Mycopathologia et Mycologia applicata, vol. 54, 1, pag. 35–46, 1974

GROWTH AND TOXIGENICITY OF FUSARIA OF THE SPOROTRICHIELLA SECTION AS RELATED TO ENVIRONMENTAL FACTORS AND CULTURE SUBSTRATES

by

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Abstract

Isolates of *Fusarium poae*, *F. sporotrichioides*, *F. sporotrichioides* var. *chlamydosporum* and *F. sporotrichioides* var. *tricinctum* made their best growth on PDA substrates at 24 °C, but good growth was also made at 18 °C and 30 °C. At 35 °C growth made by the *F. sporotrichioides* var. *chlamydosporum* was quite good, and superior to that of the other fungi. Moderate growth was made by all fungi at 12 °C and by *F. sporotrichioides* var. *tricinctum* also at 6 °C, while growth of the other fungi at that temperature was slight. At low temperatures toxic isolates of all but *F. sporotrichioides* grew better than non-toxic isolates, and growth of all isolates usually was better in light than in darkness up to temperatures of 18 °C.

F. poae and *F. sporotrichioides* produced highest toxicity on rabbit skins when grown at 5–8 °C, *F. sporotrichioides* var. *tricinctum* at 15–20 °C. Darkness always favoured toxin development at all temperatures.

In a comparison of 3 liquid substrates, overall toxin production was stronger on a starch substrate than on Czapek's or carbohydrate-peptone substrates. Among grain substrates, barley gave highest overall toxicity, which was again favoured by darkness. *F. poae* isolates were most toxic when derived from soil, *F. sporotrichioides* isolates when derived from barley.

Further tests with 8 liquid substrates confirmed that *F. poae* and *F. sporotrichioides* produce stronger toxicity at 8 °C than at 25 °C, and substrates favoured toxin production at pH 5.6 more than at pH 3.8 or 7.2. At pH 5.6 the isolates induced marked changes in the pH level of the substrate on which they grew. No relation was found to exist between the vigour of growth made by any of these fungi under various environmental conditions and the severity of the toxic reaction their extracts produced on rabbit skins.

Introduction

The toxins produced by Fusaria of the Sporotrichiella section have received widespread attention. In particular, the toxins produced by *F. poae* and *F. sporotrichioides* that cause alimentary toxic aleukia (Joffe, 1960a, 1960b, 1962, 1965, 1971) in man and those produced by *F. tricinctum* and causing disorders in a variety of animals (Marasas et al., 1969) have been studied in some detail. But little seems to have been published on the conditions under which these fungi grow and produce their toxins. This aspect has been the subject of the studies presented here.

The taxonomy of species of the Sporotrichiella section will shortly be

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Accepted for publication: 29.X.1972.

treated elsewhere (Joffe, in preparation). We distinguish between 2 species, *Fusarium poae* (Peck) Wr. and *F. sporotrichioides* Sherb. and two varieties of the latter, *F. sporotrichioides* Sherb. var. *chlamydosporum* (Wr. et Rg.) Joffe comb. nov. and *F. sporotrichioides* Sherb. var. *tricinctum* (Cda.) Raillo.

Materials and methods

Effects of environmental conditions and culture substrates on the growth of the fungi in vitro were determined as follows. Erlenmeyer flasks (150 cc) containing 10 g of sterilized wheat or other grains and 20 cc water were inoculated and the growth made by the isolates was assessed after 11 days. In one test series liquid cultures were used, and their composition is detailed in the relevant section.

In order to give growth assessment on grains quantitative expressions, they were made on the following scale of indices:

No growth		index 0.0
Very poor growth	up to 3 kernels	index 0.1
Poor growth	small groups of kernels covered superficially	index 0.5
Moderate growth	up to two thirds of the kernels' surface covered, but no penetration into deeper layers	index 1.0
Good growth	the whole surface of the kernels is covered, there is some penetration into deeper layers, but kernels at the bottom of the flask are readily distinguishable from one another	index 2.0
Very good growth	the whole surface of the kernels is covered, there is deep penetration, but kernels at the bottom are still distinguishable from one another	index 4.0
Excellent growth	the whole culture has the appearance of one solid block of thallus	index 6.0

The effect of light and darkness on growth in culture was tested by growing the fungi on PDA in Petri dishes kept at 6, 12, 18, 24, 30 and 35 °C, with the dishes under 'dark' treatment wrapped in silver paper. The diameter of colonies formed was measured after 10 days.

Effect of 3–8 liquid substrates on growth and toxicity was tested in several experimental series. These substrates were:

Substrate I:	2 g KNO_3, 1 g KH_2PO_4, 0.5 g KCl, 0.5 g $MgSO_4.7H_2O$, traces of $FeSO_4$, 10 g sucrose, 1000 cc distilled water.
Substrate II:	first 5 components as in substrate I, 5 g soluble starch, 2.5 g dextrose, 2.5 g sucrose, 1000 cc distilled water.
Substrate III:	25 g malt extract, 1000 cc distilled water.
Substrate IV:	30 g sucrose, 10 g glucose, 1 g peptone, 1000 cc distilled water.
Substrate V:	2 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4.7H_2O$, traces of $FeSO_4$, 30 g sucrose, 1000 cc distilled water.
Substrate VI:	200 g potato, 20 g dextrose, 1000 cc tap-water.
Substrate VII:	2 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4.7H_2O$, 0.001 g $FeSO_4$, 25 g soluble starch, 1000 cc distilled water.
Substrate VIII:	0.5 g K_2HPO_4, 0.25 g $MgSO_4.7H_2O$, 0.5 g KCl, 5 g glucose, 3 g yeast extract, 1000 cc distilled water.

The toxigenic properties were determined by a technique described in detail elsewhere (Joffe, 1960). This essentially involved culturing the isolate at 2–6 temperatures, making extracts from these cultures and applying the extracts to the skin of rabbits. The skin reaction produced by each culture at each temperature was assessed on the following scale of toxicity ratings:

0 = no skin reaction apparent
0.5 = slight reddening and swelling
1 = reddening and formation of slight leucocytarrhoea with or without oedema
2 = leucocytarrhoeic and oedemo-haemorrhagic reaction or leucocytarrhic oedematous reaction
3 = oedematous, haemorrhagic and necrotic reaction
4 = acute oedema and severe necrotic reaction

In the test series in which cultures grown at 7 temperatures were used for assessment of relations between fungal growth and toxicity of of environmental or substrate effects on toxicity, the toxicity ratings obtained at all the temperatures were combined in a single scale to give a value we shall term 'overall toxicity'. This value was obtained on the following scale:

0 = no skin reaction apparent from any of the extracts grown at the 6 temperatures;
1 = slight reddening and swelling (rating 0.5) produced by one of the six extracts;
2 = slight reddening and swelling produced by two of the six extracts, or incipient oedema (rating 1) produced by one of them;
3 = three of the six extracts produced slight reddening or swelling, or one or two produced these symptoms and one more produced incipient oedema;
4 = two to three of the extracts produced incipient oedema and/or some leucocytarrhea (rating 2);
5 = at least one extract produced moderately severe leucocytarrhea (rating 3) and one to two further extracts produced less severe symptoms;
6 = two to three extracts produced leucocytarrhea (rating 3), with some necrosis, and one to two further extracts produced less severe symptoms;
7 = at least three extracts produced severe leucocytarrhea and necrosis (rating 4), and two more extracts produced less severe symptoms of oedema.

Results

Effects of light, darkness and temperature on growth in culture of toxic and non-toxic isolates

Do toxic and non-toxic isolates of *Sporotrichiella* Fusaria differ in environmental relationships as well as in their toxicity? To clarify this point, toxic and non-toxic isolates were grown on PDA at six temperatures (6° to 40 °C) in light and darkness. At 40 °C there was no growth. Results obtained at the other temperatures are presented in table 1.

At 6 °C toxic isolates of three of the fungi made better growth in both light and darkness, but in the case of *F. sporotrichioides* non-toxic isolates grew better.

Similar, though less clear-cut, results were obtained at 12 °C. The ability of toxic isolates to make relatively good growth at low temperatures is important, since toxin production is favoured at these temperatures.

Table 1. Effect of light and darkness on growth (in mm) of toxic and non-toxic isolates of Fusaria of the Sporotrichiella Section on PDA

Species and varieties	Toxicity	No. of isolates	Temperature (°C)											
			6		12		18		24		30		35	
			l*	d**	l	d	l	d	l	d	l	d	l	d
F. poae	toxic	5	4.4	4.3	16.9	13.6	58.2	51.3	90.1	89.0	79.2	79.0	42.8	27.6
	non-toxic	2	0	0	14.2	9.2	51.2	61.2	93.7	95	85.5	85.0	4.0	13.7
F. sporotrichioides	toxic	6	4.0	3.3	17.1	15.0	57.8	41.1	77.9	78.4	80.0	80.3	21.0	44.4
	non-toxic	2	8.0	6.0	22.7	14.5	46.0	38.2	65.5	60.0	56.7	54.2	34.2	34.5
F. sporotrichioides var. chlamydosporum	toxic	4	18.3	13.3	24.8	18.0	50.0	38.2	57.8	70.0	37.0	40.0	30.0	20.2
	non-toxic	2	5.2	4.5	16.5	5.2	54.5	47.0	81.5	64.0	44.7	54.2	0	0
F. sporotrichioides var. tricinctum	toxic	3	7.0	6.0	16.0	24.5	56.3	52.6	90.0	88.6	81.0	82.6	50.6	58.6
	non-toxic	3	0	2.5	9.5	5.7	36.5	30.0	89.0	84.5	78.3	73.0	64.5	48.3

* light.
** dark.

At the temperatures near the optimum for growth (18 °–30 °C) differences in the development of toxic and non-toxic isolates were generally small and erratic. In some cases fairly large differences in favour of the toxic isolates were recorded, e.g. *F. sporotrichioides* at 30 °C, and *F. sporotrichioides* var. *chlamydosporum* at 18 °C. At 35 °C, the isolates of the latter two fungi did not differ much in growth, but non-toxic isolates of *F. poae* and *F. sporotrichioides* var. *tricintum* made little or no growth, while toxic isolates grew fairly well.

Growth made by all isolates at 6 °–18 °C was, with rare exceptions, somewhat better in light than in darkness. At 24 °–30 °C growth made in light and darkness was closely similar, except for results obtained with

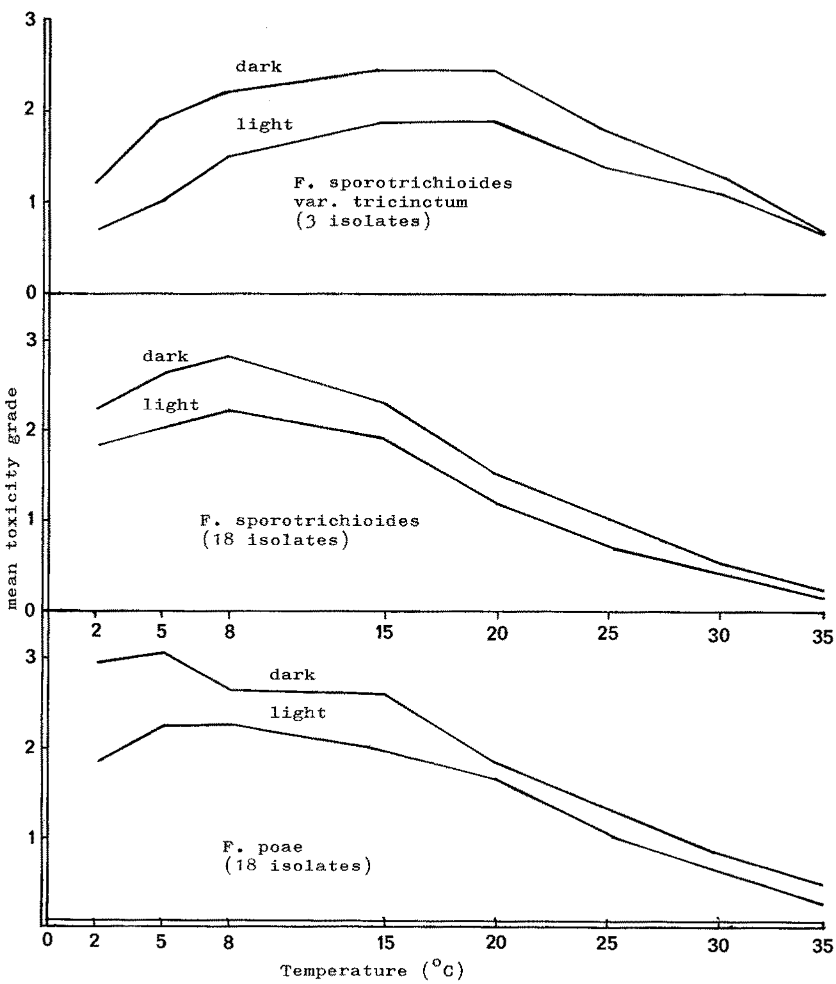

Fig. 1. Effect of temperature and light on toxicity of isolates of the Sporotrichiella Section.

F. sporotrichioides var. *tricinctum* at 24 °C. At 35 °C, light favoured the growth of toxic isolates of *F. poae* and *F. sprotrichioides* var. *tricinctum*, and that of non-toxic isolates of *F. sporotrichioides* var. *chlamydosporum*.

Effects of environment, substrate and source of isolate on toxin production

Temperature and light

The overall toxicity produced by extracts from wheat cultures grown in light and darkness at 8 temperatures, from 2 to 35 °C, is shown in fig. 1.

F. poae and *F. sporotrichioides* produced highest toxicity both in light and in darkness, at 5 ° and 8 °C, and *F. sporotrichioides* var. *tricinctum* at 15 and 20 °C. Though toxicity of the latter fungus also lessened at high temperatures, it yet developed, at 30 and 35 °C, twice as much toxicity as the other two species. Darkness clearly favoured development of toxicity of all 3 fungi at all temperatures.

Table 2. Effects of substrate and source of isolate on production of toxins (mean of results obtained at 8 temperatures)

	Mean grade of toxicity per isolate					
	F. poae		F. sporotrichioides		F. sporotrichioides var. tricinctum	
	9 isolates		9 isolates		3 isolates	
Liquid substrates	liquid	thallus	liquid	thallus	liquid	thallus
No. IV. Carbohydratepeptone	1.2*	0.5*	1.4*	0.8*	1.5**	0.7**
No. V. Czapek's	1.4	0.7	1.5	0.9	1.5	0.7
No. VII. Starch	1.6	0.8	1.8	1.0	1.7	1.0
mean	1.4	0.7	1.6	0.9	1.6	0.8
	18 isolates		18 isolates		3 isolates	
Grain substrates	light	dark	light	dark	light	dark
prosomillet	1.3	2.2	1.5	2.0	1.6	2.0
wheat	1.3	1.8	1.3	1.9	1.4	1.9
barley	1.7	2.3	1.7	2.1	1.1	1.8
mean	1.4	2.1	1.5	2.0	1.4	1.9
Sources of isolates						
prosomillet	1.3	2.0	1.4	1.9	1.5	2.1
wheat	1.7	2.2	1.2	1.7		
barley	1.2	1.8	2.2	2.8	1.1	1.4
rye	1.4	2.1	1.7	1.9		
soil	2.1	2.6	1.1	1.5		

* Each figure in this column represents the mean of 144 cultures.
** Each figure in this column represents the mean of 48 cultures.

Closely similar results regarding the temperature and light effects on production of toxicity by the species were obtained in additional test series, in which the fungi were grown on the liquid substrates desribed above.

Substrates and Sources of Isolates

Effects of substrates on overall toxicity production were first studied by growing the fungi at the above 8 temperatures on 3 grain substrates (wheat, barley, prosomillet) and on the 3 liquid substrates designated as substrates IV, V and VII (see Methods).

In the case of the liquid cultures toxicity was determined separately for the mycelial mass grown on the liquid (thallus) and for the substrate after removal of that mass. Results are summarized in table 2.

These figures show that toxicity derived from the liquid substrate was always higher tan that from the thallus grown on that substrate. Overall toxin production was constantly strongest on the starch substrate, and in the case of *F. poae* it was somewhat stronger on Czapek's than on the carbohydrate peptone substrate.

Among the grain substrates, barley yielded growth with higher overall toxicity of *F. poae* and *F. sporotrichioides* both in light and in darkness. The favorable effect of darkness on toxin production was evident on all three of these substrates.

As regards the source of isolates, those of *F. poae* were most highly toxic when isolated from soil, those of *F. sporotrichioides* when isolated from barley. Here again, darkness favoured toxin production, regardless of the source of the isolate.

Further studies with liquid substrates at 3 pH levels were carried out with *F. poae* and *F. sporotrichioides* at 8 ° and 25 °C. All the eight substrates listed in the section on methods were used, but since substrates I and II gave closely similar results, those obtained with substrate II are omitted. The purpose of these studies was to ascertain effects of the various substrates on the toxicity rating of toxic isolates, and the extent to which toxic and non-toxic isolates changed the pH level of substrates on which they were grown.

Results are summarized in table 3. They show that, with just one exception in the 24 variants of substrates tested, *F. poae* and *F. sporotrichioides* produced higher toxicity ratings at 8 °C than at 25 °C. This rating was on all substrates highest at pH 5.6, mostly lower at pH 3.8 than at 7.2. Top toxicity ratings at pH 5.6 were produced by both fungi on substrates I and VII, but *F. sporotrichioides* produced an exceptionally high rating at pH 7.2 on substrate VI.

As regards changes of pH levels induced by isolates on their substrates, the changes on substrates originally at pH 3.8 were rather small, mostly upwards up to pH 4.5, and only in a few cases (all at 25 °C) up to pH 5.0–5.2. The changes induced on substrates originally at pH 5.6 were larger, especially at 25 °C, and frequently reached pH 7.0, or on substrate III, VI and VII even pH 7.2–7.3. On substrates originally at pH 7.2, the changes induced were small, and at 25 °C reached pH 8.0–8.3 only on

Table 3. Effects of substrates and temperatures on toxicity produced by isolates of F. poae and F. sporotrichioides and the changes induced by these isolates in the pH values of the substrate (means for all isolates tested)

Substrate		F. poae						F. sporotrichioides					
		7 toxic isolates				2 non-toxic isolates		7 toxic isolates				2 non-toxic isolates	
No.*	pH	8 °C		25 °C		8 °C	25 °C	8 °C		25 °C		8 °C	25 °C
		T**	pH	T	pH	pH	pH	T	pH	T	pH	pH	pH
I	3.8	0.6	4.0	0.1	4.4	3.7	3.5	1.1	3.9	0.4	4.2	3.6	3.8
	5.6	3.7	6.3	1.9	6.7	5.6	5.5	2.1	6.5	1.2	7.0	6.0	6.3
	7.2	2.4	7.1	1.2	7.3	7.0	7.2	1.6	7.3	0.5	7.6	7.1	7.1
III	3.8	1.7	4.5	0.4	5.0	4.0	4.3	0.5	4.2	0.2	4.5	4.1	4.2
	5.6	2.2	6.6	0.5	7.0	5.6	6.1	1.4	6.3	0.5	7.3	6.0	6.2
	7.2	1.7	7.4	0.3	7.7	7.1	7.2	1.6	7.5	0.8	8.0	7.3	7.4
IV	3.8	1.1	3.8	0.3	4.3	3.3	3.4	0.6	3.7	0.3	4.1	3.6	3.2
	5.6	2.6	6.1	0.6	6.7	5.5	6.1	1.7	6.1	0.6	6.9	5.7	5.8
	7.2	1.3	7.2	0.3	7.3	6.6	7.2	1.6	7.1	1.1	7.4	6.8	7.1
V	3.8	0.8	3.5	0.5	4.1	3.5	3.4	0.9	3.6	0.5	3.9	3.7	4.0
	5.6	2.2	5.8	1.6	6.3	5.7	5.7	1.9	5.8	1.2	6.5	6.0	6.0
	7.2	1.6	7.0	0.8	7.4	6.8	6.9	1.3	7.2	0.8	7.5	7.4	7.5
VI	3.8	1.7	4.3	0.9	5.0	4.0	4.1	0.6	4.4	0.4	5.0	4.1	4.2
	5.6	2.7	6.5	2.0	7.2	6.1	6.5	1.9	6.5	1.4	7.2	6.1	6.3
	7.2	1.6	7.4	1.0	8.1	7.3	7.5	2.9	7.6	0.9	8.0	7.2	7.3
VII	3.8	2.1	4.4	1.7	4.9	4.1	4.5	1.1	4.6	0.7	5.2	4.1	4.6
	5.6	3.3	7.1	2.4	7.3	6.1	6.4	3.1	6.6	1.8	7.5	6.2	6.7
	7.2	1.7	7.9	1.5	8.3	7.3	7.4	2.2	7.9	1.6	8.7	7.5	7.8
VIII	3.8	1.0	4.0	0.4	4.4	3.7	3.8	0.9	3.9	0.2	4.4	4.3	4.5
	5.6	1.9	6.0	1.2	6.5	5.7	6.1	1.4	6.2	0.7	6.7	6.5	6.5
	7.2	1.5	7.3	1.4	7.5	6.7	6.6	1.1	7.2	0.9	7.9	7.2	7.5

* Composition of substrates is detailed in the section on methods.
** Toxicity rating (0–4).

substrates III, VI and VII, and as high as pH 8.7 for *F. sporotrichioides* on substrate VII.

Relations between vigour of growth and toxigenicity at various temperatures

Is there any relation between the growth made by these fungi *in vitro* and between the degree of toxicity of their culture extracts to rabbit skin? The results of experiments carried out at 6 temperatures supply the answer (table 4).

Table 4. Growth made in vitro by toxic isolates of Fusaria of the Sporotrichiella section at various temperatures, and toxicity to rabbit skin of the extracts from growth made made at those temperatures

Species and isolates (source and designation)	Assessment of growth made (on indices from 0 to 6) and of toxicity (grade 0 to 7)*					
	temperatures					
	6 °C	12 °C	18 °C	24 °C	30 °C	35 °C
1. F. poae (5 isolates)						
CBS, Baarn, No. 24	0.5/4	0.5/4	2/3	4/2	6/2	6/1
barley seeds, USSR, No. 792	1/4	2/3	6/3	6/2	0	0
wheat seeds, USSR, No. 958	2/4	0.5/3	6/3	6/2	2/1	0
Sambucus, Canada, No. 3918	2/4	1/3	4/3	4/2	4/1	2/0.5
oat seeds, Germany, No. 10317	1/3	0.5/2	2/2	2/1	2/0	0
mean for 5 isolates	1.3/3.8	0.9/3	4/2.8	4.5/1.8	2.8/0.8	1.6/0.3
2. F. sporotrichioides (6 isolates)						
CBS, Baarn, No. 23	2/4	2/3	6/3	6/2	6/1	0
Prosomillet, USSR, No. 738	4/4	4/3	6/3	4/2	0.5/1	0
Malus sylvestris, Germany, No. 10329	0.5/3	1/3	4/2	6/1	2/0	0
Picea abies seeds, Germany, No. 10360	1/4	1/3	4/3	4/2	2/1	0
Pinus nigra seeds, Germany, No. 10362	0.5/4	0.5/3	4/3	2/2	2/1	0
oat seeds, Germany, No. 10339	0.5/4	0.5/3	4/3	4/2	2/1	0
mean for 6 isolates	1.4/3.8	1.5/3	4.2/2.8	4.3/2.2	2.4/0.8	0
3. F. sporotrichioides var. tricinctum (1 isolate)						
field corn, FDA, Washington DC, No. M 304	1/4	1.5/3	6/3	6/2	4/1	0.5/0.5
4. F. sporotrichioides var. chlamydosporum (1 isolate)						
wheat, Canada, No. 4337	4/4	6/3	6/3	6/2	2/2	2/1

* In each pair of figures the first represents the growth index, the second the overall toxicity grade.

Three of the 4 fungi produced their best mean growth at 24 °C, and only *F. sporotrichioides* var. *chlamydosporum* grew equally well at 24 °C and 30 °C. In the latter and in *F. sporotrichioides* var. *tricinctum* only 1 isolate (out of 6 tested) was highly toxic and this is insufficient to establish a relation between growth vigour and toxicity. However, with 5 isolates of *F. poae* and 6 of *F. sporotrichioides* showing strong toxicity, relevant conclusions could be drawn. The peak for growth of both-species was at 18–24 °C, where toxicity was moderate. But toxicity was strongest at 6–12 °C, where growth was limited. At high temperatures of 30–35 °C growth was moderate and toxicity weak. Thus there seems to be no relation between vigour of growth made at any of the above temperatures and the toxigenicity of these fungi. This is clearly illustrated in fig. 2.

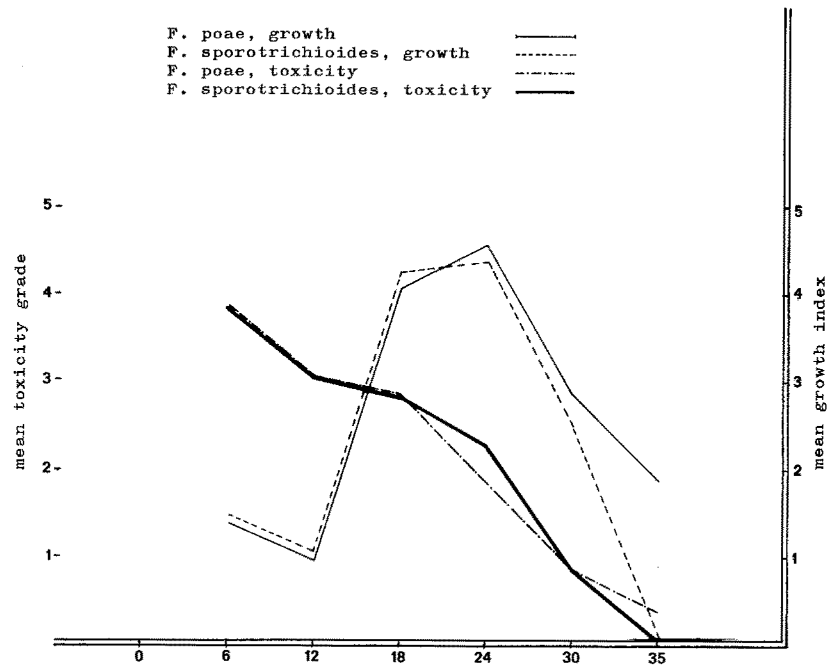

Fig. 2. Relations between growth *in vitro* and grade of overall toxicity of *Fusarium poae* (5 isolates) and *F. sporotrichioides* (6 isolates) at various temperatures.

Discussion

As far as we are aware, no studies have been published on the relationship between environmental and substrate factors and the extent of toxin production by fungi of the Sporotrichiella section.

There is hardly any literature on the relationships between toxic properties of fungal species and the temperatures under which they were grown. Only Sarkisov and Kwashnina (1948) found that cultures of *F. sporotrichioides*, when grown at 1.5 to 4 °C were more toxic than when grown at 22 ° to 25 °C.

As regards effects on growth, Lacicowa (1963) found that development of *F. poae* on potato dextrose agar and maltose agar media was best at 24–25 °C, which is in agreement with our results. This author states that the fungus developed equally well in daylight and darkness.

Seemüller (1968), working with 2–6 isolates of the following fungi determined temperature relationships as follows: *F. poae* had its minimum growth at 2.5 °C, optimum at 22.5–27.5 °C and maximum at 32.5 °C, the latter being appreciably lower than in our work (35 °C). *F. sporotrichioides* and what Seemüller called *F. tricinctum* had lower minima (0 °C), optima at 27.5 °C and 22.5 °C respectively, and maxima at 35 °C and 32.5 °C, respectively. Seemüller's *F. chlamydosporum* had a higher minimum (5 °C), an optimum at 27.5 °C and maximum at 35 °C or a little above that value. This agrees with our observation that *F. sporotrichioides* var. *chlamydosporum* made better growth at 35 °C than any of the other fungi.

Acknowledgement

The author would like to thank Dr. W. Gerlach and Dr. W. C. McDonald for providing the cultures used in this study.

Support given to this study by the Central Research Fund of the Hebrew University is gratefully acknowledged.

Zusammenfassung

Stämme von *F. poae*, *F. sporotrichioides*, *F. sporotrichioides* var. *chlamydosporum* und *F. sporotrichioides* var. *tricinctum* wuchsen am besten auf PDA Nährboden bei 24 °C, aber Wachstum war auch gut bei 18 °C und 30 °C. Bei 35 °C war das Wachstum der *F. sporotrichioides* var. *chlamydosporum* noch recht gut und erheblich besser als das der anderen Pilze. Alle Pilze wuchsen mässig bei 12 °C, *F. sporotrichioides* var. *tricinctum* auch bei 6 °C, während bei dieser Temperatur Wachstum der anderen Stämme niedrig war. Bei niedrigen Temperaturen wuchsen toxische Stämme besser als nicht-toxische, mit Ausnahme von *F. sporotrichioides*. Das Wachstum im Licht war meistens besser als im Dunkeln bei Temperaturen bis 18 °C.

F. poae und *F. Sporotrichioides* erreichten die höchste Toxizität in Proben auf Kaninchenhaut wenn sie bei 5–8 °C wuchsen, *F. sporotrichioides* var. *tricinctum* bei 15–20 °C. Dunkelheit förderte Entwicklung von Toxinen bei allen Temperaturen.

Bei einem Vergleich von 3 flüssigen Nährböden war im allgemeinen die Toxin Produktion von höherem Gehalt auf einem an Stärke reichen Nährboden als auf Czapek's oder Carbohydrate Pepton Nährboden. Von den Getreide-Nährböden förderte Gerste die Toxizität am meisten, besonders im Dunkeln. *F. poae* Stämme ergaben die höchste Toxizität wenn von Bödenarten isoliert, während die Stämme von *F. sporotrichioides* auf Gerste-Nährboden am meisten toxisch waren.

Weitere Experimente auf 8 flüssigen Nährböden bestätigten, das *F. poae* und *F. sporotrichioides* viel stärkeres Toxin bei 8 °C als bei 25 °C entwickelten, und dass pH 5.6 günstiger für Toxin Produktion ist als pH 3.8 oder pH 7.2. Bei pH 5.6 bewirkten die Pilze erhebliche Aenderungen in den pH Werten der Nährböden auf denen sie wuchsen.

Die Stärke des Wachstums dieser Pilze unter verschiedenen Umweltsbedingungen zeigte keinen Zusammenhang mit der Intensität der toxischen Reaktion, die ihre Extrakte auf Kaninchenhaut bewirkten.

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