

FOUR SPECIES OF EXOBASIDIUM IN PURE CULTURE

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INTRODUCTION

The purpose of this investigation was to study the possibilities of obtaining pure cultures of *Exobasidium*.

The following species of *Exobasidium* were studied:

Exobasidium japonicum Shir., parasitic on *Azalea indica*.

Exobasidium rhododendri Cram., parasitic on *Rhododendron ferrugineum* and *Rh. hirsutum*.

Exobasidium vaccinii (Fuck.) Wor., parasitic on *Vaccinium vitis-idaea*.

Exobasidium vexans Mass., parasitic on *Thea sinensis*, causing the blister-blight.

The manner of attack of their hosts is the same for the four species: the affected part of the plant becomes hypertrophic and after some time basidiospores are formed on the exterior of the hypertrophy. When the basidiospores are mature, they are shot from the basidia with some force.

E. japonicum, *rhododendri* and *vaccinii* form conidia (= sporidia = secondary spores) after the emergence of the germ-tubes from the basidiospores; the conidia are small, fusiform cells, formed in branched chains.

E. vexans differs in its manner of germination from the other species studied. In this species long germ-tubes grow out from the basidiospores, but no conidia are formed.

In the collection of the "Centraalbureau voor Schimmelcultures", Baarn, Netherlands the genus *Exobasidium* was represented by one species viz. *Exobasidium japonicum*, an isolation from *Azalea indica*, received from Dr. BLUMER (Switzerland).

Living cultures of the species dealt with in this paper have been deposited with the collection of the "Centraalbureau voor Schimmelcultures".

SHORT SURVEY OF THE LITERATURE

The genus *Exobasidium* was founded by WORONIN in 1867 (10). WORONIN gave an excellent description of the type species, *E. vaccinii*, and also studied the germination of the basidiospores and the manner of infection of this fungus. He saw that fallen basidiospores could germinate on practically any substratum in a humid atmosphere. Within an hour after placing a slide under a hypertrophy, basidiospores are found on the glass; twenty-four hours later the glass is covered with a white powder of spores. The spores, when shed, often form a septum.

If the air is saturated with water vapour the spores germinate; germ-tubes are formed at both ends of the spore, the contents of the spore move into the germ-tubes and the number of septa in the spore increases to three or four. The germ-tubes continue to grow, occasionally they branch, after some time they begin to form the one-celled, fusiform conidia. The conidia, formed in mass, are loosely connected. The germination on a slide may continue for three to five days, then no further growth is observed.

WORONIN also describes the germination of the basidiospores on the leaf of the host *Vaccinium vitis-idaea*, which is quite different from the germination observed on a slide. The germ-tubes do not develop conidia, but penetrate into the leaf, either by the stomata or by piercing the cuticle and the epidermis. Within the leaf a mycelium is formed and the affected area of the host reacts by forming a hypertrophy.

BREFELD (1) is the first investigator, who cultivated an *Exobasidium*. He describes and draws a culture of *E. vaccinii* growing in liquid medium. When the tips of the outgrowing germ-tubes had reached the boundary-line between liquid and air, conidia were formed. The development of conidia continued until the medium was exhausted. No basidia were observed. The conidia which Brefeld obtained in his cultures could be used for inoculation of the host.

RICHARDS (8) reports that his attempts to cultivate *Exobasidium* on agar media were not successful.

LAUBERT in "Sorauer's 'Handbuch der Pflanzenkrankheiten'" (5) reports on the genus *Exobasidium* a germination of the spores identical to the germination of *E. vaccinii* as is described by WORONIN.

GADD and LOOS (2) describe the germination of the basidiospores of *E. vexans* in hanging drops. In about five hours germ-tubes grow out from both ends of the spore, sometimes they also develop laterally. Likewise in this species the contents of the spore flow into the germ-tubes, leaving behind an emptied spore-wall.

In another publication the same authors (3) give some more details about the germination of the spores. They observe the development of appressoria on the germ-tubes. These are small, half-spherical organs, which firmly attach the fungus to the leaf. This adherence is of importance for the fine hyphal thread, which, growing out of the appressorium, has to force its way through cuticle and epidermis.

The most favourable conditions for the germination are obtained when the spores are allowed to germinate on a glass slide. This slide is covered by a very thin film of water formed by condensation.

GADD and LOOS in this paper also describe the germination of *E. rhododendri*, which is comparable to that of *E. vaccinii*, as in both species small conidia are produced.

REITSMA and VAN EMDEN (6) also report the germination of basidiospores of *E. vexans*. In a later publication (7) the authors state that they succeeded in cultivating germinated basidiospores on togé-agar during seven days. Germ-tubes of 60 μ length were formed. Unfortunately the culture was lost.

Loos (4) considers *E. vexans* as an obligate parasite, that will never grow on dead organic material, therefore, according to Loos, all experiments to culture this fungus will be doomed to fail.

In a recent paper WOLF and WOLF (9) report their experiments with *E. camelliae* var. *gracilis* Shir., parasitic on *Camellia sasanqua*. They successfully grew this fungus in pure culture. The isolations were made either by placing fragments of affected leaves on maltagar or by transferring mature basidiospores to agar media. Within a week greyish to brownish, rough, yeastlike colonies had developed.

MATERIAL

E. vaccinii was the first species studied. Material of this fungus growing on *Vaccinium vitis-idaea* was collected at the Veluwe (Netherlands).

Twigs of *Rhododendron ferrugineum* and *Rh. hirsutum* attacked by *E. rhododendri* were received from Switzerland.

Later, several specimen of cultivated *Azalea* (*A. indica*) were obtained, which were infected by *E. japonicum*.

Several times tea leaves attacked by blister-blight were received from Ceylon and Indonesia. This material, however, was often in a very poor state and could rarely be used for making isolations of *E. vexans*. Then, some infected tea seedlings were received, which continued to grow in a hot-house, they became the base for the study of living material of *E. vexans* in the Netherlands, as from these seedlings a few young healthy tea plants could be inoculated.

METHODS

The methods generally used for isolating a fungus such as putting small portions of disinfected hypertrophic areas of the plant on agar plates or plating out a spore suspension, or by streaking scratched off particles on agar, did not give any result with these fungi. Either no growth was obtained or saprophytes covered the plate after a few days.

Finally, good results were obtained by starting from germinated basidiospores discharged on a solid surface.

A. *Exobasidium japonicum*, *rhododendri* and *vaccinii*

Hypertrophic parts of the hosts were kept in sterile Petri dishes; by means of some wet cotton wool a relative humidity of 100 % was

secured. After 24 hours a white spot was clearly visible under the hypertrophy. When observed under the microscope this white spot proved to consist of germinated basidiospores, which had already begun to produce conidia. By means of a firm needle with a flattened tip a little of this spore substance was transferred to agar slants. After about 6 days the cultures — which will be described below — began to grow.

A second method, giving the same results, was carried out as follows: Small fragments of a hypertrophy were fixed with vaseline to the inside of the lid of a Petri-dish, filled with agar. By turning the lid somewhat during the first hours of spore discharge, the outgrowing colonies were spread over the agar surface. A disadvantage of this method is that sometimes a plant fragment will fall on the agar thus causing contaminations which outgrow the culture of *Exobasidium*.

B. *Exobasidium vexans*

The first method of isolation described above rarely gave a result. As this fungus does not produce conidia, the germ-tubes were too much damaged when they were scratched from the glass surface. *E. vexans* was therefore always isolated by the second method.

CULTURE MEDIA

Exobasidium vaccinii was cultivated on a series of 25 different solid culture media and on 5 liquid media. The best growth was obtained on the ordinary media such as cherry, potato-dextrose, malt and maltsaleb*agar.

Cultures on maltsalebagar showed characteristic growth forms. The other three *Exobasidium* species also grew well on these media. Attempts to grow the fungus on an agar medium, prepared with a decoction of leaves of *Vaccinium vitis-idaea* were not very successful, the growth on this medium was very poor.

E. vaccinii cultures were used to study the influence of the temperature on the growth of this fungus. Low temperatures as well as a constant temperature of 28° C. proved to be unfavourable. The normal room temperature (17° — 20° C.) with a slight change between day and night, proved to be the optimal temperature for the growth of *E. vaccinii*. In its cultural requirements, *E. vaccinii* therefore does not differ from the common moulds.

DESCRIPTION OF CULTURES

I. *Exobasidium vaccinii*

Basidiospores discharged in a Petri dish are transferred with a needle to maltsaleb, malt, cherry and potato-dextroseagar in tubes. After 6 days a slight growth becomes visible. The colonies grow concentrically and become somewhat raised, forming half spherical heaps, not unlike bacteria colonies. The colony very soon acquires the shape of a small crater when its border becomes less regular; later, when the culture ages this shape becomes less marked;

* Saleb is a starch obtained from the corms of orchids growing in the Netherlands.

scattered colonies are growing together, the growth then resembles a coral, showing a yellowish-white, granular and wrinkled surface (fig. 1). Transfers of cultures always show the coral-shaped form of growth only. On malt agar the colour of the aging culture turns dark yellow till brown. Cultures of *E. japonicum* (strain Blumer) show similar growth characters; it therefore seemed very probable that the isolates were indeed those of *E. vaccinii*. Later the identity could be proved by successful inoculations of healthy plants of *Vaccinium vitis-idaea* with conidia from these cultures (see page 522).

A mount made from a culture shows numerous fusiform cells, the conidia, measuring $15-20 \times 2-3 \mu$. Sometimes also larger mycelial fragments are observed. Always the particles are connected, though very loosely.

The occurrence of the larger fragments appeared to be influenced by the culture medium. On Acer-twigg, lupine-stem and tomato-stem mycelial fragments were produced of 30μ . They also occurred on an agar medium prepared with a decoction of leaves of *Vaccinium vitis-idaea*.

II. *Exobasidium japonicum*

Young cultures of this fungus exactly resemble those of *E. vaccinii*. On further development, however, they show a more regular cerebri-form folding of the surface and are never as irregularly wrinkled as those of *E. vaccinii*. The colour on all media is greyish-brown, often the medium covered by the mould discolours; potato-dextrose agar e.g. turns brown.

This fungus also produces conidia about 25μ long and 2μ broad.

III. *Exobasidium rhododendri*

At first the cultures are directly comparable to those of *E. vaccinii* but soon the feature of the culture shows a rather flat structure which can best be characterized by comparing it to folded cultures as seen by yeasts.

Mounts of these cultures again show *Exobasidium* conidia, measuring $10 \times 2 \mu$.

IV. *Exobasidium vexans*

As *E. vexans* was considered to be an obligate parasite (Loos 4) which cannot be grown on dead substances, the cultures obtained from hypertrophic tea leaves were at first very critically watched.

From the fact, however, that identical cultures were obtained from the Ceylon material as well as from Indonesian tea leaves and from the material obtained here in the hot-house through inoculation by the seedlings from Ceylon, it was concluded that the cultures were indeed isolates of *E. vexans*.

Moreover the dull spots on agar media caused by the spore discharge of *E. vexans* were examined microscopically. They always proved to consist of germinated basidiospores. The spores had already

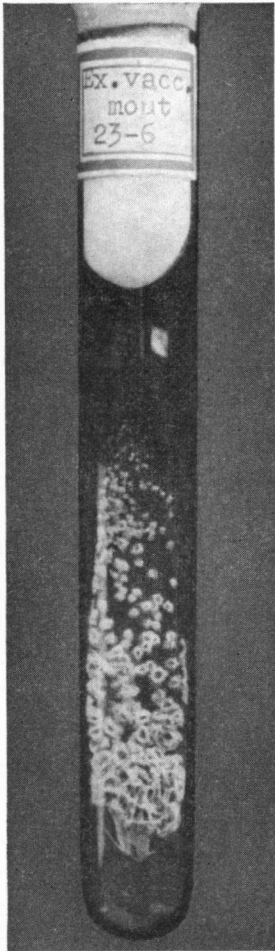


Fig. 1

Fig. 1. Culture of *Exobasidium vaccinii* (Fuck.) Wor. on maltagar.

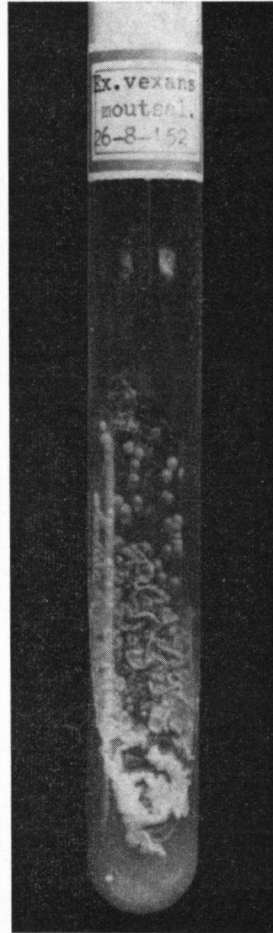


Fig. 2

Fig. 2. Culture of *Exobasidium vexans* Mass. on maltsalep agar.

emptied their contents into the irregularly growing germ-tubes. In later stages also, empty spores could often be found amongst the hyphae. As it is very difficult to cultivate tea plants in a moderate climate, the absolute proof of the identity of the cultures viz. by demonstrating their parasitic power to cause blister blight, could not yet be given. Experiments concerning this point are still in progress.

Young transfers also show the typical feature of scattered half-spherical colonies. By growing together, these separate colonies then form the typical much folded culture (fig. 2).

Conidia are never observed in *E. vexans*. The culture consists of loosely connected mycelium fragments of different lengths. The width of the hyphae is 1—2 μ .

INOCULATION EXPERIMENTS WITH *EXOBASIDIUM VACCINII*

To obtain a spore suspension the ordinary method of shaking a culture with water proved unsuccessful as the conidia have a strong tendency to clump. Good results were obtained by crushing a culture in mortar; most of the conidia remain uninjured in this process.

A suspension, thus obtained, was sprayed on some healthy plants of *Vaccinium vitis-idaea* which had been previously well moistened. After 8 days small white spots became visible on many of the young leaves, after 16 days several hypertrophies had developed, identical to those formed under natural conditions.

From these hypertrophies cultures could again be isolated, identical to those which had been used for the inoculation.

Two conditions are of importance for obtaining positive inoculations:

- a. The plants to be inoculated should have young sprouting shoots.
- b. The culture used for the inoculation should not be older than a few weeks.

SUMMARY

Some methods are described for isolating and culturing *Exobasidium species*. Detailed descriptions are given of cultures of

Exobasidium japonicum Shir.

Exobasidium rhododendri Cram.

Exobasidium vaccinii (Fuck.) Wor.

Exobasidium vexans Mass.

Successful inoculation experiments of *Exobasidium vaccinii* are reported, the fungus being re-isolated from the hypertrophies it had caused on *Vaccinium vitis-idaea*.

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