

MYC 8005, an antibiotic against spider mites. 2. Growth inhibition and pseudo-sterilization in insects and mites

J. MELTZER

NV Philips-Duphar, 's-Graveland

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Abstract

The antibiotic MYC 8005, known as an acaricide, appeared to possess strong growth inhibiting properties, not only in immature stages of spider mites but also in larvae of several insect species. Adult mites and insects seemed not to be affected. However, the fecundity of female *Tetranychus cinnabarinus* was strongly reduced by deposits of MYC 8005 on the leaves of bean plants. The feeding of adult houseflies and Colorado potato beetles with MYC 8005 resulted in complete or almost complete sterilization of the insects. The sterilizing effect appeared to be caused by inhibition of maturing processes, in particular of yolk formation in the eggs, whereas follicles looked normal. These facts, and the strong reduction of the fat body in treated insects, suggest that the lipid and protein metabolism are disturbed by MYC 8005.

Introduction

In a recent paper (Den Admirant et al., 1972) the microbiological, fungicidal, and acaricidal properties of MYC 8005, an antibiotic isolated from *Streptomyces exfoliatus* var. *echinosporus* were described. The most obvious property with respect to a possible practical use appeared to be the considerable mite larvicidal activity. Likewise it was remarkable that neither adult mites nor eggs were affected, and that the larvae seemed to die by starvation rather than by poisoning. At 24°C and 70% r.h. the larvae normally moult into protonymphs within two days. On plants treated with MYC 8005, however, they remained in the larval stage until they died after 10-14 days. These facts suggest that MYC 8005 might act as a growth inhibitor.

In the above mentioned paper it was also shown that adult house flies (*Musca domestica* L.), aphids (*Aphis fabae* Scop.), third stage larvae of the large cabbage white (*Pieris brassicae* L.) and of the Colorado potato beetle (*Leptinotarsa decemlineata* Say) were not seriously affected by MYC 8005. Larvae of *Aedes aegypti* L. and *Drosophila melanogaster* Meig., however, were killed by concentrations of 10 and 3 ppm respectively.

The present paper describes a number of experiments, which demonstrated growth inhibiting effect on some insect species. Besides, histological studies revealed also that development in the ovary was inhibited by MYC 8005.

The methods as far as they are not described in this paper, are dealt with in Meltzer and Welle (1969) and Den Admirant et al. (1972).

Growth inhibition by MYC 8005

Leptinotarsa decemlineata. When third stage larvae of the Colorado potato beetle were used, only 43% were killed after 5 days by the deposit on potato leaves that had been sprayed with 100 ppm of MYC 8005. It was observed, however, that consumption of the leaves was remarkably reduced by the treatment.

To study more closely the behaviour of the larvae after treatment an experiment was made with dipped excised potato leaves in Petri dishes of 12 cm diameter. Each dish was provided with 10 just-hatched larvae. After 3 days all the larvae which were still alive were weighed. The results are summarized in Table 1. From this table it appears that all treatments of the first trial resulted in a considerable reduction of the weight of the larvae after 3 days and complete kill after 4 days. In a second experiment with lower concentrations it appeared that at 10 ppm the larvae survived, but still a considerable reduction in growth rate occurred. As can be seen in Fig. 1, the damage to the treated leaves is limited to very small holes. The untreated leaf shown in the picture was the fourth one that was consumed by the larvae, because the first three leaves were eaten completely. At first sight the impression is obtained that the antibiotic might be acting as a repellent or an antifeedant. This is not so, however, for there was never the slightest indication that the larvae tried to escape from the treated leaves. Moreover they readily began to feed, but feeding stopped very soon at the higher concentrations. On account of these results it seems reasonable to assume that the third stage larvae, of which only 43% were killed after 5 days, might have died later, if we had watched them longer. At 10 ppm, feeding as well as growth was decreased, but no mortality occurred. These facts, too, indicate that there is no real anti-feeding effect but that the reduced feeding is the result of intoxication.

Pieris brassicae. In the usual screening test with third stage caterpillars of *Pieris brassicae*, only 14% mortality was caused by the deposit on cabbage plants that had been sprayed with MYC 8005 at a concentration of 100 ppm. Also in this case a remarkable reduction of feeding was observed. In Petri dish trials with first stage

Table 1. Growth inhibition and mortality of first stage larvae of the Colorado potato beetle, *Leptinotarsa decemlineata* Say, after foliar treatment with MYC 8005.

Concentration (ppm)	Experiment 1		Experiment 2	
	weight of 10 larvae after 3 days (mg)	mortality after 4 days (%)	weight of 10 larvae after 4 days (mg)	mortality after 6 days (%)
1,000	6.0	100		
300	7.5	100		
100	9.5	100	8.0	100
30			9.9	100
10			30.8	0
0	45.0	0	85.0	0

Tabel 1. Groeiremming en sterfte van larven van het eerste stadium van de Coloradokever, *Leptinotarsa decemlineata* Say, na behandeling van de bladeren met MYC 8005.

Fig. 1. Growth inhibition by MYC 8005 in larvae of *Leptinotarsa decemlineata* on potato leaflets treated with 10, 30 and 100 ppm respectively as compared with normal development on untreated foliage.

Top: right untreated; left treated with 100 ppm.

Bottom: right treated with 10 ppm; left treated with 30 ppm.



Fig. 1. Groeiremming door MYC 8005 bij larven van *Leptinotarsa decemlineata* op aardappelblaadjes, behandeld met 10, 30 en 100 dpm in vergelijking met normale ontwikkeling op onbehandeld blad.

Boven: rechts onbehandeld; links behandeld met 100 dpm.

Onder: rechts behandeld met 10 dpm; links met 30 dpm.

caterpillars, performed in the same way as described above for *Leptinotarsa*, 100% mortality occurred at a concentration of 100 ppm (Table 2).

In another experiment, with lower concentrations also the weight of the caterpillars was determined. The results, listed in Table 2, show that growth is seriously inhibited by the MYC 8005 treatments. Fig. 2 illustrates the reduced feeding and the growth inhibition. The treated leaves were still the first ones to which the caterpillars were exposed; the untreated leaf shown in the picture was the fifth in succession, because the 4 leaves offered before were consumed completely.

Table 2. Growth inhibition and mortality of first stage caterpillars of *Pieris brassicae* L. after foliar treatment with MYC 8005.

Concentration (ppm)	Weight of 10 caterpillars after 4 days (mg)	Mortality after 6 days
1000	—	100
300	—	100
100	—	100
30	27.3	100
10	70.7	0
0	101.8	0

Tabel 2. Groeiremming en sterfte van rupsen van het eerste stadium van *Pieris brassicae* L. na behandeling van de bladeren met MYC 8005.

Fig. 2. Growth inhibition by MYC 8005 in larvae of *Pieris brassicae*. Left: untreated leaf. Right: leaf treated with 100 ppm MYC 8005.



Fig. 2. Groeiremming door MYC 8005 bij larven van *Pieris brassicae*. Links: onbehandeld blad. Rechts: blad behandeld met 100 ppm MYC 8005.

Adoxophyes orana. Just hatched first-stage caterpillars of the summer fruit tree leaf roller were used in tests on a wheat-germ casein diet. Small glass tubes of about 15 ml volume were filled for one third with the rearing medium, and provided with 2 larvae. For each treatment 10 tubes, with 20 caterpillars in all, were used.

From the control tubes 14 pupae out of 20 larvae were obtained, with a mean weight of 29 mg. When MYC 8005 was added to the diet at a concentration of 3 ppm only 8 out of 20 larvae completed development, and the mean pupal weight was 20

mg only. At 10 ppm only 1 pupa was obtained. Thus also in this species the growth rate of the surviving larvae is appreciably decreased.

Aedes aegypti. When third stage larvae of *Aedes aegypti* were exposed to MYC 8005, 3 ppm appeared to be lethal, whereas 1 ppm did not affect development. Eggs of *Aedes aegypti*, however, were killed at a concentration as low as 0.1 ppm. At 0.03 ppm the eggs hatched but the young larvae died within a few days. At 0.01 ppm hatching was not affected and the life cycle was completed normally.

Musca domestica. Larvicidal tests with the housefly were performed in plastic ice cream beakers of 100 ml, which were provided with 30 ml of rearing medium containing yeast and milk powder. Each beaker was provided with 20 eggs. MYC 8005 caused a high mortality and the growth of the surviving larvae was considerably reduced, as is demonstrated by the pupal weight (Table 3).

Drosophila melanogaster. When the Petri dish method was used with agar containing malt extract and nutrient salts (Den Admirant et al., 1971), eggs and young larvae were killed by MYC 8005 at concentrations as low as 1 ppm. However, when added to the usual diet of maize and living yeast even 100 ppm showed no detrimental effect at all. Whether this discrepancy is caused by the difference in antagonizing substances or by the fact that in the Petri dish method the antimicrobial activity of MYC 8005 is stronger because of the lack of the yeast, remains uncertain.

Table 3. Growth inhibition and mortality by exposing larvae of *Musca domestica* to MYC 8005 in the rearing medium.

Concentration (ppm)	Mortality* (%)	Mean weight of surviving pupae (mg)
0	0	27.3
100	26	20.1
300	93	17.8
1000	100	—

*Control mortality 32%. Listed percentages are calculated according to Abbott's formula.

Table 3. Groeiremming en sterfte na blootstelling van larven van *Musca domestica* aan MYC 8005 in de voedingsbodem.

Sterilizing activity of MYC 8005

Tetranychus cinnabarinus. In a previous paper (Den Admirant et al., 1971) it was shown that MYC 8005 possessed strong mite larvicidal activity. Neither adult mites nor the eggs were affected, but growth of the larvae was severely inhibited, and after 7–14 days they died before moult or in the protochrysalis stage.

After the spraying of plants with high concentrations it appeared that the fecundity of the females was reduced, and therefore some special experiments were designed. In one of these experiments bean leaves infested with deutonymphs were dipped in

Table 4. Fecundity of females of *Tetranychus cinnabarinus*, which had been dipped in the deutonymph stage in aqueous suspensions of MYC 8005.

Dip concentration (ppm)	Total number of eggs laid on untreated leaf discs during 4 days	Total number of eggs laid on leaves dipped in the same concentrations during 4 days
0	28.8	
10	31.4	21.0
100	24.3	17.0
1000	20.7	8.0

Tabel 4. Vruchtbaarheid van wijfjes van *Tetranychus cinnabarinus*, die in het deutonymf-stadium gedompeld zijn in waterige suspensies van MYC 8005.

aqueous suspensions of MYC 8005. Three days later, when all deutonymphs had developed into females, they were transferred to leaf discs of 22 mm diameter, placed on a wet cotton pad in Petri dishes. Each leaf disc was provided with one female only. After four days the number of deposited eggs was determined.

From the results, summarized in Table 4, it appears that the fecundity of the females which had been removed to untreated leaves was not affected by 10 ppm, whereas the 100 and 1000 ppm treatments resulted in a clear reduction of the number of eggs. More severe still was the reduction when the females remained on treated leaves.

When females were dipped in suspensions of MYC 8005 at concentrations of 10–1000 ppm and immediately transferred to untreated leaves, no reduction of fecundity was observed. Apparently contact with the antibiotic alone was not capable of inducing sterility. In another experiment, however, in which untreated females were exposed to treated leaves, a considerable reduction in egg deposition occurred (Table 5). In this experiment leaf discs of 22 mm diameter were brushed with an acetonc solution of MYC 8005. On each leaf disc 3 females of 0–2 days old were placed and every 3 days transferred to new leaf discs. For each treatment 10 discs were used. Although 10 ppm caused a considerable reduction in egg numbers, it is remarkable

Table 5. Egg production of females of *Tetranychus cinnabarinus* on leaf discs treated with MYC 8005.

Concentration (ppm)	Egg production during day 1–3		Egg production during day 4–6		Egg production during day 7–9	
	total	mean number per female per day	total	mean number per female per day	total	mean number per female per day
0	507	7	631	10	334	8
10	341	5	544	7	218	5
100	197	3	185	3	102	3
1000	66	1	0	0	0	0

Tabel 5. Eiproduktie van wijfjes van *Tetranychus cinnabarinus* op bladschijfjes, die behandeld zijn met MYC 8005.

that even at 100 ppm a number of eggs were still laid. The highest concentration was the only one that inhibited oviposition completely after one or two days. All the eggs hatched normally, though the larvae died later on account of the residual larvicidal activity of the deposit.

Leptinotarsa decemlineata. In order to study the possible sterilizing properties of MYC 8005 on the Colorado potato beetle, use was made of newly emerged beetles. They were placed on cut potato haulms standing in water. Over the plants a plexiglass cylinder of 13 cm diameter and 20 cm height was placed. The potato haulms were treated by dipping into the appropriate concentrations, and not before the plants had dried were the beetles put on the leaves. Each cylinder contained 5 female and 5 male beetles. There were two replicates.

As can be seen in Table 6, egg production was considerably reduced by the treatments with 1 and 3 ppm and inhibited completely by 10 ppm. Food consumption also was considerably reduced. This might suggest that MYC 8005 was acting like an antifeedant or a repellent, but during the whole period of 20 days there was not the slightest evidence that the beetles tried to escape from the treated leaves or to search for other food.

On dissection of the treated female beetles it appeared that the ovaries were not or but poorly developed, and also the fat body was strongly reduced.

Table 6. Sterilizing effect of MYC 8005 on newly emerged Colorado potato beetles, *Leptinotarsa decemlineata*. Number of eggs after 20 days per 5 females.

Concentration (ppm)	Food consumption	Number of eggs
0	normal	> 1.000
1	less	± 300
3	slight	23
10	nearly none	0

Tabel 6. Steriliserend effect van MYC 8005 op pas uitgekomen Coloradokevers, *Leptinotarsa decemlineata*. Aantal eieren na 20 dagen per 5 wijfjes.

Musca domestica. Sterilization tests with *Musca domestica* were performed in triplicate in plexiglass cylinders of 13 cm diameter and 20 cm height, covered with nylon gauze. Each cylinder was provided with 10 male and 10 female newly emerged flies. They were offered sugar, water, and a milk powder suspension as food. After 4 days the milk suspension was removed for examination and replaced by a new one. After another 4 days the second portion was examined.

MYC 8005 appeared to be harmless to the adult flies, for after 8 days there was in general no mortality observed in the treatments, which varied from 100–10,000 ppm MYC 8005 in the milk powder suspension. In one case, 4–8% mortality occurred in the 1% treatment.

Egg deposition was more or less normal in the 100 ppm treatment, but the 1,000–10,000 ppm treatment prevented oviposition to a high degree. At 10,000 as well as

Fig. 3. Ovaries of 4-day old *Musca domestica*. Top: untreated females. Middle: untreated females fed with sugar water only. Bottom: females fed with 0.1% MYC 8005, incorporated in a full-milk powder suspension. Magnification $\times 10$.

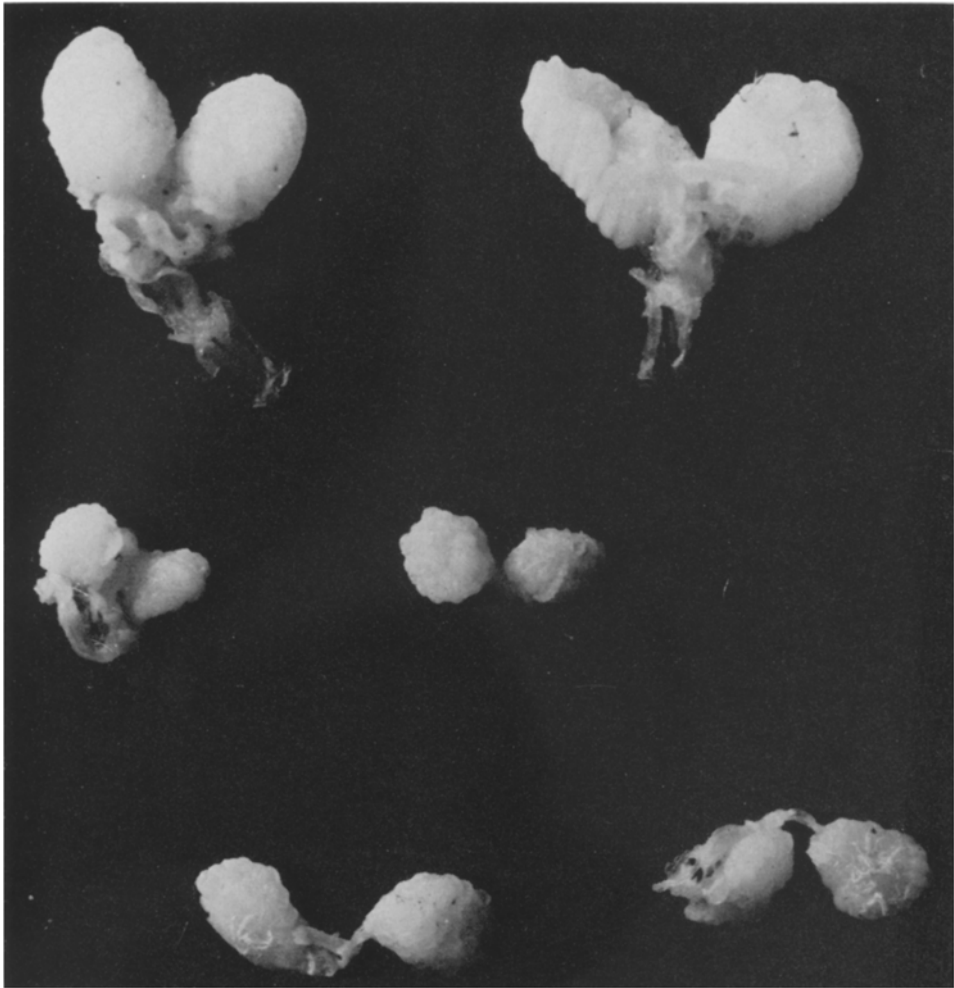


Fig. 3. Ovaria van *Musca domestica* van 4 dagen oud. Boven: onbehandelde wijfjes. Midden: wijfjes gevoerd met uitsluitend suikerwater. Onder: wijfjes gevoerd met 0,1% MYC 8005, verwerkt in een suspensie van volle-melkpoeder. Vergroting ca. $10\times$.

in 1,000 ppm only a few eggs were laid representing less than 0.1% of the number of eggs laid by untreated flies.

On dissection, the ovaries of the treated flies appeared undeveloped, like those of flies which had been fed with sugar water only. Fig. 3 shows the enormous difference in size between the ovaries of normal and treated flies. Histological examination showed that the follicles in the ovaries of the treated flies were normally formed but that yolk formation did not occur. This is clearly demonstrated in Fig. 4 and 5.

Fig. 4. Section of the ovary of an untreated 4-day old *Musca domestica*. Note the abundant yolk formation. Magnification $\times 300$.



Fig. 4. Doorsnede van het ovarium van een onbehandelde *Musca domestica* van 4 dagen oud. Let op de sterke dooivorming. Vergroting ca. $300\times$.

Discussion

The antibiotic MYC 8005 appears to inhibit growth in developing stages of mites and insects. Even at low dosages this inhibition of immature stages results in eventual death. In the adult mites and insects the compound inhibits the maturing of eggs, and as a consequence oviposition is reduced or prevented. If it had not been known that MYC 8005 was a potent toxicant, the impression might be gathered that both the observed growth inhibition and the sterilization were the result of starvation, in view of the observation that feeding of mites and insects on treated plants was strongly reduced.

Several authors mention the antifeedant and sterilizing properties of organotin compounds (among others Ascher and Meisner, 1969). Objections can be raised against the use of the terms antifeedant and sterilant for compounds that are cell poisons. Besides, it is well known that any poisoning may lead in the end to loss of

Fig. 5. Section of the ovary of a 4-day old *Musca domestica* fed with 0.1% MYC 8005 in a full-milk powder suspension. Note the absence of yolk formation. Magnification $\times 300$.

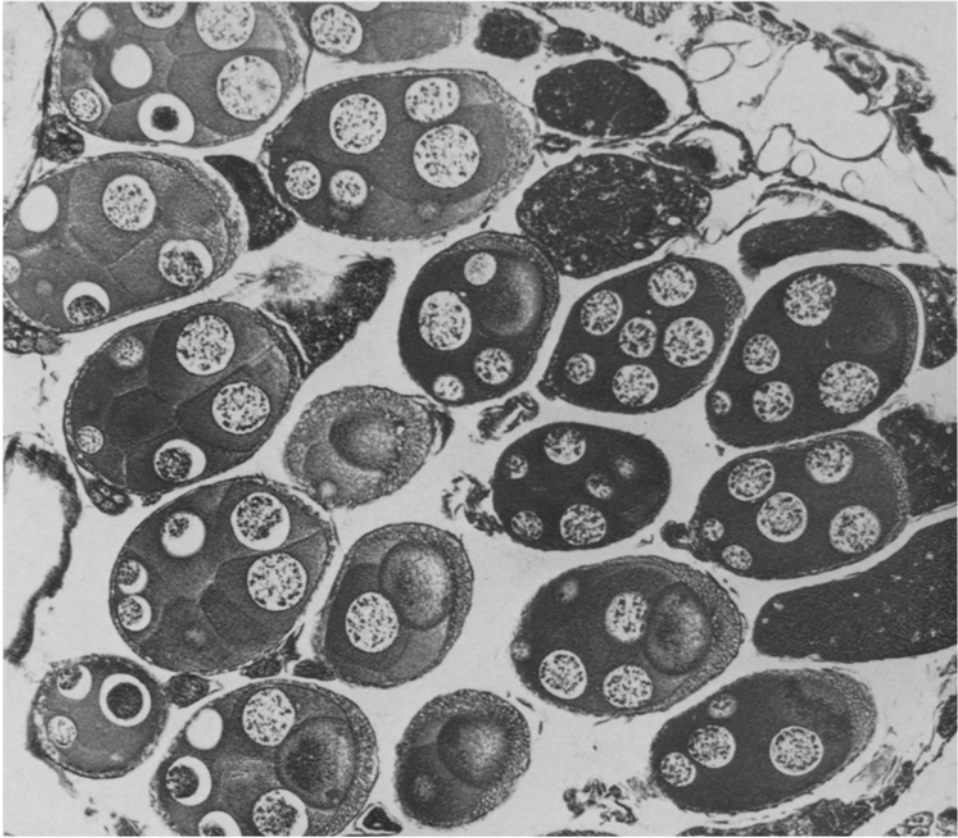


Fig. 5. Doorsnede door het ovarium van *Musca domestica* van 4 dagen oud, gevoerd met 0,1% MYC 8005 in een volle-melkpoedersuspensie. Let op de afwezigheid van dooivorming. Vergroting ca. 300 \times .

appetite and weight, growth inhibition and inhibition of oviposition. Therefore the term antifeedant should be reserved for those compounds that make food unacceptable to insects without poisoning them. Whenever a real antifeedant is involved, it is observed that the insects will search for alternative food. Food plants treated with MYC 8005, however, are not rejected by insects and mites. On the contrary they are normally accepted, but soon the insects and mites become intoxicated and then stop feeding. Hence death in the immature stages is due to intoxication and not to starvation as a result of an antifeedant effect.

Adult insects and mites are not killed by foliar applications of MYC 8005, the only obvious symptom of intoxication being an inhibition of oviposition. As has already been mentioned, yolk formation in the house fly is prevented by MYC 8005. As a matter of fact this could be a secondary effect of intoxication, and it seems logical to compare this situation with that of the organotin compounds mentioned above. In these latter compounds there is a very narrow margin, if any, between the lethal con-

centration and the concentration that prevents oviposition. Moreover the non ovipositing females distinctly show other symptoms of intoxication.

As both larval growth and growth of the oocytes are affected by MYC 8005, it seems reasonable to assume that these phenomena are correlated. It is likely that MYC 8005 acts as an antimetabolite, interfering with lipid and protein metabolism. This is supported by the fact that treated adult insects show, on dissection, strongly reduced fat bodies and inhibition of yolk formation.

If the use of the term sterilization is restricted to those compounds that act specifically on the reproductive organs, MYC 8005 cannot be considered as a real sterilant. Therefore the term pseudo-sterilization is suggested, in order to emphasize the most obvious phenomenon caused in adult insects and mites.

Samenvatting

MYC 8005, een antibioticum tegen spintmijten. 2. Groeiremming en pseudo-sterilisatie bij insecten en mijten

Het antibioticum MYC 8005 blijkt niet alleen voor mijten een sterke groeiremming te zijn, maar ook voor insectenlarven. Larven van *Leptinotarsa decemlineata* werden gedood door een residu van een bladbehandeling met een concentratie van 30 dpm MYC 8005 en hoger. De groei werd door deze lethale concentraties evenals door 10 dpm sterk geremd (Tabel 1, Fig. 1). Larven van *Pieris brassicae* werden door dezelfde concentraties gedood, resp. geremd in groei (Tabel 2, Fig. 2).

Rupsen van *Adoxophyes orana* werden gedood als aan de kunstmatige voedingsbodem 10 dpm MYC 8005 werd toegevoegd. Bij een concentratie van 3 dpm wogen de overlevende poppen slechts 20 mg tegenover 29 mg van de onbehandelde.

Larven van het derde stadium van *Aedes aegypti* werden door 3 dpm MYC 8005 gedood, de eieren zelfs door 0,1 dpm. Bij een concentratie van 0,03 dpm kwamen de eieren wel uit, maar de jonge larven waren binnen enkele dagen dood.

Bij larven van *Musca domestica* werd meer dan 90% sterfte verkregen als aan de voedingsbodem 300 dpm MYC 8005 werd toegevoegd. Bij een concentratie van 100 dpm overleefde een hoog percentage de behandeling, maar het popgewicht was aanzienlijk lager dan van de onbehandelde (Tabel 3).

De vruchtbaarheid van *Tetranychus cinnabarinus* werd aanzienlijk verminderd door MYC 8005. Desalniettemin werd een absolute sterilisatie eerst verkregen bij een behandeling van de bladeren met 1000 dpm (Tabel 4 en 5).

De vruchtbaarheid van *Leptinotarsa decemlineata* werd aanzienlijk gereduceerd door behandeling van aardappelblad met 1 en 3 dpm MYC 8005; een totale sterilisatie werd met 10 dpm verkregen (Tabel 6). Bij het histologisch onderzoek van de behandelde kevers bleken de ovaria slecht ontwikkeld te zijn en het vetlichaam was sterk gereduceerd.

Hoewel MYC 8005 niet schadelijk was voor volwassen *Musca domestica*, veroorzaakten concentraties van 1.000 tot 10.000 dpm in het voedsel een totale sterilisatie. De ovaria van de behandelde vliegen waren aanzienlijk kleiner dan van de onbehandelde. De follikels ontwikkelden zich normaal, maar de dooivorming vond niet plaats (Fig. 4 en 5).

In de discussie worden de remming van de groei en van de vruchtbaarheid verge-

leken met de in de literatuur genoemde 'antifeeding' en steriliserende eigenschappen van organische tinverbindingen. Zowel de organische tinverbindingen als MYC 8005 zijn geen 'antifeedants', omdat zij de insecten en mijten vergiftigen. Men mag pas van 'antifeedant' spreken, als het voedsel door behandeling met de stof niet meer wordt geaccepteerd, zonder dat vergiftiging optreedt.

Daar zowel de groei van onvolwassen stadia als de dooiervorming door MYC 8005 wordt geremd, worden beide verschijnselen met elkaar in verband gebracht. MYC 8005 werkt als een anti-metaboliët, die in de vet- en eiwitstofwisseling ingrijpt, hetgeen gesteund wordt door het feit dat bij behandelde volwassen insecten het vetlichaam sterk is gereduceerd en dooiervorming uitblijft.

Indien de term sterilisatie beperkt moet blijven tot verbindingen, die specifiek op de geslachtsorganen werken, kan MYC 8005 niet als een sterilisant worden beschouwd. Daarom wordt de term pseudosterilisatie voorgesteld ten einde de nadruk te leggen op het meest opvallende verschijnsel, dat de stof in volwassen insecten en mijten veroorzaakt.

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Present address

Jan Steenlaan 20, Nieuw-Loosdrecht, the Netherlands