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STUDIES ON THE BIOLOGY OF *BOTRYTIS ALLII* ON *ALLIUM CEPA*[1]

Waarnemingen betreffende de biologie van Botrytis allii *op* Allium cepa

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Botrytis allii Munn is capable of attacking young onion plants without impeding the growth of the plants, provided conditions for infection are favourable. Infected green leaves are symptomless since the mycelium remains restricted to the epidermal cells which lack chlorophyll. At a certain physiological age of the leaves the fungus penetrates the underlying parenchyma tissue and spreads further through the leaves into the bulb. Apparently healthy bulbs can carry mycelium. A macroscopically invisible infection can be demonstrated readily with methyl-red. Young and old plants proved to be equally susceptible to the disease.

INTRODUCTION

In the Netherlands grey mould neck rot is one of the most important diseases in onions, in some years causing losses up to 60%. Neck rot is usually referred to as a "storage" disease, as in general symptoms are evident only after a certain period of storing the bulbs, although very rarely an affected bulb is found in the field.

According to WALKER (1925) the fungi that have been recorded as causing neck rot are *Botrytis squamosa* Walker, *Botrytis byssoidea* Walker and *Botrytis allii* Munn. In the Netherlands *B. allii* is generally found associated with the disease (VAN DOORN *et al.*, 1962).

Control of the disease by using fungicides and/or haulm killers was unsuccessful. Good control can be obtained by drying the bulbs artificially at an air temperature of 30 °C. This method is based on a more rapid drying of the neck tissue, thus preventing the fungus from entering the bulb. Artificial drying reduces losses considerably but in a proportion of the affected onions the fungus has already spread so far in the fleshy scales that artificial drying is of no use.

The results obtained with the various control methods indicate that infection tends to occur much earlier in the growing season than is generally assumed. In order to improve control of the disease it was important to investigate the stage of plant development at which infection takes place, the infection process, and the spread of the mycelium through the leaf into the bulb.

MATERIALS AND METHODS

In all inoculation experiments onion sets var. 'Rijnsburger' of about one month old, all with green leaves, were used.

B. allii was isolated from diseased onions and cultured on potato-dextrose-agar (PDA) in petri dishes. Radio-active conidia were obtained by growing the fungus on PDA to which the isotope ^{32}P was added at a rate of 100 μCu per

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15 ml nutrient agar. After transferring the mycelium to the PDA the dishes were incubated for 10 days at 23 °C.

Inoculations were carried out by putting a drop of spore suspension on the leaf, covering it with cotton wool fixed in position with adhesive tape, or by spraying the whole plant with the spore suspension. The first method was used for inoculations with labelled suspension. In all experiments undamaged, intact leaves were inoculated .The treated plants were kept in a saturated atmosphere in normal daylight and at a temperature of 20–25 °C.

To study the infection process, leaf samples were taken at intervals after inoculation. Autoradiographs were made from leaves inoculated with the labelled suspension. At the same time small disks were punched out of these leaves for microscopic examination. From leaves sprayed with the suspension, small parts (1 cm^2) were taken and cleared in a mixture of dioxan and propionic acid, 95:5 v/v, for 24 hours at 60 °C, after which they were washed in running tap water and stained with cotton blue-lactophenol. The staining method described by PREECE (1959) was also used. The same methods of staining were used for tracing mycelium in the neck tissue and in bulb scales.

Infection of the bulbs was also demonstrated by treating halved bulbs with 0.02% methyl-red in 95% ethanol.

RESULTS

The inoculated plants developed normally without any leaf spots. Microscopic examination of the leaves showed that the germ tubes of the conidia enter the leaf through the stomata. This is usually preceded by the formation of an appressorium and is actually effected by an infection hypha which penetrates the guard cells, the adjacent epidermal cells and the substomatal cavity. During the green leaf stage, the fungus may either remain quiescent in the epidermis or, if moisture conditions are favourable, may grow further over the leaf surface occasionally entering a stoma (Fig. 1).

Growth through the underlying mesophyll does not occur until the leaves become senescent. In infected leaves which have wilted and become yellowish, the fungus spreads rapidly intracellularly. Under high humidity conditions grey mouldy growth appears on the leaves, consisting mainly of conidiophores.

Autoradiographs of inoculated leaves, made one week after inoculation, showed mycelium growing from the point of inoculation to the leaf tip and in the direction of the bulb. Depending upon the site of inoculation on the leaf, the fungus could be traced in the neck tissue and in bulb scales 4 to 7 weeks after inoculation (Figs. 2, 3, 4, 5).

Once it was clear that *B. allii* was capable of attacking young onion plants, the next question was whether plants of different ages, in different stages of development, are equally susceptible. For this purpose, groups of plants of the same age were sprayed with a spore suspension at various times after planting. After the whole crop had ripened, the bulbs were examined for rot after four weeks storage. The results are shown in Table 1.

The results indicate that all plants were infected, no matter at what age the spores were applied. There is a slight tendency, however, for older plants to be somewhat more susceptible.

In diseased bulbs the affected scales take on an amber colour and become

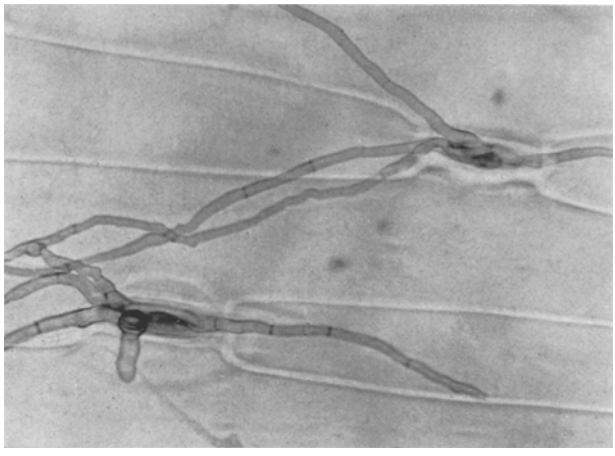

FIG. 1. Formation of an appressorium. Magnification × 300.
Vorming van een appressorium. Vergroting 300 ×.

FIG. 2. Autoradiograph of an inoculated leaf; mycelium growing from the site of inoculation (black circle) towards both sides.
Autoradiogram van geïnoculeerd blad; mycelium groeiend vanuit inoculatieplek (zwarte cirkel) naar beide kanten.

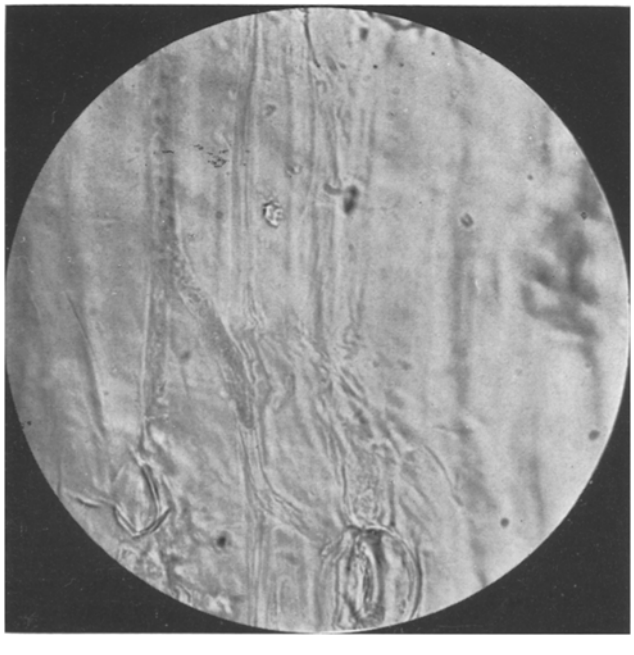

FIG. 3. Punched out area of the leaf of Fig. 2, showing mycelium. Magnification × 400.
Ponsstukje uit blad van fig. 2 met mycelium. Vergroting 400 ×.

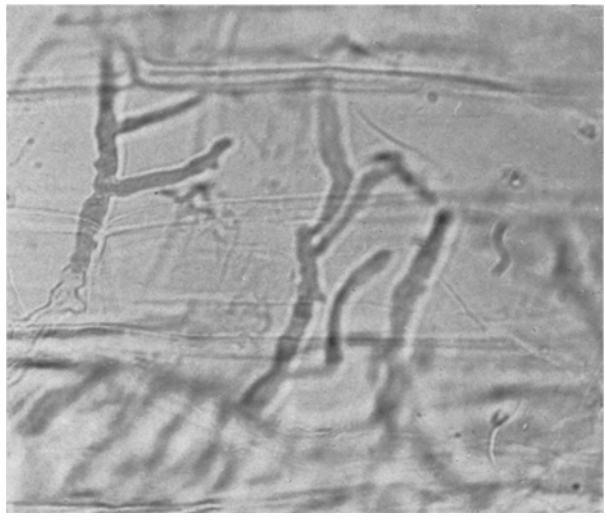

FIG. 4. Mycelium of *B. allii* in bulb 6–7 weeks after inoculation of the leaf. Magnification × 300.

Mycelium van B. allii *in bol 6–7 weken na inoculatie van het blad. Vergroting 300 ×.*

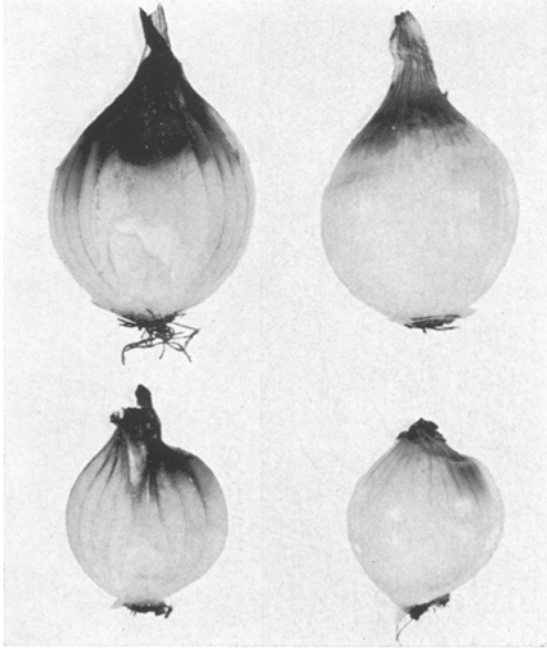

FIG. 5. Reddening (black on the photograph) of the diseased tissue after treatment with methyl-red; note the sharp demarcation line between the diseased and healthy tissue on the two onions on the right.

Roodkleuring (op de foto zwart) van het zieke weefsel na behandeling met methylrood; op de twee uien rechts is de scheidingslijn tussen ziek en gezond weefsel zichtbaar.

TABLE 1. Neck rot development in bulbs of plants inoculated with *B. allii* at different times after planting.

Het optreden van koprot in bollen van planten die op verschillende tijdstippen na het planten met B. allii *zijn geïnoculeerd.*

Number of inoculated plants	Age at inoculation in days	Bulbs with neck rot	
		Number	%
23	17	17	74
24	31	19	79
23	45	20	87
16	67	14	88
18	76	16	89

depressed with a fairly sharp line of demarcation between the diseased and healthy tissue. When an infected onion is cut into halves and methyl-red is applied, the affected tissue turns crimson, because of the acid reaction (pH 4.2). The healthy part of the scale takes on a yellow-orange colour (pH 6.6). Mycelium could always be traced in the crimson part but was never observed in the healthy tissues. There exists a mycelium-free zone in the crimson tissue of about 10 mm, measured from the demarcation line. This zone is obviously the result of enzymatic activity by *B. allii*, showing that it kills in advance of hyphal penetration.

DISCUSSION

The inoculation experiments proved that *B. allii* is capable of infecting young onion plants. Though symptoms of the disease only become visible during storage, infection actually takes place in the field. The same phenomenon occurs with *Botrytis cinerea* infection of strawberries (POWELSON, 1960), raspberries (JARVIS, 1962) and tomatoes (WILSON, 1962). The lack of symptoms may be because the mycelium remains restricted to the colourless epidermal cells in the initial stages of infection. In all experiments *B. allii* failed to cause leaf spots. This is in contradiction to the results of other workers (HANCOCK, 1963; SEGALL & NEWHALL, 1960).

Since a young onion crop is liable to attack, control measures should be started early in the growing season. Fungicidal sprays applied at regular intervals will protect the crop until it ripens and the leaves fall. From then on, complete fungicidal coverage of the leaves is not possible and they are thus exposed to infection. However, there is a chance that regular sprays will keep the inoculum potential in the field low.

According to WALKER (1926) the fungus overwinters as sclerotia, sometimes as mycelium or conidia, in the soil or on decayed bulbs. This is in agreement with the experience of the author who, in February 1967, isolated *B. allii* from organic particles in soil which had been cropped with onions the previous summer. Observations showed that the fungus is not restricted to the onion plant but that it can live saprophytically on decaying plant materials (cereals, flax, lucerne, bean, pea).

Other important sources of infection are the large piles of diseased onions which are left lying around storehouses. The fungus sporulates profusely in the upper layer of these piles during spring until June. The spores are dispersed by

the wind and thus are able to colonize plant debris, giving rise to new sources of inoculum. For sanitary reasons these piles should be cleared.

Inoculum is present throughout the growing season. Early infection of the young crop always leads to neck rot of the bulbs while the consequences of a late infection of the ripening crop depend on weather conditions during and after lifting time.

During the initial stage of infection the fungus can establish itself in the bulb without there being any evidence of rot. At this stage the presence of mycelium can only be demonstrated macroscopically by the use of methyl-red. With this staining method it is even possible to predict the losses to be expected after storage. This method is, however, of no practical value since, for a reliable estimation of the losses, many onions have to be cut and, where the incidence of the disease is low, losses due to sampling would exceed the losses due to *Botrytis* infection.

SAMENVATTING

Botrytis allii is in staat om onder voor infectie gunstige omstandigheden jonge uieplanten aan te tasten. Geïnfecteerde jonge bladeren vertonen geen symptomen omdat het mycelium zich uitsluitend tot de chlorofylloze epidermiscellen beperkt. Pas bij het ouder worden van de bladeren dringt de schimmel het parenchymatisch weefsel binnen waar het verder kan uitgroeien tot in de bol. Uitwendig gezond lijkende bollen kunnen geïnfecteerd zijn. Met methylrood kan een met het oog niet waarneembare besmetting worden aangetoond. Jonge en oudere planten zijn in gelijke mate vatbaar voor de ziekte.

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