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The Influence of Temperature on the Antagonistic Effect of *Trichoderma viride* Fr. on *Fomes annosus* (Fr.) Cke.

Den antagonistiska effektens temperaturberoende hos Trichoderma viride Fr. gentemot Fomes annosus (Fr.) Cke.

by

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The Influence of Temperature on the Antagonistic Effect of *Trichoderma viride* Fr. on *Fomes annosus* (Fr.) Cke.

Fomes annosus, the widespread basidiomycete causing root rot of Norway spruce and Scots pine in Sweden, is an extensively studied fungus in single culture by a. o. ROBAK (1933), TRESCHOW (1938), ROLL-HANSEN (1940) and RENNERFELT (1946, 1952). BJÖRKMAN (1949) surveyed, as the first, the problem from microecological views and isolated from forest soils a couple of organisms antagonistic to *Fomes annosus*. RISHBETH (1951) studied the growth of *Fomes annosus* and *Trichoderma viride* and found that at high temperatures only, *Trichoderma viride* inhibited the growth of *Fomes annosus*. RENNERFELT and PARIS (1952—1953) confirmed this effect on malt agar culture at 12° and 22° C.

The antagonistic effect is however known already from WEINDLING's (1932) work on *Rhizoctonia solani* KÜHN, *Phytophthora parasitica* DASTUR and *Pythium spp.* Similar effects have later been reported by ALLEN and HAENSELER (1935) and JAARSVELD (1944) against *Rhizoctonia sp*, by DAINES (1937) against *Actinomyces scabies* (THAX). WAKSM. and HENR. and by BLISS (1951), AYTOUN (1952) and GARRETT (1958) against *Armillaria mellea* (VAHL EX FR.) QUÉL.

The toxic principle of *Trichoderma viride* reported by WEINDLING (1934) is composed of two distinctly different substances, gliotoxin active both as a bactericide and a fungicide, and viridin exclusively a fungicide. A single strain of *Trichoderma viride* produces either gliotoxin or viridin (BRIAN and HEMMING 1945).

Material and methods

Fomes annosus S 1 isolated from spruce, Bogesund, Sweden, and *Trichoderma viride* T 211 from ICI, England producing gliotoxin cultivated on 2 % slant malt extract agar.

Nutrient solution 1: Modified SHIVE completed with 1 % glucose and 0.5 % yeast extract (Difco).

KH_2PO_4	0.062 g/l	NH_4NO_3	0.029 g/l
$MgSO_4 7\,H_2O$	0.110	Trace elements	1 ml Stand. sol.
$CaCl_2 6H_2O$	0.197	Distilled water	1000 ml
$FeCl_3 6H_2O$	0.005		

Nutrient solution 2: WEINDLING

Glucose	25.00 g/l
Bacto-Peptone	2.00
$KH_2 PO_4$	2.00
$MgSO_4$	1.00
$FeCl_3$	0.01
Distilled water	1000 ml

The growth was determined on malt agar plates as mm radius daily and in nutrient solutions as dry weights of mycelia. As a rule the calculations were made from four replicates. The nutrient solutions were buffered with phosphate according to PAECH and SIMONIS (1952).

Experimental

Small pieces of *Trichoderma viride* were inoculated at one side of each four Petri dishes and at the opposite side pieces of *Fomes annosus*. The controls included only inoculates of one species arranged as above. The dishes were incubated at the following temperatures: 5°, 8°, 12°, 16°, 20°, 25° and 30° C. After twentyeight days of determining the radius this experiment was finished. Some of the characteristic trends at the chosen ranges of temperature are graphed in figure 1 a and b.

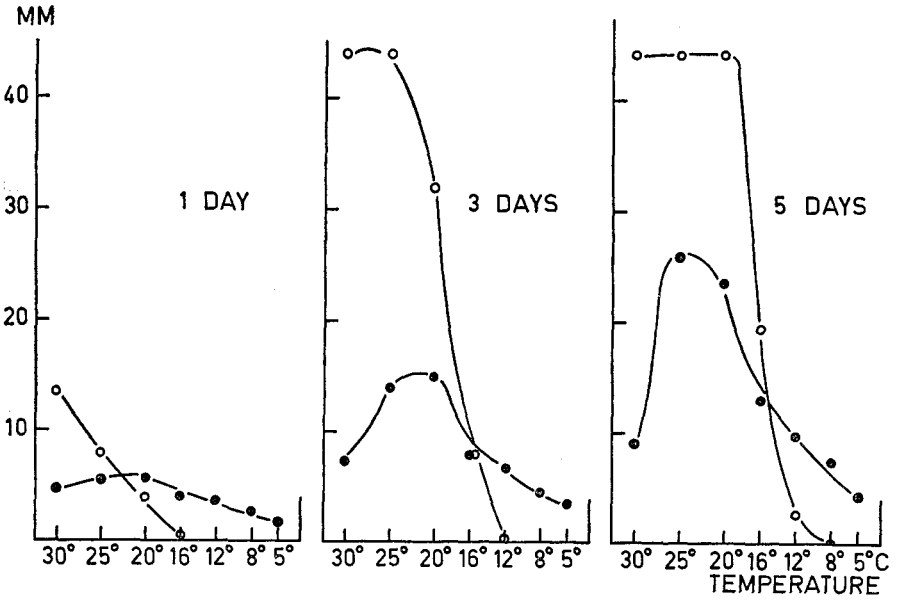

Fig. 1 a. Radius growth of *Fomes annosus* ● and *Trichoderma viride* ○ at different temperatures on malt agar dishes.

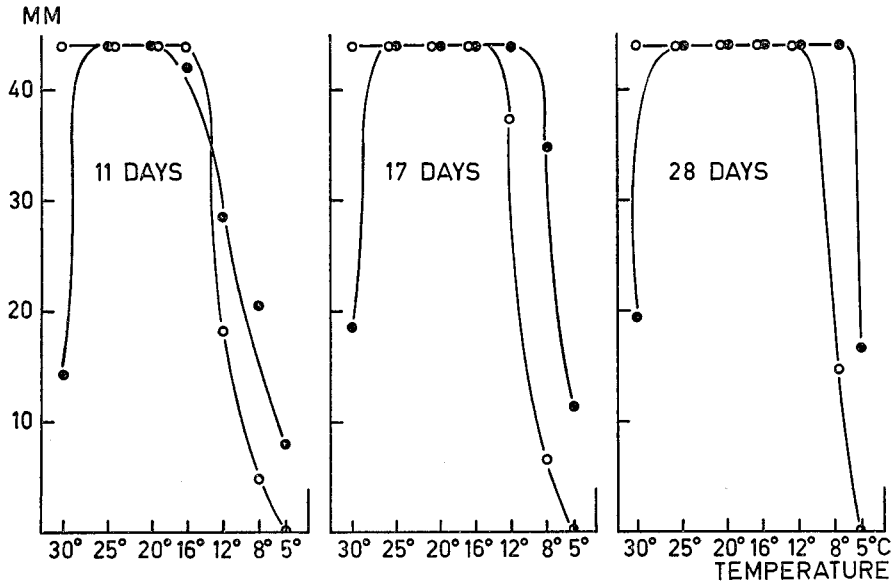

Fig. 1 b. Radius growth of *Fomes annosus* ● and *Trichoderma viride* ○ at different temperatures on malt agar dishes.

From these graphs the influence of the temperature on the growth rate is quite obvious concerning *Trichoderma viride* already after three days. *Fomes annosus* is growing slowly and reaches at the optimal temperature the same numerical values after eleven days as *Trichoderma viride* after three.

There are, however, still some other characteristic differences between the fungi investigated. *Trichoderma viride* has an optimal growth rate at 25°—30° C, but at 5° no growth at all occurs. At 30° *Fomes annosus* has reached its maximal range of growth, though at 5° there is still a continuing growth.

In figure 2 a and 2 b the growth at different temperatures is presented. After 11 days the sporulation of *Trichoderma viride* is quite evident at temperatures above 12° C.

Besides the results obtained on solid media the experiment was repeated in WEINDLING's nutrient solution at the same temperatures as used above. The mixed culture was, however, excluded here. Principally the results from growth on solid media and in nutrient solution agree at the temperatures chosen. The sparse growth of *Fomes annosus* at 30° on solid media is more pronounced in solution. *Trichoderma viride* is delayed at low temperatures also in nutrient solutions. See figure 3.

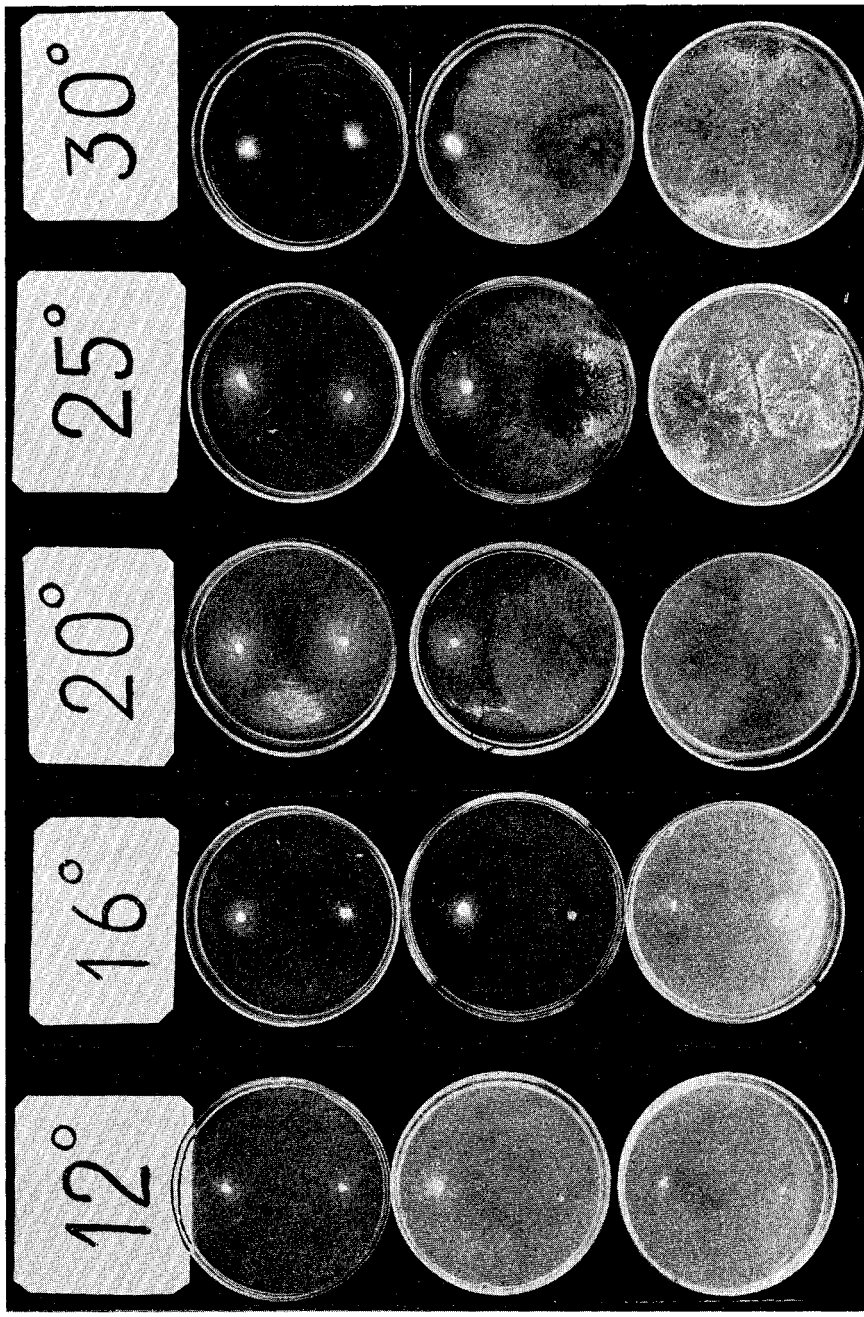

Fig. 2 a. Growth after 3 days at different temperatures.
Left row: *Fomes annosus*
Middle row: *Fomes annosus* (left) and *Trichoderma viride*
Right row: *Trichoderma viride*

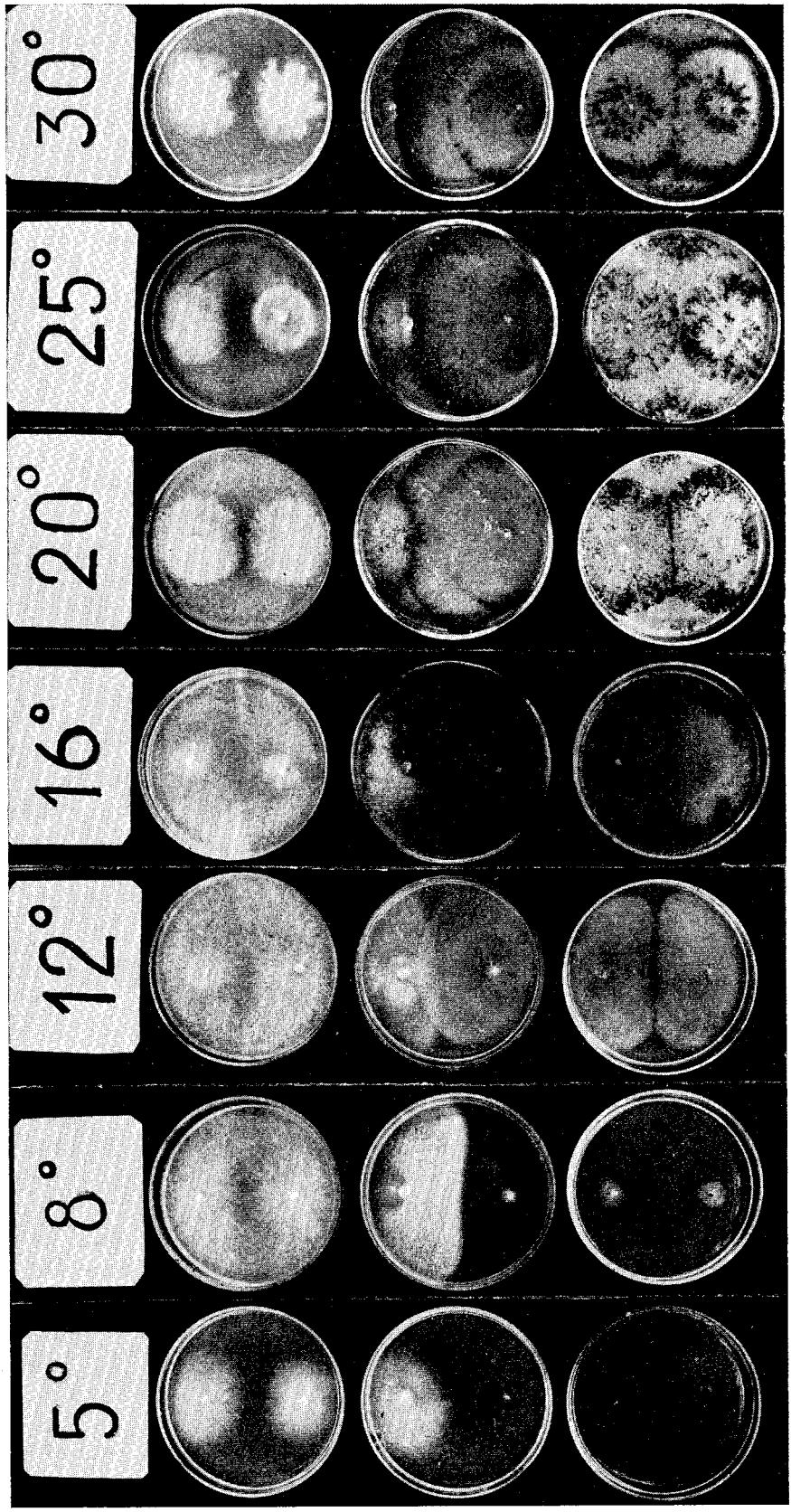

Fig. 2 b. Growth after 15 days at different temperatures. The Petri-dishes are arranged in the same order as in the preceding figure.

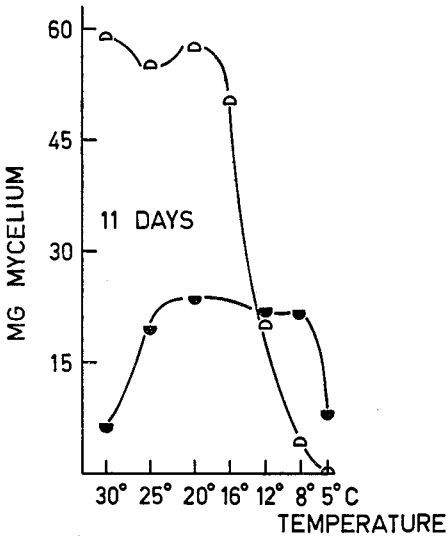

Fig. 3. Growth of *Fomes annosus* ● and *Trichoderma viride* □ at different temperatures in nutrient solutions. Every legendrepresents 4 flasks.

According to WEINDLING (1934) and WARD and HENRY (1961) the growth of *Trichoderma viride* is considerable in a pH-range from 3.1 to 6.9 with an optimum between 3.5—5.5. AYTOUN (1952) reports that *Trichoderma viride* is strongly active against *Armillaria mellea* at pH 3.4 but with increasing pH values the antagonistic activity decreases. For *Fomes annosus* ROBAK (1933) reported an pH optimum on saw-dust in the range 5.3—5.8. Later TRESCHOW (1938) found a wide and flat optimum depending on the volume of culture medium. RENNERFELT (1952) establishes the optimum pH value ranging from 5.1 to 5.8 both on malt agar and in a nutrient solution. PERSSON (1957) cultivated an isolate of the fungus from Scots pine in RAULIN's medium and proved the optimal growth occurring at pH 4.5—5.5. Finally WARD and HENRY (1961) found a distinct pH optimum at 5.1.

In order to investigate the importance of pH on the growth of the strain T 211 of *Trichoderma viride* and *Fomes annosus* strain S 1 in nutrient solutions an experiment was started with the following pH values chosen: 3.6, 4.7, 5.6, 6.8, 7.4 and 8.0. After autoclaving they were changed thus: 4.2, 4.7, 5.2, 6.4, 7.2 and 8.0. Every flask was inoculated with one piece of mycelium, average dry weight of 20 pieces was 3.5 mg, from a slant culture of *Trichoderma viride* and one, averaging 5.9 mg, from *Fomes annosus*. Half the number of flasks were incubated at 10° and half at 20° C. Harvests were performed after 4, 8, 16 and 24 days of growth.

The average dry weights of mycelia from 4 flasks each are graphed in figure 4, page 7. The variation is tolerably low.

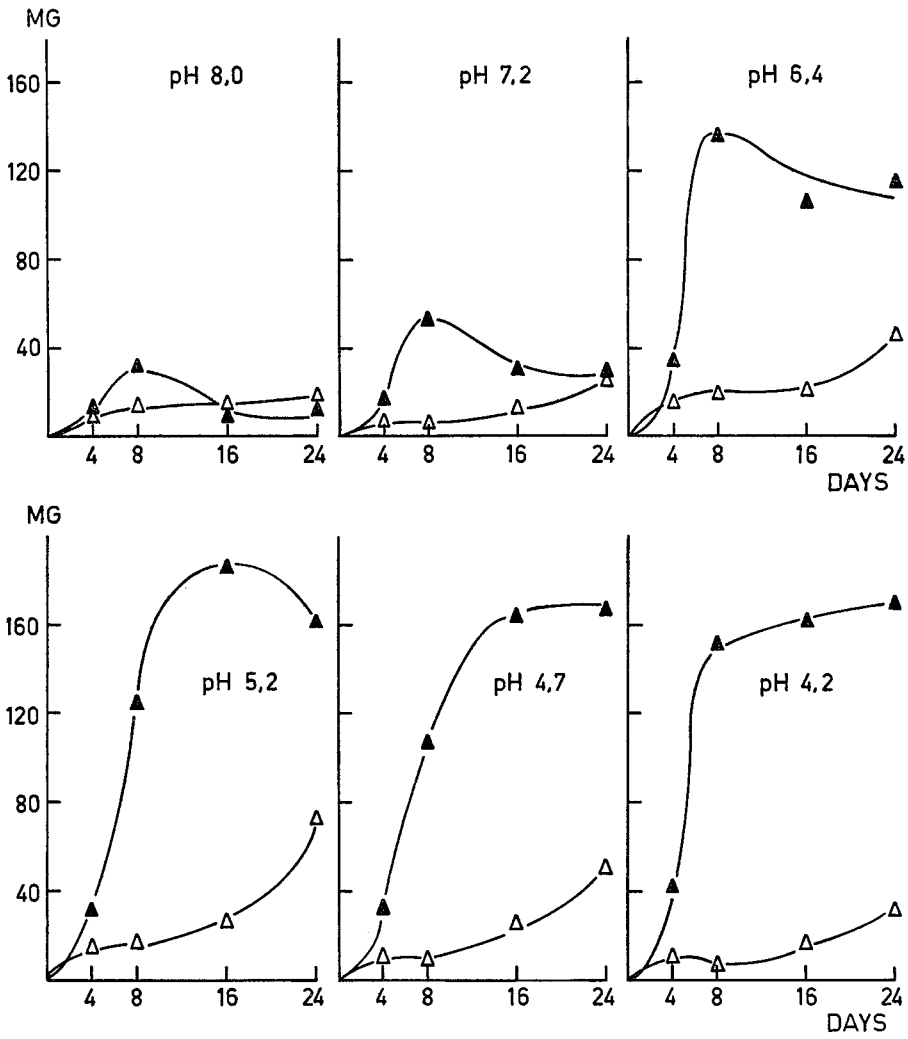

Fig. 4. Influence of pH on growth of mixed culture of *Fomes annosus* and *Trichoderma viride* in SHIVE's nutrient solution. Every legend represents 4 flasks.
△ 10° C.
▲ 20° C.

Among the pH values chosen that of 5.6 yielded optimal mycelial growth in either of the temperatures. For the following experiments this pH value was used.

With the experiences reported above, the antagonistic effect exerted by *Trichoderma viride* on *Fomes annosus* was studied in SHIVE's nutrient solution

described elsewhere in this paper.The inoculations of the fungi were performed simultaneously and the following temperatures were used: 8°, 10°, 12°, 14° and 18° C. Harvests were made after 4, 8, 16, 26, and 46 days of growth.

Table 1. Growth of Fomes annosus and Trichoderma viride at different temperatures cultivated single and in mixture in SHIVE's nutrient solution.

Days	Mycelial weights mg					
	8° C	10° C	12° C	14° C	18° C	Species
4	6.0	6.6	8.3	5.5	12.6	*Fomes annosus*
8	11.1	8.7	13.2	17.7	30.2	S 1
16	14.6	57.8	46.4	92.4	140.5	
26	31.7	85.9	134.4	185.7	170.2	
46	160.1	161.5	162.3	192.3	127.7	
4	10.5	10.9	8.8	10.2	35.2	
8	9.0	10.3	17.9	49.2	283.8	*Trichoderma viride*
16	21.0	22.8	76.5	267.0	264.4	211
26	28.5	30.5	265.2	251.3	241.4	
46	31.7	84.2	242.4	190.9	192.0	
4	11.8	5.1	5.8	9.6	32.0	
8	18.3	13.2	18.2	53.1	292.0	*Fomes annosus* S 1
16	34.7	47.4	127.7	304.8	284.8	and *Trichoderma vi-*
26	83.6	89.6	269.0	304.7	297.7	*ride* 211 mixed
46	180.5	126.7	222.7	216.1	201.4	

In the table above the mycelial dry weights are summarized. Within the pure culture of *Trichoderma viride* a marked limit exists between the yield at 10° and that at 12° C. At the former the yield is quite discrete, at the latter it is important, particularly with increasing time of culture. *Fomes annosus* does not display any such distinct gradient caused by temperature. With increasing temperature there exists a corresponding response of growth.

By comparing the mycelial yields from mixed culture of the actual fungi with the conjoined yields of the two single fungi, with two exceptions of insignificant magnitude, the former are inferior to the latter yields, indicating the occurrence of inhibiting effects in the mixed cultures. Though *Trichoderma viride* is growing poorly at high pH values in buffered nutrient solutions, the fungus in the solution of SHIVE rapidly increases the pH whereas *Fomes annosus* normally decreases it.

The pH values of the mixed cultures closely correspond to those of the single culture of *Trichoderma viride.* The initial value of 5.6 for this fungus is changed to 8.1 after 26 days of growth at 18°, but at 8° the pH increased only slightly to 5.8 during the same time of experiment. The corresponding pH values of *Fomes annosus* are 5.0 at 18° and 5.6 at 8° C. Obviously *Tricho-*

derma viride in the mixed cultures at favourable temperatures controls the development both by producing excess of mycelia and by conducting the processes of antagonistic interaction.

Discussion

The present investigation forms part of experimental work on interaction of the root rot fungus *Fomes annosus* and microorganisms antagonistic to that fungus under field conditions and in the laboratory. Some basic experiments are performed concerning the influence of temperature and pH on the growth of *Fomes annosus* and *Trichoderma viride* in single cultures and in mixed ones. The results confirm satisfyingly those found by RISHBETH (1951), RENNERFELT (1952) and WARD and HENRY (1961) on the main effect of temperature on the growth of the actual fungi. Under the present conditions a pronounced limit of temperature was established for *Trichoderma viride*, situated between 10° and 12° C. At 10° the growth of the fungus was scanty but at 12° it was considerably abundant. RISHBETH (1951) reports growth of his isolates of *Trichoderma viride* from East Anglia still at 5°. At 10° the daily radius growth is 4.5 mm. His strains of *Fomes annosus* are still active at 0° C averaging 0.4 mm daily.

From the ecological point of view the temperature of the soil may be a selective factor determining the micro-organisms present. RISHBETH states the average soil temperatures from Breckland, England, during January to 3.6° C. and during August to 16.9° C at a depth of 30 cm. TROEDSSON (1956) found at the same depth in July a maximum of 12.8° in unsorted moraine of Middle Sweden. At a depth of 10 cm the maximum was slightly higher, 13.6° C. Only in June, July and August the temperature in Swedish moraine soils equals or exceeds 10° C. Under such conditions *Trichoderma viride* should be active against *Fomes annosus* only during a short period of the growth season, while the latter fungus should be in a growth phase during eight months. These remarks indicate the complicated cases that arise when only two species are concerned. By introducing *Trichoderma viride* into a fertile forest soil not only the temperature but also another couple of complicated interactions may appear.

Summary

The influence of temperature on the growth of *Fomes annosus* isolated from spruce and *Trichoderma viride* yielding gliotoxin cultivated separately and mixed is studied. The fungi have different demands of temperature, *Trichoderma viride* growing rapidly still at 30° C, at which temperature *Fomes*

annosus ceased. On the other hand at temperatures below 12° *Trichoderma viride* developed very slowly in contrast to *Fomes annosus*.

The growth of the mixed fungi is, however, affected of pH too. At and above the point of neutrality the growth is only slightly influenced by temperature. High yields of mycelia are obtained at high temperatures and low pH values.

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Sammanfattning

Den antagonistiska effektens temperaturberoende hos *Trichoderma viride* Fr. gentemot *Fomes annosus* (Fr.) Cke.

Temperaturens inflytande på tillväxten hos en ras av *Fomes annosus*, isolerad från gran, och en gliotoxinproducerande ras av *Trichoderma viride* isolerad från jord har studerats på såväl fasta som i flytande substrat. Temperaturer under 12° C är kritiska för utvecklingen av *Trichoderma viride*, under det att *Fomes annosus* växer suboptimalt i detta temperaturområde.

Beroendet av pH har undersökts vid två distinkta temperaturer 10° och 20° C. Vid och ovan neutralpunkten var påverkan av temperaturen obetydlig och mycelmängderna små. I det sura området dirigerar temperaturen utvecklingen av samkulturerna.

Med utgångspunkt från erhållna resultat diskuteras möjligheterna att introducera denna värmesensibla ras av *Trichoderma viride* i svensk skogsmark i avsikt att biologiskt influera *Fomes annosus*.