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Contribution to the taxonomy and pathogenicity of fungicolous Verticillium species. II. Pathogenicity

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Abstract

In recent years in the Netherlands a second mushroom species, *Agaricus bitorquis*, which prefers higher temperatures than *A. bisporus* and is less susceptible to certain diseases, is often commercially grown. *Verticillium fungicola* var. *fungicola*, the causal agent of dry bubble, is responsible for considerable damage in crops of *A. bisporus*. In *A. bitorquis*, however, dry bubble has hardly been noticed, but brown spots due to *V. fungicola* var. *aleophilum* resulted in inferior mushroom quality. The latter variety also caused brown spots in *A. bisporus*, but to a minor degree. In variety Les Miz 60 of *A. bisporus*, however, it also induced fruit-body deformation in a way different from dry bubble.

Verticillium psalliotae, isolated from *A. bitorquis* in England, induced more confluent brown spots in *A. bitorquis*. In the Netherlands, where more *A. bitorquis* is grown than in other countries, *V. psalliotae* has not yet been encountered in crops of *A. bitorquis*. *V. psalliotae*, which has a high temperature optimum for mycelial growth, like *V. fungicola* var. *aleophilum* and *A. bitorquis*, did not infect *A. bisporus* in our trials.

Artificial infection of *A. bisporus* or *A. bitorquis* could not be accomplished with the following related and/or fungicolous fungi: *Verticillium lamellicola*, *V. fungicola* var. *flavidum*, *V. biguttatum*, *Nectriopsis tubariicola*, *Acremonium crotocinigenum* and *Aphanocladium album*.

Additional keywords: mushroom diseases, dry bubble, mycoparasites, brown spots, *Agaricus bisporus*, *Agaricus bitorquis*, *Verticillium fungicola*, *V. psalliotae*, *V. biguttatum*, *V. lamellicola*, *Nectriopsis tubariicola*, *Acremonium crotocinigenum*, *Aphanocladium album*.

Introduction

Dry bubble in *Agaricus bisporus* (Lange) Imbach, one of the most important mushroom diseases, is caused by *Verticillium fungicola* (Preuss) Hassebr. var. *fungicola* (syn. *V. malthousei* Ware; for nomenclature see Gams, 1971, and Gams and Van Zaayen, 1982; for description of symptoms see Ware, 1933). Since 1974, *Agaricus bitorquis* (Quél.) Sacc. (syn. *Psalliota edulis* Vitt.) is grown commercially in the Netherlands. Varieties of this species are immune to mushroom virus disease (Van Zaayen, 1976) and are suitable for cultivation in summer, requiring higher growing temperatures than *A. bisporus*.

A. bitorquis was reported by Poppe (1972) to be more tolerant to *'Verticillium'* than *A. bisporus*. By *'Verticillium'* the author probably meant *Verticillium fungicola*. Over

the years, dry bubble was hardly noticed, if at all, in commercial crops of *A. bitorquis* and inoculation with *V. fungicola* did not or hardly lead to infection; this was explained by the higher growing temperature (Dieleman-van Zaayen, 1975).

In November 1979, brown-spotted mushrooms were observed in some commercial crops of *A. bitorquis* in Helden, Netherlands. The yield was not reduced, but affected mushrooms were inferior in quality. The causal agent was isolated and appeared to be a *Verticillium* species (Van Zaayen, 1981). It could be distinguished from *V. fungicola* var. *fungicola,* the cause of dry bubble, mainly by its higher temperature optimum (24 – 27 °C) for mycelial growth. It was named *Verticillium fungicola* var. *aleophilum* W. Gams & Van Zaayen, type strain CBS 357.80 (Gams and Van Zaayen, 1982). Trials on its pathogenicity to *A. bitorquis* and *A. bisporus* are described in the present study. The pathogenicity of some other fungicolous *Verticillium* species recently described by Gams and Van Zaayen (1982) was also tested. A number of *Verticillium* species and other phialidic hyphomycetes, isolated in the course of some years from cultivated mushrooms showing various symptoms, were also included in this study.

Materials and methods

Isolation and mycelial growth. The isolates of the various fungi were either obtained from diseased fruit-bodies of *A. bitorquis* or *A. bisporus,* or were provided by the Centraalbureau voor Schimmelcultures (CBS), Baarn. *Verticillium psalliotae* (CBS 194.79) and *V. fungicola* var. *fungicola,* both isolated from *A. bitorquis* (Upstone and Carter, 1979), were kindly supplied by Mrs Margaret Carter of the Agricultural Development and Advisory Service in Bristol, England.

Mycelium was usually grown on mushroom tissue agar (MTA; Schisler et al., 1968) at 25 °C, except for *Verticillium fungicola* var. *flavidum,* which was grown on malt agar (Oxoid CM 59) at 20 °C.

To suppress bacterial growth, oxytetracyclin (commercially available as Vendarcin) was added to both media at a concentration of 50 mg l^{-1}.

Trials in mushroom growing-rooms. Mushrooms of *A. bisporus* and *A. bitorquis* were grown as described by Bollen and Van Zaayen (1975), and Van Zaayen and Van der Pol-Luiten (1977), respectively. The growing temperatures were 25 °C during mycelial growth and 16 – 18 °C during harvesting for *A. bisporus,* and 30 °C during mycelial growth and 25 °C during harvesting for *A. bitorquis.* In the trials, different varieties of both *Agaricus* species were used.

Trays with a growing area of 0.27 m^2 were inoculated with the fungi to be tested by atomizing a conidial suspension over the trays (usually *c.* 1.5×10^7 conidia per m^2). This was done nine to ten days after casing, unless otherwise stated. Conidia were obtained from cultures preferably grown on MTA at 25 °C (in case of *V. fungicola* var. *flavidum* on malt agar at 20 °C) for two to three weeks. During inoculation and for about 1 h afterwards, uninoculated (control) trays were covered with plastic sheets and ventilation and circulation in the growing-room were switched off to prevent contamination.

Mushrooms appeared 19 to 21 days after casing. For the next four to five weeks they were harvested every two to three days and weighed; the yields were adjusted for 100 kg compost per m^2. Diseased mushrooms were not weighed but counted. Trials 1

Fig. 1. *Agaricus bitorquis* inoculated with *Verticillium fungicola* var. *aleophilum:* brown spots (A) and ruptured cap (B).

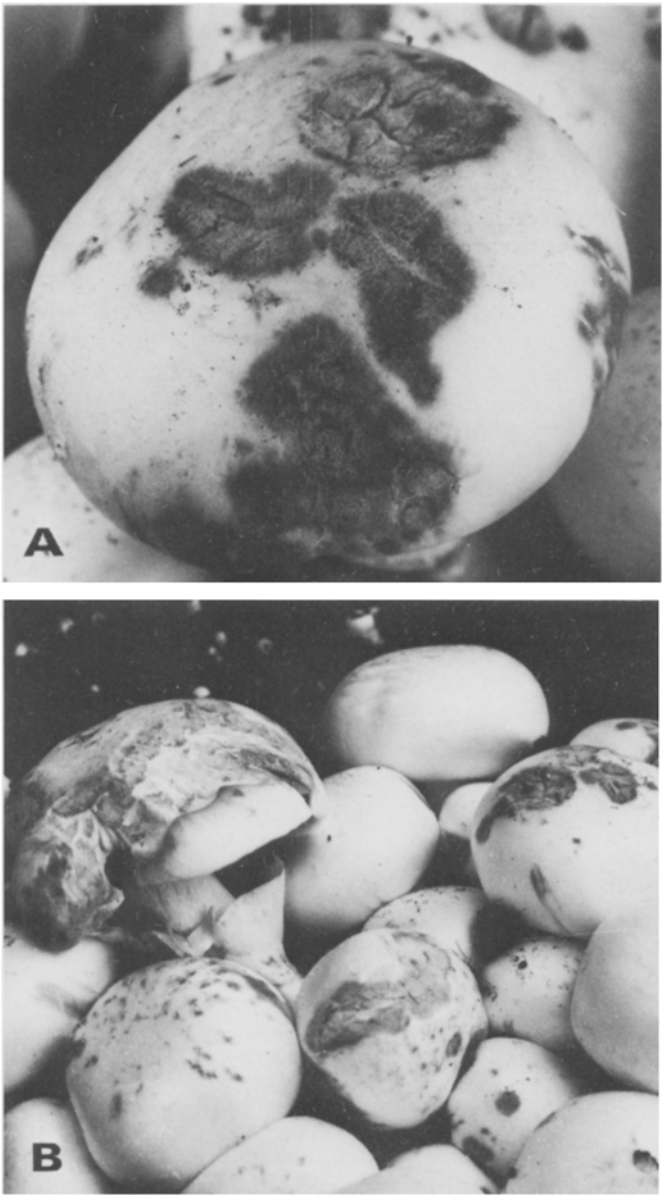

Fig. 1. Bruine vlekken (A) en gebarsten hoed (B) in A. bitorquis na inoculatie met V. fungicola var. aleophilum.

and 4 and supplementary trials were in five replicates, and 2 and 3 were in nine replicates. All trials were in isolated growing-rooms for disease experiments.

To prevent other growing-rooms of the Experimental Station from becoming contaminated, precautions were observed as described by Dieleman-van Zaayen (1972).

Results

1. Verticillium fungicola var. aleophilum

a. Pathogenicity to A. bitorquis. Inoculation in trial 1 *(A. bitorquis)* with 1.5×10^6 or 1.5×10^7 conidia per m^2 resulted in spotted mushrooms (Fig. 1) and consequently yield loss (Table 1). Inoculation with 1.5×10^7 conidia per m^2 caused more severe infection than with the lower concentration. Variety Horst K32 was more sensitive to infection than Horst K26 (Table 1). From the induced brown spots, *V. fungicola* var. *aleophilum* was reisolated. Inoculation of *A. bitorquis* consistently led to severe infection in trials 2 and 3 and resulted in decreased yield by the presence of many inferior, spotted mushrooms (Table 2). CBS 300.70A, isolated before 1970 from a subtropical soil near Brisbane (Gams, 1971) and showing a temperature maximum above 30 °C (Gams and Van Zaayen, 1982), appeared to be similar to isolate 357.80 of *V. fungicola* var. *aleophilum* with regard to its pathogenicity to *A. bitorquis* in trial 3.

Table 1. Average yields of two varieties of *A. bitorquis* and incidence of brown spots after inoculation with *Verticillium fungicola* var. *aleophilum,* CBS 357.80 (trial 1).

Mushroom variety	Inoculum (conidia m^{-2})	Mushroom yield[1] (kg m^{-2})	Spotted mushrooms (average number/tray)
Horst K26	0 (control)	19.3	0
	1.5×10^6	15.7	32
	1.5×10^7	13.5	83
Horst K32	0 (control)	18.2	0
	1.5×10^6	15.0	55
	1.5×10^7	8.5	124

[1] After 5 weeks of harvesting

Tabel 1. Gemiddelde opbrengsten van twee rassen van A. bitorquis en optreden van bruine vlekken na inoculatie met Verticillium fungicola var. aleophilum, CBS 357.80.

Description of symptoms in A. bitorquis. Brown, often sunken spots were present on caps and sometimes on stipes of mushrooms from the first flush onward on inoculated trays. In the first flush, many spots were circular and about 1.5 cm in diameter on mushrooms with a cap diameter of about 5 cm. Later in the crop, brown spots scattered over the caps, sometimes discolouring the entire caps; deeper sunken large spots even caused rupture of the cap (Fig. 1B). Very often, the brown spots were covered with a greyish mycelial film, especially in the centre. All spots, even sunken ones, only affected the outermost layer of the cap (or stipe).

Table 2. Average yields of *A. bitorquis* and *A. bisporus* and symptoms after inoculation with various varieties of *V. fungicola* and some other species (trials 2–4).

Fungal isolate		*A. bitorquis*, variety Horst K32				*A. bisporus* – trial 4			
		trial 2		trial 3		variety Le Lion B92		variety Les Miz 60	
CBS-number	name	yield[1]	symptoms[2]	yield	symptoms	yield	symptoms	yield	symptoms
—	(control)	13.0	–	11.3	–	16.0	–	16.6	–
CBS 648.80	*V. fungicola* var. *fungicola*	n.t.[3]	–	n.t.	–	0.1[4]	ooo	2.9[4]	ooo
CBS 357.80	*V. fungicola* var. *aleophilum*	4.9	+++	7.8	++	6.2	+++	6.9	o/+++
CBS 300.70A	*V. fungicola* var. *aleophilum*	n.t.	–	9.5	++	n.t.	–	n.t.	–
CBS 290.80	*V. fungicola* var. *flavidum*	n.t.	–	12.2	–	17.4	–	18.7	–
CBS 342.80		13.6	–	n.t.	–	16.5	–	18.1	–
CBS 879.73		n.t.	–	n.t.	–	17.7	–	17.7	–
CBS 116.79	*Nectriopsis tubariicola*	12.8	–	n.t.	–	14.4	–	15.1	–
CBS 360.77	*V. biguttatum*	13.7	–	n.t.	–	n.t.	–	n.t.	–
CBS 525.80	*Acremonium crotocinigenum*	13.3	–	n.t.	–	n.t.	–	n.t.	–

[1] Yields in kg m^{-2} after 5 weeks of harvesting in trials 2 and 3, and 4 weeks in trial 4.
[2] Symptoms: assessed as average number of infected mushrooms per tray: + = spots, o = bubble. +/o = 1–10 infected mushrooms per tray, ++ = 10–100 infected mushrooms per tray, +++/ooo = more than 100 infected mushrooms per tray.
[3] n.t. = not tested
[4] Severely infected trays were removed after one week of harvesting.

Tabel 2. Gemiddelde opbrengsten van A. bitorquis en A. bisporus en optreden van symptomen na inoculatie met verschillende variëteiten van V. fungicola en enkele andere soorten.

b. Pathogenicity to A. bisporus. Inoculation in trial 4 *(A. bisporus)* caused infection (Table 2), spotted mushrooms and consequently loss of yield by inferior quality. Infection by CBS 357.80 was also obtained in two other trials with *A. bisporus.* In a supplementary trial CBS 519.81, a *V. fungicola* var. *aleophilum* isolated from brown spots in *Agaricus bisporus,* caused brown spots and consequently a yield loss of *c.* 32% in two varieties of *A. bisporus* after inoculation.

In the three trials with *A. bisporus, V. fungicola* var. *aleophilum* (CBS 357.80) caused average yield losses of 50% or less. The damage caused by this fungus in crops of *A. bitorquis* was usually greater. The damage caused in varieties of *A. bisporus* by *V. fungicola* var. *fungicola* was much greater than by var. *aleophilum* (Table 2).

Description of symptoms in A. bisporus. The most common symptoms were irregular, greyish to brown spots that varied in size and often speckled the mushroom cap (Fig. 2A). The spots had fringed margins and were very similar to those caused by

Fig. 2. *A. bisporus,* inoculated with *V. fungicola.*
A. Brown spots in variety Le Lion H_1 inoculated with *V. fungicola* var. *aleophilum.*
B. and C. Variety Les Miz 60 inoculated with *V. fungicola* var. *aleophilum.* B: lumps with remnants of small caps in the first flush. C: deformed mushrooms in the second flush.
D. Characteristic symptoms of dry bubble in a white variety, inoculated with *V. fungicola* var. *fungicola.*

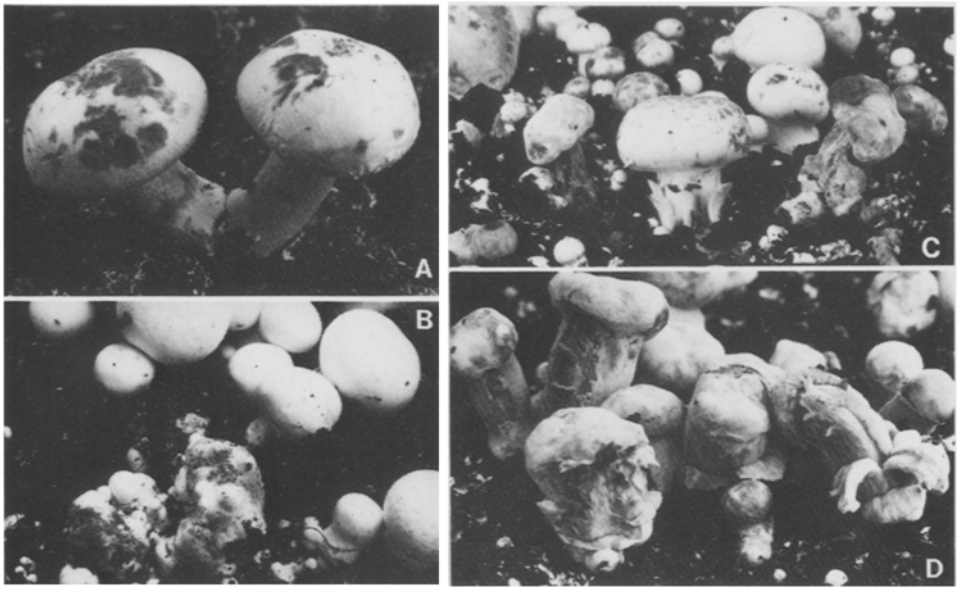

Fig. 2. A. bisporus, geïnoculeerd met V. fungicola.
A. Bruine vlekken op het ras Le Lion H_1, na inoculatie met V. fungicola var. aleophilum.
B. en C. Ras Les Miz 60 geïnoculeerd met V. fungicola var. aleophilum. B: molletjes met resten van kleine hoedjes in de eerste vlucht. C: misvormde champignons in de tweede vlucht.
D. Kenmerkende symptomen van droge mollen in een wit ras, na inoculatie met V. fungicola var. fungicola.

Verticillium fungicola var. *fungicola* on *A. bisporus* (in the early stage of an affected crop, preceding the symptoms of dry bubble).

In trial 4 (Table 2), but also in two later supplementary trials, variety Les Miz 60, which is rather tolerant to *V. fungicola* var. *fungicola,* not only showed spots after inoculation with *V. fungicola* var. *aleophilum,* but also some deformed mushrooms. The mis-shapen fruit-bodies resembled the 'dry bubble' symptom, but differed in the following characteristics: small amorphous masses of fungal tissue appearing mainly in the first flush, 1 – 6 cm in diameter, often bearing remnants of small caps, *c.* 0.5 cm in diameter (Fig. 2B). The lumps somewhat resembled the onion-shaped young mushrooms associated with dry bubble. These small lumps turned from white via greyish (i.e. covered with a grey mycelial film) to brownish. Inside, the lumps were hollow, spongy, brown and rotting. Sometimes they had a flat, truncated top with rifts. Later in the crop, in the second flush, some very deformed mushrooms occurred (Fig. 2C), *c.* 5 – 6 cm high. They differed conspicuously from mushrooms with 'dry bubble' (Fig. 2D), which can be much bigger. Such mis-shapen fruit-bodies showed a swollen base of the stipe, a bent stipe and a deformed cap. Cap and sometimes even stipe were covered with a grey film of mycelium. Deformed mushrooms were discoloured brown due to a secondary bacterial rot, which is never observed in the case of 'dry bubble'. Larger mushrooms might look quite normal, apart from torn off strips of the stipe (Fig. 2C), one of the symptoms characteristic of 'dry bubble' (Fig. 2D).

From mushrooms with the above described symptoms *V. fungicola* var. *aleophilum* was reisolated.

2. Verticillium fungicola var. fungicola

Trays with *A. bitorquis* (variety Horst K26) were inoculated with the isolate from *A. bitorquis* in England (Upstone and Carter, 1979) at a concentration of 1.5×10^6 conidia per m^2, nine days after casing. Infection did not occur. The conidial load may have been too low, but 1.5×10^6 conidia per m^2 of *V. fungicola* var. *fungicola,* applied nine days after casing, always results in a severe infection of *A. bisporus.*

3. Verticillium psalliotae Treschow

An isolate from *A. bitorquis* with brown spots (Upstone and Carter, 1979) was inoculated onto four varieties of *A. bisporus* nine days after casing and again in the third flush (6×10^6 to 1×10^8 conidia per m^2) without success. Inoculation onto variety Horst K26 of *A. bitorquis* only succeeded, after three unsuccessful attempts in between the flushes of a trial, when 2×10^8 conidia per m^2 were applied in the fourth flush. The earlier inoculations had been done with less conidia per m^2. The symptoms already appeared two days after inoculation and consisted of brown, irregular and often confluent spots over the entire cap (Fig. 3). The symptoms corresponded with the detailed description given by Upstone and Carter (1979). *V. psalliotae* was reisolated from the brown spots in this trial.

4. Verticillium lamellicola (F.E.V. Smith) W. Gams

An isolate (CBS 150.82) was made in February 1979 from mushrooms in a commer-

Fig. 3. Results of excessive inoculation of *A. bitorquis* with *V. psalliotae.*

Fig. 3. A. bitorquis, overdadig geïnoculeerd met V. psalliotae.

cial crop of *A. bitorquis* in Beesel; the caps were covered with small brown specks and pits and consequently were inferior in quality.

No infection was obtained by repeated inoculations with 1.5×10^6 to 1.5×10^8 conidia per m^2 at various times after casing in two trials in ten replicates with three varieties of *A. bitorquis*. Inoculation with mycelium and conidia of this fungus immediately after casing in a third trial did not lead to infection. Creation of conditions that are supposed to favour the growth of *V. lamellicola*, such as temporary increase of relative air humidity and temperature, was not successful.

5. Aphanocladium album (Preuss) W. Gams

Two isolates of this species were used in inoculation trials: (a) An isolate from compost and casing soil on which a crop of variety Somycel 2,017 of *A. bitorquis* gave a decreased yield, without conspicuous symptoms on the fruit-bodies. Inoculation of the same variety of *A. bitorquis* with 1.5×10^6 conidia per m^2 at spawning or at casing had no effect; (b) An isolate from variety Le Lion H_1 of *A. bisporus* with dark brown, circular spots of unknown origin. Inoculation of three *A. bisporus* varieties with mycelium or conidia (7.5×10^6 to 1×10^8 per m^2) at various times did not lead to symptoms or yield decrease.

6. Other species tested for pathogenicity

Neither visible symptoms nor yield decreases were caused in crops of *A. bitorquis* or

A. bisporus after inoculation with the following fungi (Table 2): *Verticillium fungicola* var. *flavidum* W. Gams & Van Zaayen, CBS 290.80, CBS 342.80 and CBS 879.73; *Nectriopsis tubariicola* W. Gams, CBS 116.79; *Verticillium biguttatum* W. Gams, CBS 360.77; and *Acremonium crotocinigenum* (Schol-Schwarz) W. Gams, CBS 525.80.

Discussion

Although some of the tested fungi had been isolated from (decaying) mushrooms or toadstools, only *V. fungicola* var. *aleophilum* and *V. psalliotae* were pathogenic in our trials to *Agaricus* species, and in particular to *A. bitorquis.*

V. fungicola var. *aleophilum* is a newly discovered pathogen in mushroom cultivation. The large-scale cultivation of a new mushroom species at higher temperatures may imply the appearance of new diseases. *V. psalliotae* has not yet been isolated from commercial crops of *A. bitorquis* in the Netherlands; the occurrence of *V. fungicola* var. *aleophilum,* however, is increasing. This variety may have occurred, although unnoticed, in crops of *A. bisporus* for many years. The spots caused in *A. bisporus* by this fungus resemble those caused by *V. fungicola* var. *fungicola* at the onset of an infection. CBS 300.70A was isolated earlier than 1970 (Gams, 1971).

Both *V. fungicola* var. *aleophilum* and *V. psalliotae* show a high temperature maximum (33 °C) of mycelial growth on agar (Gams and Van Zaayen, 1982) and thermal death points of conidia of 42 °C (Van Zaayen and Rutjens, 1981). *V. fungicola* var. *fungicola,* a pathogen of *A. bisporus,* has a lower temperature maximum (27 °C) and also a lower thermal death point of conidia: 38 – 39 °C.

This may explain why *V. fungicola* var. *fungicola* did not cause any infection in *A. bitorquis* in our trials, although such an infection has been reported by Poppe (1972) and by Upstone and Carter (1979). Whereas *A. bitorquis* was grown in our trials at the proper temperatures, Poppe (1972) reported a temperature of 22 – 26 °C after casing, and of 22 – 24 °C during cropping, which is not optimal for *A. bitorquis.* This may have facilitated infection by var. *fungicola.* Similarly, Upstone and Carter (1979) mentioned temperatures in the range of 24 – 27 °C during growing.

V. fungicola var. *aleophilum* can also infect *A. bisporus;* presumably the damage would increase if *A. bisporus* was grown at higher – detrimental – temperatures.

Varieties of *A. bisporus* showed remarkably varying symptoms after inoculation with *V. fungicola* var. *aleophilum.* The deformed mushrooms in variety Les Miz 60 resembled symptoms of dry bubble but differed by showing smaller lumps and smaller deformed and brown discoloured mushrooms. Considerable differences, both in susceptibility to infection and sensitivity to fungicides have also been noticed in varieties of *A. bisporus* inoculated with *V. fungicola* var. *fungicola* (Gandy and Spencer, 1978).

Benomyl and other MBC-fungicides do not control *V. fungicola* var. *fungicola* since this variety is often tolerant to such fungicides (Bollen and Van Zaayen, 1975). Benomyl does not control *V. fungicola* var. *aleophilum* either (Van Zaayen, 1981; see also Gams and Van Zaayen, 1982). Chlorothalonil (at 2.2 g a.i. m^{-2}) controls the latter fungus satisfactorily (Van Zaayen, 1981), as it does *V. fungicola* var. *fungicola.*

Varieties of *A. bisporus* have been reported to react differently upon inoculation with *V. psalliotae.* Infection only succeeded in brown mushroom varieties and at a

cropping temperature above 20 °C (Treschow, 1941). This may account for our failure to infect several white varieties of *A. bisporus* with *V. psalliotae,* and also for the fact that *V. psalliotae* has not been recorded in a national survey of mushroom diseases in England (Gaze and Fletcher, 1975), as nowadays white varieties are prevalent. Cropping temperatures of *A. bisporus* are moreover usually below 20 °C and environmental conditions are much better controlled than in the past.

Upstone and Carter (1979) obtained infection of *A. bitorquis* with 2×10^7 conidia per m^2 of *V. psalliotae,* applied at casing (M.E. Upstone, pers. comm., 1979). In our trials infection only succeeded in a fourth attempt with 2×10^8 conidia per m^2. Consequently we assume that this isolate has a very low pathogenicity. The comparison of different isolates of this fungus would be required for a more decisive judgement of its pathogenicity; it is, obviously, of little importance in mushroom cultivation, but occurs very commonly on many kinds of other substrates (Gams, 1971).

Verticillium lamellicola was reported to cause 'gill mildew' when cropping temperatures were high (23 – 25 °C; Smith, 1924), or dark brown spots (Atkins, 1966, p. 130), but we have not been able to establish infection in *A. bitorquis.* Perhaps sub-optimal growing conditions of *A. bitorquis* (lower temperatures) might favour infection; *V. lamellicola* has a temperature maximum of 27 °C (Gams and Van Zaayen, 1982). The symptoms of gill mildew may develop in large, open mushrooms only. In our trials, however, mushrooms were picked before they opened.

Similarly, no infection was obtained in repeated trials with *Aphanocladium album.* This fungus has been reported to infect mushroom fruit-bodies and to reduce mushroom yield in Australia; a greyish white fluffy growth was observed on the cap and sometimes on the gills of large open mushrooms. The pathogen, however, was also observed on sporophores already infected with *V. fungicola* (Nair et al., 1980). The warm climate and the practice of growing large open mushrooms in Australia may explain the discrepancy in results with this fungus.

Earlier reports mentioned the association of symptoms in *A. bisporus* with *Sporotrichum roseolum* (Bels-Koning and Bels, 1958: pp. 175 and 192) and *Verticillium nanum* (CBS 165.45), which were both considered to be *A. album* (Gams, 1971), and with *Cephalosporium* sp. (Storey, 1974), which was also identified as *A. album* by W. Gams in 1975. However, trials on the pathogenicity to *A. bisporus* of all of these fungi have not been performed. In addition, *A. album* is commonly found as a parasite of *Myxomycetes,* rusts and powdery mildews (Gams, 1971: p. 226; Biali et al., 1972; and unpublished observations by Gams).

None of the other fungicolous fungi tested showed pathogenicity to *Agaricus* species under the usual conditions; they had, however, never been isolated from such species, except the recent isolate of *Acremonium crotocinigenum* (CBS 525.80). Loss of pathogenicity, inoculation with too few conidia or unfavourable temperatures could have influenced the behaviour of these fungi, but it is more likely that they do not attack young fruit-bodies of *Agaricus* species. No loss of pathogenicity could have occurred with the freshly isolated *A. crotocinigenum,* and in infection experiments with dry bubble we still use *Verticillium fungicola* var. *fungicola,* CBS 733.74, which was isolated in 1974 (Bollen and Van Zaayen, 1975). *V. fungicola* var. *aleophilum,* CBS 300.70A was isolated earlier than 1970 but was still infectious in our trials.

Acknowledgements

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Samenvatting

Een bijdrage aan de taxonomie en pathogeniteit van met schimmels geassocieerde Verticillium-soorten. II. Pathogeniteit

Vooral in Nederland wordt sinds een aantal jaren naast *Agaricus bisporus* ook de warmteminnende champignonsoort *Agaricus bitorquis* geteeld, die minder vatbaar is voor bepaalde ziekten. Terwijl *Verticillium fungicola* var. *fungicola* in de teelt van *A. bisporus* 'droge mollen' en daardoor veel schade veroorzaakt, komen in de teelt van *A. bitorquis* geen droge mollen voor maar wel bruine vlekken, die tot kwaliteitsverlies en dus schade leiden. De vlekken bleken veroorzaakt te worden door *V. fungicola* var. *aleophilum.* Deze schimmel veroorzaakte ook in *A. bisporus* bruine vlekken, hoewel in minder ernstige mate, maar in het ras Les Miz 60 van *A. bisporus* bovendien misvormde champignons, die wel op 'droge mollen' leken, maar daaraan niet gelijk waren.

Ook *V. psalliotae,* in Engeland geïsoleerd van *A. bitorquis* met vlekken, veroorzaakte wat meer samenvloeiende, bruine vlekken in *A. bitorquis.* In Nederland, waar meer *A. bitorquis* geteeld wordt dan in andere landen, is *V. psalliotae* nog niet aangetroffen in teelten van *A. bitorquis.* In *A. bisporus* kon geen kunstmatige infectie worden verkregen met *V. psalliotae,* die net als *V. fungicola* var. *aleophilum* en *A. bitorquis* warmteminnend genoemd zou kunnen worden.

Met de volgende *Verticillium*-achtige of van paddestoelen geïsoleerde schimmels kon evenmin op kunstmatige wijze een infectie worden opgeroepen in *A. bisporus* of *A. bitorquis: Verticillium lamellicola, V. fungicola* var. *flavidum, V. biguttatum, Nectriopsis tubariicola, Acremonium crotocinigenum* en *Aphanocladium album.*

References

Atkins, F.C., 1966. Mushroom growing to-day. Faber and Faber Ltd., London, 188 pp.

Bels-Koning, H.C. & Bels, P.J., 1958. Handleiding voor de champignoncultuur. Proefstn Champ.cult., Horst (L.), 295 pp.

Biali, M., Dinoor, A., Eshed, N. & Kenneth, R., 1972. *Aphanocladium album,* a fungus inducing teliospore production in rusts. Ann. appl. Biol. 72: 37 – 42.

Bollen, G.J. & Zaayen, A. van, 1975. Resistance to benzimidazole fungicides in pathogenic strains of *Verticillium fungicola.* Neth. J. Pl. Path. 81: 157 – 167.

Dieleman-van Zaayen, A., 1972. Spread, prevention and control of mushroom virus disease. Mushr. Sci. 8: 131 – 154.

Dieleman-van Zaayen, A., 1975. *Agaricus bitorquis* en mollen. Jversl. Proefstn Champ.cult. 1974: 20.

Gams, W., 1971. *Cephalosporium*-artige Schimmelpilze (Hyphomycetes). G. Fischer, Stuttgart, 262 pp.

Gams, W. & Zaayen, A. van, 1982. Contribution to the taxonomy and pathogenicity of fungicolous *Verticillium* species. I. Taxonomy. Neth. J. Pl. Path. 88: 57 – 78.

Gandy, D.G. & Spencer, D.M., 1978. The interaction between mushroom strains and fungicides in the control of dry bubble caused by *Verticillium fungicola.* Ann. appl. Biol. 90: 355 – 360.

Gaze, R.H. & Fletcher, J.T., 1975. ADAS survey of mushroom diseases and fungicide usage 1974/5. Mushr. J. 35: 370 – 376.
Nair, N.G., Letham, D.B. & Walker, J., 1980. Incidence of *Aphanocladium album* on the cultivated mushroom, *Agaricus bisporus,* in New South Wales. Pl. Dis. Surv. Dep. Agric. Biol. Br. 1978-79: 20 – 22.
Poppe, J.A., 1972. Un excellent *Agaricus* tétra-sporique cultivable commercialement avec succès. Mushr. Sci. 8: 517 – 525.
Schisler, L.C., Sinden, J.W. & Sigel, E.M., 1968. Etiology of mummy disease of cultivated mushrooms. Phytopathology 58: 944 – 948.
Smith, F.E.V., 1924. Three diseases of cultivated mushrooms. Trans. Br. mycol. Soc. 10: 81 – 97.
Storey, I.F., 1974. *Cephalosporium* sp. attacking mushrooms. Mushr. J. 20: 1 – 3.
Treschow, C., 1941. The *Verticillium* diseases of cultivated mushrooms. Dansk bot. Ark. 11(1): 1 – 31.
Upstone, M.E. & Carter, M.A., 1979. The occurrence of *Verticillium psalliotae* on *Agaricus bitorquis* in Surrey. Mushr. J. 73: 38 – 40.
Ware, W.M., 1933. A disease of cultivated mushrooms caused by *Verticillium malthousei* sp. nov. Ann. Bot. 47: 763 – 785.
Zaayen, A. van, 1976. Immunity of strains of *Agaricus bitorquis* to mushroom virus disease. Neth. J. Pl. Path. 82: 121 – 131.
Zaayen, A. van, 1981. *Verticillium* sp., a pathogen of *Agaricus bitorquis.* Mushr. Sci. 11(1): 591 – 595.
Zaayen, A. van & Pol-Luiten, B. van der, 1977. Heat resistance, biology and prevention of *Diehliomyces microsporus* in crops of *Agaricus* species. Neth. J. Pl. Path. 83: 221 – 240.
Zaayen, A. van & Rutjens, A.J., 1981. Thermal death points for two *Agaricus* species and for the spores of some major pathogens. Mushr. Sci. 11(2): 393 – 402.

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