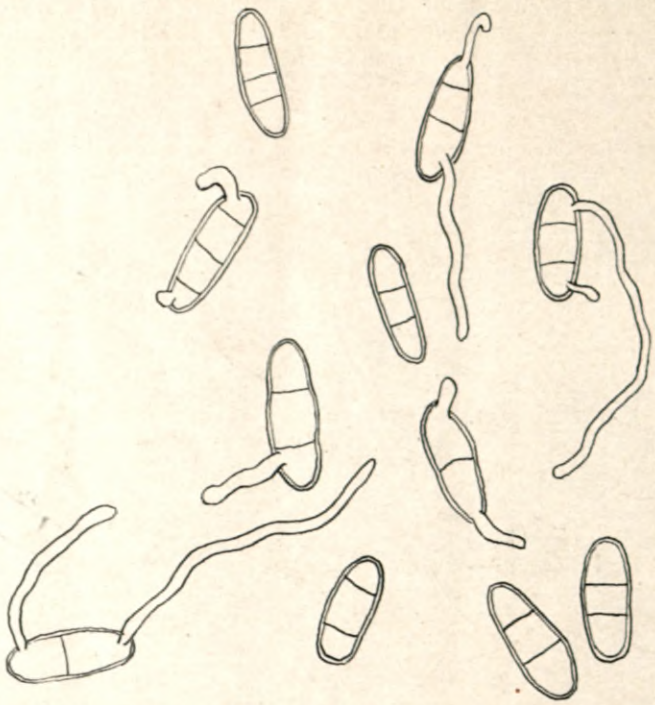

Fig. 5.
Conidia after 24 hours at 25° C.

The plants which were inoculated with conidia soon showed signs of infection. The tissues around the incision became discoloured and sunken; in some cases the affected area slowly increased in size for a few weeks until it was about 1 cm. in diameter, and then its progress was arrested. Two trees became completely girdled, the whole stem was invaded and killed, and after six months typical conidia were produced. The pycnidia and conidia on these artificially infected trees were identical with those found on twigs in the orchard.

Pathological Histology.

The fungus appears to be a wound parasite. In the cortical tissues of infected twigs there is a rather coarse brownish mycelium about 3·5 μ thick. The cell walls are discoloured, but there is no very prominent deposit in any of the cells. At certain spots there are masses of densely woven hyphae in the epidermal and hypodermal cells, which rupture the cuticle,

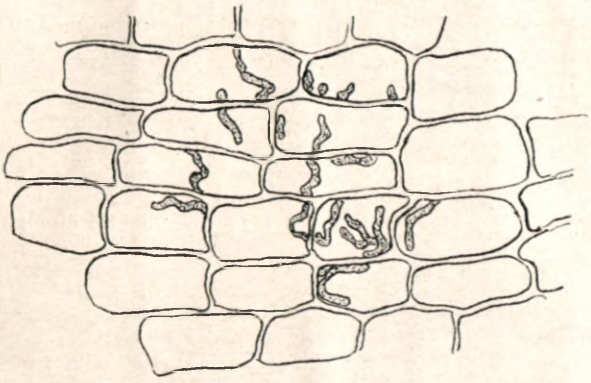

Fig. 6.

Section through twig 6 months after inoculation, showing mycelium in cells of cortex.

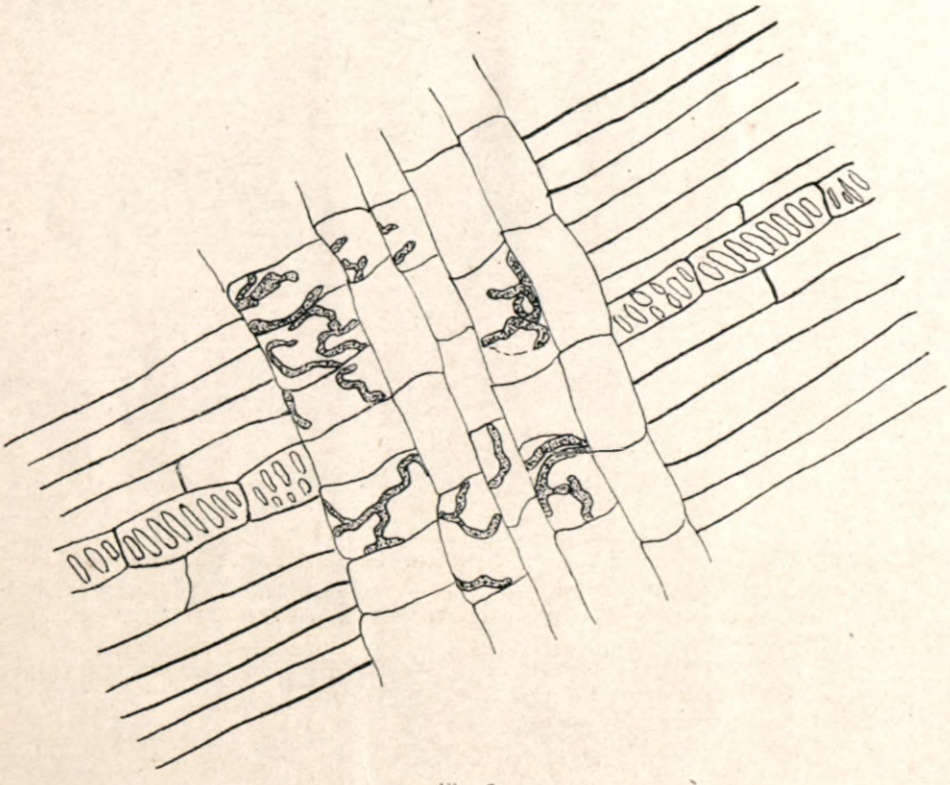

Fig. 7.

Section through xylem region of same twig, showing mycelium confined to cells of medullary rays.

and are the early stages in the development of the pycnidia and perithecia. The mycelium is intracellular, it penetrates through the cortical cells to the cambium, and invades the xylem region, where it is apparently confined to the cells of the medullary rays. In a large number of sections examined no hyphae were observed in the xylem vessels.

Preventive Measures.

In its early stages the disease could probably be arrested by surgical methods and spraying with bordeaux mixture. As in dealing with other cankers all diseased twigs should be removed, cankers on larger limbs excised, and all wounds painted with some disinfectant and protective substance. In its later stages it is difficult and almost impossible to arrest the progress of the disease.