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MYC 8005, an antibiotic against spider mites. 1. Description of the producing organism (Streptomyces exfoliatus var. echinosporus var. nov.) and properties of the antibiotic

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Abstract

An antibiotic substance, MYC 8005, was found to be produced by a *Streptomyces* strain, D197. It proved to possesss strong antibiotic activities against bacteria, fungi and spider mites. It might be similar to antibiotic 323/58 reported in 1962 by Kruglyak *et al.* This antibiotic, however, is no longer available for comparison. The producing organism resembles *Streptomyces exfoliatus* (Waksman & Curtis) Waksman & Henrici but differs from this species in the surface structure of the spores. For this reason it is proposed to give D 197 the name *Streptomyces exfoliatus* var. *echinosporus* var. nov.

The nature of the symptoms observed with spider mites and the susceptibility of various acaricide-resistant strains to this antibiotic suggest a new mode of action.

Introduction

During a screening of *Actinomycetes* for their capacity to produce antibiotics a strain, D 197, was isolated and selected for further study on account of its ability to produce a substance, designated MYC 8005, with activity against some fungi (e.g. *Cladosporium cucumerinum* Ellis & Arth.) and bacteria (e.g. *Mycobacterium tuberculosis* Lehmann & Neumann). The isolated antibiotic, on account of its fungicidal activity, was subsequently tested in a standard screening programme against other plant-pathogenic fungi, insects and mites. It was discovered that this antibiotic was strongly active as an acaricide with a mode of action being quite different from that of the common acaricides.

In a second paper more details regarding the mode of action will be published (Meltzer, in press).

Material and methods

Composition of some special culture media

Corn steep – glucose – salts medium: 30 g glucose; 5 g $(NH_4)_2SO_4$; 0.2 g KH_2PO_4; 1 g corn steep solids; 8 g $CaCO_3$ in 1 l of tap water; pH 6.0 (prior to the addition of $CaCO_3$).

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Malt – yeast extract – corn steep medium: 20 g malt paste; 10 g yeast extract; 5 g corn steep solids in 1 l of tap water; pH 7.0.

Carbon-free inorganic salts agar medium (*C⁻-agar*): 2 g $(NH_4)_2SO_4$; 1 g K_2HPO_4; 1 g KCl; 0.1 g $MgSO_4$. 7 H_2O; 0.1 g $FeSO_4$.7 H_2O; 15 g agar in 1 l of distilled water; pH 7.6.

Malt – peptone agar: 10 g peptone (Difco); 15 g malt extract; 5 g NaCl; 15 g agar in 1 l of distilled water; pH 7.0. Other microbiological media mentioned in this paper were prepared according to the recipes given in Waksman (1961).

Test methods

MIC determinations[1]. The activity in vitro against bacteria and most fungi was determined by the micro serial dilution test. The MIC determinations of *Fusarium culmorum* (W. G. Smith) Sacc. and of *Venturia inaequalis* (Cooke) Wint. were performed in a spore germination test..

Determination of the protective activity against foliar fungal diseases. Tests were performed on *Podosphaera leucotricha* (Ell. & Everh.) Salm. on apple seedlings, *Sphaerotheca fuliginea* (Schlecht.) Salm. on cucumber seedlings. *Piricularia oryzae* Cav. on rice seedlings, *Botrytis cinerea* Pers. ex Fr. on detached lettuce leaves, *Phytophthora infestans* (Mont.) de Bary on detached tomato leaves, *Plasmopara viticola* (Berk. & Curt.) Berl. & De Toni on detached vine leaves and *Venturia inaequalis* (Cooke) Wint. on shoots of clonally raised apple rootstocks (type MII). Finely ground suspensions of MYC 8005 were sprayed on the leaves of the test plants which were allowed to dry and subsequently inoculated with spores of the fungi. These methods are described in more detail [Anon., 1970]

Test methods used with insects and spider mites. Tests were performed on the house fly (*Musca domestica* L.), the vinegar fly (*Drosophila melanogaster* Meig.), the yellow-fever mosquito (*Aëdes aegypti* L.), the Colorado potato beetle (*Leptinotarsa decemlineata* Say), the caterpillar of the large cabbage white (*Pieris brassicae* L.), the black bean aphid (*Aphis fabae* Scop.), the carmine spider mite (*Tetranychus cinnabarinus* Boisd.), and the two-spotted spider mite (*Tetranychus urticae* Koch).

Musca domestica was tested in the Petri dish method, using 10 adults per dish. *Leptinotarsa decemlineata* and *Pieris brassicae* were used as third stage larvae on cut potato haulm or cauliflower leaves respectively. Each replicate contained 10 larvae. *Aphis fabae* and the *Tetranychus* spp. were used on potted plants of broad beans or French beans, respectively. Each replicate was provided with 10 adult females.

In the ovo-larvicidal tests with the *Tetranychus* spp. 7 adult females were confined to the leaf by means of a plexiglass cage for two days and then removed. In that time the females produced 100–150 eggs.

Full particulars of the test methods used with *Musca domestica*, *Aphis fabae*, *Leptinotarsa decemlineata*, *Pieris brassicae* and *Tetranychus* spp. can be found in

[1] MIC = minimum inhibitory concentration, i.e. concentration just sufficient to prevent the growth of the test organism.

Meltzer and Welle (1969); the mite ovicidal tests in particular have been described by Meltzer (1955), and the systemic tests on aphids and mites by Tempel *et al.* (1968).

The *Drosophila* ovo-larvicidal tests were performed in plastic Petri dishes with a diameter of 9 cm. The dishes were provided with an agar rearing medium containing 46% of Pearl and Penniman solution (Pearl *et al.*, 1926), 8% malt extract, 2% agar and the antibiotic in the required concentration. Five female flies, 7–14 days old, were placed in each dish and anaesthetized with CO_2, and then stored in an incubator at 24 °C. The flies were removed after 24 h and the eggs were counted under the binocular microscope. The dishes were subsequently kept in the incubator for another ten days before the final control took place.

The tests on *Aëdes aegypti* were performed in small plastic cups, filled with 100 ml of tap-water to which MYC 8005 was added. Each cup was provided with 20 third-stage larvae. The larvae were fed daily with a mixture of ground bread and brewer's yeast. The larvae were checked for mortality after storage at 25 °C in a climatically conditioned cabinet for six days.

All tests were carried out in triplicate. The mortality percentages were corrected by incorporation of the natural mortality according to the formula of Abbott: $(a-b)/a \times 100\%$, in which *a* indicates the number of survivors of the control and *b* the number of survivors of the object concerned.

Description and identification of strain D 197

Description

The organism showed good development in all media tested (malt peptone agar, Emerson's agar, nutrient agar, oatmeal agar, starch agar, Czapek saccharose agar, Czapek glycerol agar, glucose calcium malate agar and glucose asparagine agar). Except malt peptone agar all these media have been described by Waksman (1961) (pp. 328–334). The colonies were either pasty and highly folded (e.g. malt peptone agar) or tough and hardly folded at all (e.g. starch agar).

The colour of the vegetative mycelium varied from colourless (e.g. Czapek saccharose agar) to light greenish yellow and reddish brown (Czapek glycerol agar). The aerial mycelium was pinkish and developed in a few media only (starch agar, Czapek saccharose agar, oatmeal agar). It consisted of sporophores sympodially branched to form tufts. The spore chains were rather long, straight or weakly flexuous upon short main axes (rectus flexibilis) (see Fig. 1A). The spores were oval to almost cylindrical and varied considerably in size (average 0.7–1.0 μm by 1.2–1.8 μm). The surface was spiny, as was found with the electron microscope (see Fig. 1B).

Gelatin was readily broken down (liquefaction of gelatin medium). No melanoid pigments were produced on melanin-formation agar. The growth in litmus milk was not accompanied by coagulation, peptonisation or acid formation. Hydrogen sulfide was not produced (peptone yeast extract iron agar). Nitrate was reduced (nitrate reduction medium; Griess-Romeyn reagent). Hydrolysis of starch was slow.

Identification

By comparison of the growth characteristics and biochemical properties of strain D 197 with those of the species described in the handbooks of Waksman (1961) and Hütter (1967) it was found that D 197 possessed a strong likeness to *Streptomyces ex-*

Fig. 1A. Aerial mycelium of *Streptomyces* spec. D 197, grown on C⁻ agar *(Streptomyces exfoliatus* var. *echinosporus)* (× 700).

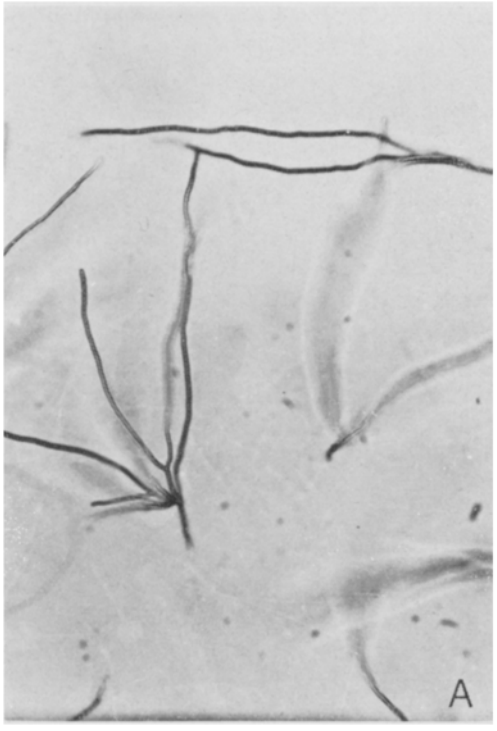

Fig. 1B. Electron micrograph of spores of *Streptomyces* spec. D 197 *(Streptomyces exfoliatus* var. *echinosporus)* (× 7800).

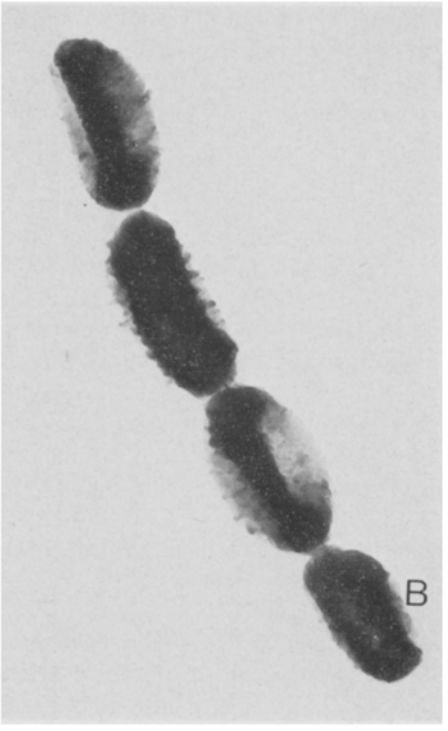

Fig. 1A. Luchtmycelium van Streptomyces spec. D 197, gegroeid op C⁻ agar (Streptomyces exfoliatus var. echinosporus) (700 ×).

Fig. 1B. Sporen van Streptomyces spec. D 197 (Streptomyces exfoliatus var. *echinosporus) (7800 ×).*

Table 1. Comparison of strain D 197 with *Streptomyces exfoliatus* ATCC 12627.

	Strain D 197	ATCC 12627
colour vegetative mycelium	colourless to light yellow and reddish brown	light brown beige to dirty red-brown
pigment production	Czapek glycerol agar: light brown	Czapek glycerol agar: reddish brown
	oatmeal agar: light yellow	oatmeal agar: no pigment produced
colour aerial mycelium	pink	pink to light carmine
structure aerial mycelium	tufts, straight to flexuous	tufts, straight to flexuous
(on C⁻agar)	spore chains (Fig. 1A)	spore chains (Fig. 2A)
spore morphology	usually oval; *spiny* (Figure 1B)	usually oval; *smooth* (Figure 2B)
melanoid pigment formation	negative	negative

Tabel 1. Vergelijking van stam D 197 met Streptomyces exfoliatus ATCC 12627.

Fig. 2A. Aerial mycelium of *Streptomyces exfoliatus* (Waksman & Curtis) (Waksman & Henrici, ATCC 12627, grown on C⁻ agar (× 700).

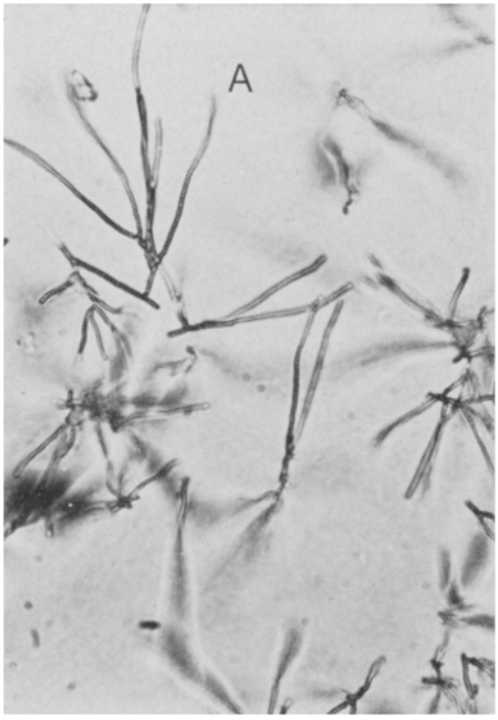

Fig. 2B. Electron micrograph of spores of *Streptomyces exfoliatus* (Waksman & Curtis) Waksman & Henrici, ATCC 12627 (× 5250).

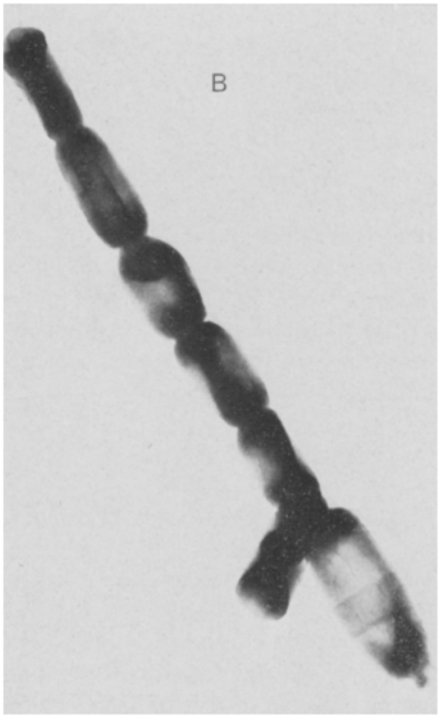

Fig. 2A. Luchtmycelium van Streptomyces exfoliatus (Waksman & Curtis) (Waksman & Henrici, ATCC 12627, gegroeid op C⁻ agar (700 ×).

Fig. 2B. Sporen van Streptomyces exofliatus (Waksman & Curtis) (Waksman & Henrici, ATCC 12627 (5250 ×).

foliatus (Waksman & Curtis) Waksman & Henrici. A strain of that species has been obtained from the ATCC collection (ATCC 12627) and in Table 1 the experimental data of both this strain and strain D 197 are listed together. It follows from these data that the only real difference between these strains lies in the surface structure of the spores. The spores of *S. exfoliatus* ATCC 12627 are smooth, whereas those of D 197 are spiny (Fig. 1B and 2B). In view of the importance at present attributed to this morphological property (Shirling and Gottlieb 1966, Hütter 1967), D 197 cannot be regarded as a true representative of the species *S. exfoliatus*. The other differences are small and it is therefore proposed to call D 197 a variety of *S. exfoliatus* with the name *Streptomyces exfoliatus* var. *echinosporus* den Admirant. The strain has been deposited as number CBS 418.70 with the Centraalbureau voor Schimmelcultures at Baarn.

Antibiotic MYC 8005

Formation

Spores of strain D 197 obtained from an oatmeal agar culture were inoculated into 500 ml Erlenmeyer flasks containing 100 ml malt peptone water. Following incubation

of this culture on a rotary shaker (250 rpm) at 26 °C for three days, a 5% transfer was made to the production medium: corn steep – glucose – salts medium or malt-yeast extract-corn steep medium. The formation of the antibiotic was determined daily by measuring the activity of a 10% methanolic extract of the culture liquid against *Bacillus subtilis* Cohn ATCC 6633. In about three days the production of the antibiotic activity had reached its maximum value.

Isolation

Antibiotic activity was observed mainly in the mycelium from which it could be extracted with aqueous methanol or aqueous acetone. The organic solvent was removed by distillation, water being added from time to time. The residual aqueous concentrate was extracted with *n*-butanol. The butanol extract was concentrated *in vacuo* below 50 °C. The active fraction was precipitated and was purified further by treatment with ammonia, washing with water and acetone and treatment with activated charcoal. Finally the pure substance (MYC 8005) was recrystallized from aqueous butanol.

Properties and identification

The UV and IR absorption spectra are shown in Fig. 3 and 4. The $E^{1\%}_{1cm}$ at 260 nm is 118. A search of the literature on antibiotics revealed that mycospocidin (Nakamura, 1957) and the antibiotic 323/58 (Kruglyak *et al.*, 1962) had a similar UV absorption spectrum. The composition of the hydrolysate of MYC 8005 resembles closely that of 328/58. Glycine, reported to be present in the hydrolysate of mycospocidin, could not be detected in that of MYC 8005. In Table 2 the analytical and chemical data of MYC 8005 and 323/58 are compared. There is a slight but not significant difference in the elementary analysis of the two compounds.

Antibiotic 323/58 is no longer available at the Institute of New Antibiotics, Moscow where this substance was first isolated. According to the description (Kruglyak *et al.*,

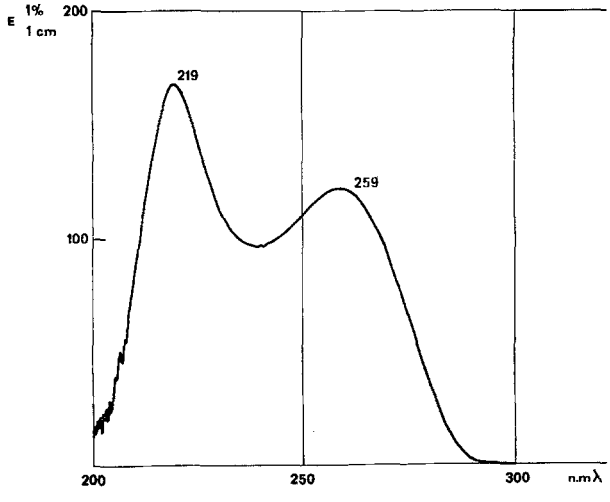

Fig. 3. UV absorption spectrum of MYC 8005.

Fig. 3. UV-absorptiespectrum van MYC 8005.

Fig. 4. IR absorption spectrum (in KBr) of MYC 8005.

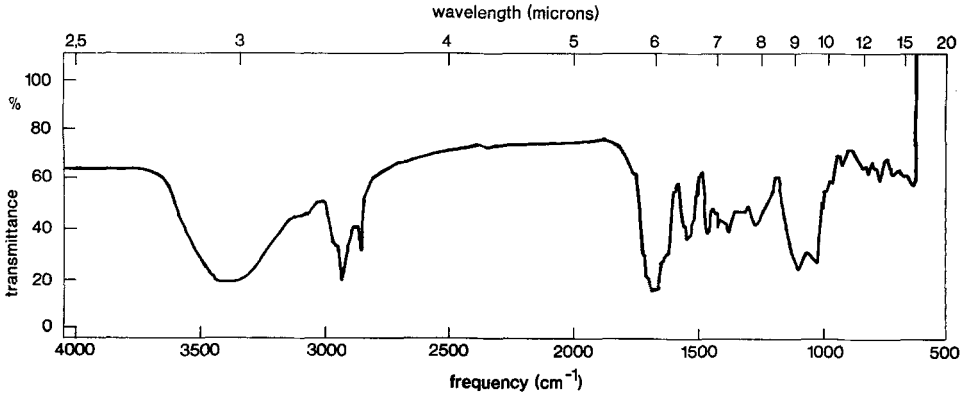

Fig. 4. IR-absorptiespectrum (in KBr) van MYC 8005.

Table 2. Comparison of the antibiotics MYC 8005 and 323/58.

	MYC 8005	323/58 (data from the paper by Kruglyak *et al.*, 1962)
UV spectrum (methanol)	maximum 259–260 nm	259–260 nm
	$E^{1\%}_{1\,cm}$ 260 nm = 118	130
	minimum 240 nm	240 nm
	strong absorption 220 μm	ibid
IR spectrum (KBr)	720, 760, 810, 840, 920, 960, 1020, 1090, 1270, 1310, 1380, 1420, 1460, 1540, 1670, 2850, 2920, ± 3400 cm^{-1}	720, 767, 811, 837, 915, 959, 1023, 1028, 1097, 1221, 1257, 1316, 1336, 1337, 1446, 1545, 1558, 1635, 1654, 1674, 1703, 2857, 2930, 3068, 3300–3306 cm^{-1}
isolation method	extraction from the mycelium by acetone; treatment with activated charcoal leads to the removal of a yellow substance	ibid
decomposition point	235–240 °C	226–229 °C
stability	between pH 2 and 9	ibid
benzoyl chloride test	positive	positive
biuret test	negative	negative
Fehling's reagent	negative	negative
ferric chloride	no colour	no colour
ninhydrin test	negative	negative
solubility: insoluble in	acetone, chloroform, ether, carbon tetra-chloride, petroleum ether	ibid
moderately soluble in	———	aqueous acetone
well soluble in	pyridine, 10% alkali, warm methanol and ethanol, aqueous acetone	ibid except aqueous acetone
elementary analysis	C: 55.3%; H: 8.0%; N: 6.4%	C: 51.3%; H: 7.7%; N: 6.2%
products of hydrolysis	uracil, glucosamine	uracil, glucosamine
$[\alpha]_D^{25}$	60° (0.5% in pyridine) 59° (5% in 0.03 N NaOH)	74° (0.5% in pyridine)
UV irradiation	leads to inactivation	unknown

Tabel 2. Vergelijking van het antibioticum MYC 8005 met 323/58

1962) the 323/58 producing organism might be *Streptomyces exfoliatus* – or a very similar organism.

Biological activity of MYC 8005

Activity in vitro against fungi and bacteria. In Table 3 the activity (minimum inhibitory concentration) is given of the purified preparation against a number of bacteria and fungi.

Table 3. Antibiotic activity of MYC 8005 against some test strains.

	MIC (μg/ml)
Bacillus subtilis Cohn ATCC 6633	0.3
Staphylococcus aureus Rosenbach ATCC 6538 P	> 5
Mycobacterium tuberculosis Lehmann & Neumann H 37 R^V	3
M. tuberculosis 5582	1
M. tuberculosis A 407 a	0.3
Saccharomyces cerevisiae Meyen ex Hansen ATCC 9763 A	2.5
Candida albicans (Robin) Berkhout	5
Cladosporium cucumerinum Ellis & Arth., N 1	0.3
Fusarium spec. Link ex Fries, N 3	50
Trichophyton mentagrophytes Blanch, R 177	50
Verticillium dahliae Kleb., N 2	> 50
Fusarium culmorum (W. G. Smith) Sacc.	40
Venturia inaequalis (Cooke) Wint	5

Tabel 3. Antibiotische werkzaamheid van MYC 8005 tegen enkele teststammen.

Table 4. Protective fungicidal activity of MYC 8005 against foliar diseases (infection in % control).

Disease caused by	Period between spraying and inoculation (h)	Concentration of MYC 8005 (ppm)					Standard fungicide concentration (ppm)					common name
		300	100	30	10	3	300	100	30	10	3	
Podosphaera leucotricha	1–2	95					5		45			dinocap
Sphaerotheca fuliginea	1–2	71						0		23		dinocap
Piricularia oryzae	1–2	63		82					11			blasticidin
Botrytis cinerea	1–2			5	29	51			4	20	30	thiram
Phytophthora infestans	1–2	16		87			3		8			fentin hydroxide
Plasmopara viticola	1–2	0	2	3	28		0	3	82	59		captan
Venturia inaequalis	24	15		35			13		24			captan
Venturia inaequalis	72	4		35			19		35			captan
Venturia inaequalis	144	97		100			43		88			captan

Tabel 4. Beschermende fungicide werkzaamheid van MYC 8005 op bladziekten (infectie in % controle).

Activity against foliar fungal diseases in vivo. The results of a number of tests, in which the compound was applied on plant leaves to determine the protective action against a number of leaf attacking fungi, are given in Table 4. The results obtained with standard fungicides in the same tests are also included in this table. The antibiotic showed no activity against powdery mildew of apple and cucumber, and against *Piricularia oryzae*, but it did show activity against the other four pathogens.

The activity against apple scab under natural conditions was still present when inoculation occurred 3 days after the application; on the sixth day, however, no residual activity of MYC 8005 could be detected anymore with this test.

Activity on insects. The test results on insects are summarized in Table 5. There was some activity in the *Drosophila* ovo-larvicidal test and in the *Aëdes*-larvae test; a slight activity on Colorado potato beetle was also observed. Thus the level of the insecticidal activity can be regarded as low.

Table 5. Insecticidal activity of the antibiotic MYC 8005. + = 90–100% mortality; − = < 50% mortality.

Insect	Concentration (ppm)				
	100	30	10	3	1
Musca domestica (adulticidal test)	−	−	−		
Drosophila melanogaster (ovo-larvicidal test)			+	−	−
Aedes aegypti (larvicidal test)			+	+	−
Leptinotarsa decemlineata (larvae)	−*	−	−		
Pieris brassicae (caterpillars)	−	−	−		
Aphis fabae	−	−	−		

*43% mortality

Tabel 5. Insecticide werkzaamheid van het antibioticum MYC 8005. + = 90–100% sterfte; − = < 50% sterfte.

Acaricidal activity

Normal foliar treatment. Adult females of *Tetranychus cinnabarinus* Boisd. were not killed by MYC 8005 in a concentration as high as 1000 ppm. In the ovo-larvicidal test, however, low concentrations proved to be active (Table 6). The substance showed exclusively larvicidal action, since all treated eggs hatched normally. The growth of the larvae thus was inhibited. Whereas the first moult into protonymphs proceeds normally within two days, it failed to take place after treatment with MYC 8005. The first few deaths, however, did not occur until after about a week and only after a fortnight was mortality complete.

Some resistant strains of the two-spotted spider mite, *Tetranychus urticae* Koch, viz rSt (a tetradifon-resistant strain), rCp (a parathion-resistant strain), and rCk (a dicofol-resistant strain) were tested (Table 7).

The remarkable larvicidal action, exhibited by the extension of the larval stage for

Table 6. Larvicidal activity of MYC 8005 on *Tetranychus cinnabarinus*. Mortality percentages of the larvae.

Concentration (ppm)	A (Dipped eggs)	B (Eggs laid on dry deposit)
10	100	100
3	100	100
1	92	91
0.3	21	33

Tabel 6. Larvicide werkzaamheid van MYC 8005 op Tetranychus cinnabarinus. Percentage sterfte van de larven.

Table 7. Action of MYC 8005 on the resistant strains rCk, rCp and rSt of *Tetranychus urticae*. Mortality percentages of the larvae.

Concentration (ppm)	Strain					
	rCk		rCp		rSt	
	A[1]	B[2]	A	B	A	B
10	100	100	100	100	100	100
3	100	100	100	100	100	100
1	99	75	95	90	98	99
0.3	90	20	65	80	97	89

[1] A: dipped eggs
[2] B: eggs laid on dry deposit

Tabel 7. Werking van MYC 8005 op de resistente stammen rCk, rCp en rSt van Tetranychus urticae. Percentage sterfte van de larven.

several days before death occurs, as well as the similar activity on various resistant strains, suggests an entirely new mode of action.

Residual activity of MYC 8005. The residual activity was determined on normal *Tetranychus cinnabarinus* as well as on the Tedion-resistant strain rSt of *T. urticae*.

The results summarized in Tables 8 and 9 indicate that some loss of activity takes place. This is detectable only when a concentration of 1 ppm was used. At higher concentrations clearly sufficient material (more than 1 ppm) is left for as long as 4 weeks to cause a complete or nearly complete kill.

Systemic activity. The resistance of the eggs of *Tetranychus cinnabarinus* to the high concentration of 100 ppm, as mentioned above, raised some doubt whether the larvicidal properties were to be attributed to contact action. If not a contact poison, MYC 8005 may only act as a stomach poison. In that case, however, the antibiotic must be capable of penetrating the leaf in order to be available for oral uptake by the larvae.

The systemic activity of MYC 8005 was tested in an experiment with French beans in water cultures. Per pot with 150 ml of nutrient solution 12.5 mg of MYC 8005 was

Table 8. Residual activity of MYC 8005 on French bean. Infection with *Tetranychus cinnabarinus*.

Conc. (ppm)	Initial activity		Residual activity[2]. Re-infection after			
	A[1]	B[2]	6 d	13 d	20 d	27 d
30	99	100	100	100	100	100
10	100	100	100	97	100	100
3	99	100	100	99	98	97
1	94	71	92	46	54	4
0.3	4	3				

[1] dipped eggs [2] eggs laid on dry deposit

Tabel 8. Werkingsduur van MYC 8005 op stambonen. Infectie met Tetranychus cinnabarinus.

Table 9. Residual activity of MYC 8005 on French bean. Infection with a Tedion-resistant strain of *Tetranychus urticae*.

Conc. (ppm)	Initial activity		Residual activity[2]. Re-infection after			
	A[1]	B[2]	7 d	14 d	21 d	28 d
30	100	100	100	99	98	100
10	100	100	99	99	97	100
3	100	100	98	97	83	94
1	98	99	87	79	49	29
0.3	97	89				

[1] dipped eggs [2] laid on dry deposit

Tabel 9. Werkingsduur van MYC 8005 op stambonen. Infectie met een Tedion-resistente stam van Tetranychus urticae.

added. No mortality of spider mites or larvae on the plants occurred; the development of the eggs into adults proceeded normally. It is therefore concluded that MYC 8005 does not possess systemic activity. In order to investigate the possible foliar penetration, only one side of bean leaves of about 25 cm^2 was smeared with an acetonic solution containing 1 mg of MYC 8005. Female spider mites were allowed to deposit eggs either on the treated side or on the non-treated side. The experiment is

Table 10. Test for foliar penetration of MYC 8005. 1 mg of the substance was smeared on a bean leaf with a surface of about 25 cm^2.

Treated surface	Surface with eggs	Effect (mortality)
upper side	upper side	+
upper side	lower side	–
lower side	upper side	–

Tabel 10. Toets voor bladpenetratie van MYC 8005. 1 mg van de stof werd uitgestreken op een boneblad van ca 25 cm^2.

summarized in Table 10. From the results it must be concluded that the mortality is the result of a contact action and that the substance does not penetrate into the leaf.

This test does not exclude the possibility of the penetration of the antibiotic into the leaf, followed by decomposition inside. The residual activity of the deposit (shown above), however, lasts extraordinarily long and therefore a rapid penetration followed by a rapid decomposition seems highly improbable.

Preferential adsorption. The comparison of the dipping method with the spraying method demonstrated a considerable difference in effect. Dipping of the bean plants into aqueous dilutions, obtained by mixing a concentrated solution of MYC 8005 in acetone with various amounts of water, resulted in a higher mortality rate than spraying until just before dripping off. In Table 11 this difference is clearly shown. From this result the conclusion must be drawn that the leaf has a strong affinity for the active substance.

Table 11. Preferential adsorption of MYC 8005 by leaves.

Concentration (ppm)	Mortality percentage on			
	dipped leaves		sprayed leaves	
	A[1]	B[2]	A	B
10	100	100	100	100
3	99	100	82	88
1	97	95	49	20
0.3	42	15	2	2

[1] A: eggs either dipped or sprayed [2] B: eggs laid on dry deposit

Tabel 11. Preferentiële adsorptie van MYC 8005 door bladeren.

Table 12. MYC 8005 irradiated for 24h with a sun lamp, 100,000 lux, compared with non-irradiated substance. Percentage mortality of larvae of *Tetranychus cinnabarinus*.

Concentration (ppm)	Irradiated		Non-irradiated	
	A[1]	B[2]	A	B
10	97	99	100	100
3	25	64	99	100
1	2	4	60	82
0.3	5	11	2	11

[1] A: dipped eggs [2] B: eggs laid on dry deposit

Tabel 12. MYC 8005, na 24 uur bestraling met een zonlamp, 100.000 lux, vergeleken met niet-bestraalde stof. Percentage sterfte van larven van Tetranychus cinnabarinus.

Photochemical stability. To determine the light stability a thin layer of MYC 8005 was placed on a glass slide at the dosage of 100 $\mu g/cm^2$ and exposed to a 300 W Philips MLU

irradiation lamp at a light intensity of 100,000 lux for 24h. This lamp can be considered as a sun lamp because, besides visible light, also UV radiation as well as infrared radiation is emitted. The activity of the irradiated substance was compared with that of the untreated substance.

It appears from the data in Table 12 that the irradiated substance had only about one third of the initial activity. Such a decrease in activity does not occur when MYC 8005 is used in other laboratory tests. The climatically conditioned cabinets in which the incubation occurs are equipped with fluorescent light tubes that do not emit UV light. It seems reasonable, therefore, to assume that the loss of activity which occurs during irradiation with the sun lamp mentioned, is due to the UV fraction of the spectrum.

Toxicity to mice and fish. MYC 8005 is to be considered as highly toxic for mice. The LD_{50} of the purified antibiotic when administered orally to mice was 37 mg/kg (fiducial limits: 27 to 50 mg/kg) and when administered intraperitoneally to mice it was 1.5 mg/kg (fiducial limits: 0.7 to 2.5 mg/kg). MYC 8005 also proved toxic to *Lebistes reticulatus* Peters (guppy) when it was added to the water. A quantity of 2 μg/ml was lethal.

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Samenvatting

MYC 8005, een antibioticum tegen spintmijten. 1. Beschrijving van het producerende organisme (Streptomyces exfoliatus var. echinosporus var. nov.) en van de eigenschappen van het antibioticum

Tijdens een onderzoek van Actinomyceten op antibiotische werking werd een stam D 197 geisoleerd. De uit deze stam geïsoleerde stof, die MYC 8005 werd genoemd, bleek ook een sterke werkzaamheid tegen mijten te bezitten.

Stam D 197 vertoonde een sterke overeenkomst met *Streptomyces exfoliatus* (Waksman & Curtis) Waksman & Henrici (Tabel 1), maar week daarvan vooral af door het gestekelde oppervlak van de sporen. Daarom werd de stam D 197 als een afzonderlijke variëteit beschouwd. Voorgesteld werd hiervoor de naam *Streptomyces exfoliatus* var. *echinosporus* Den Admirant. De stam werd bij het Centraalbureau voor Schimmelcultures te Baarn gedeponeerd onder nr CBS 418.70.

Het antibioticum was vnl. aanwezig in het mycelium. De fysisch-chemische eigenschappen van MYC 8005 vertoonden veel overeenkomst met het antibioticum 323/58 (Kruglyak *et al.*, 1961; vergelijk Tabel 2). In de elementairanalyse werd een niet belangrijk verschil gevonden.

In vitro bleek MYC 8005 werkzaam te zijn tegen een aantal bacteriën en schimmels (Tabel 3). *In vivo* werden bladeren van verschillende planten door bespuiting beschermd tegen een aantal schimmelziekten (Tabel 4). Terwijl de werkzaamheid tegen insekten en volwassen mijten gering bleek te zijn, was de werking op spintlarven zeer

sterk (Tabel 5 en 6). De werkzaamheid op dicofol-,parathion- en tetradifon-resistente stammen was minstens even sterk (Tabel 7).

De werkingsduur onder laboratoriumomstandigheden was zowel t.o.v. gevoelige als van resistente stammen tenminste 4 weken (Tabel 8 en 9).

MYC 8005 vertoonde geen systemische werking en ook penetratie in het blad kon niet worden aangetoond (tabel 10). Het antibioticum bleek sterk aan het bladoppervlak te worden geadsorbeerd (Tabel 11). Bestraling van een dunne laag MYC 8005 met een zonlichtlamp met een intensiteit van 100.000 lux gedurende 24 uur had een aanzienlijk verlies van de werkzaamheid ten gevolge, vermoedelijk als gevolg van de ultraviolette straling (Tabel 12).

MYC 8005 bleek zeer giftig te zijn voor muizen en vissen. De LD 50 voor muizen bedroeg oraal 37 mg/kg; intraperitoneaal 1,5 mg/kg. Voor vissen, i.c. guppen was 2 mg MYC 8005 per liter water dodelijk.

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