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Susceptibility of *Oryctes rhinoceros* Adults to *Metarrhizium anisopliae*

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The susceptibility of *Oryctes rhinoceros* adults to infections due to entomopathogenic fungi (*Beauveria bassiana, Beauveria tenella, Metarrhizium anisopliae, Paecilomyces fumoso-roseus,* and *Spicaria rileyi*) was studied by spraying titrated spores suspensions on the insect integument. The results show a definite susceptibility of the adults to *Metarrhizium anisopliae* strains of the major type only.

The susceptibility of *Oryctes* sp. (Coleoptera, Scarabaeidae) larvae to the mycosis caused by *Metarrhizium anisopliae* (Fungi Imperfecti, Moniliales) has been reported several times since Friederichs's (1919,1920) observations. Until recent years, a detailed biological study of this injurious insect of palms has been difficult because of the high natural mortality caused by this fungus during the rearing of the insect. It is only recently (Hurpin and Mariau, 1966; Hurpin and Fresneau, 1966, 1967, 1970) that these difficulties have been overcome, which has encouraged the development of research to find a means of control most adapted to the particular ethology of this coleopteran.

If the introduction of a viral disease caused by *Rhabdionvirus* appears to be a promising biological control method (Marschall, 1970; Hammes, 1971; Monty, 1972; Zelazny, 1973), the research on the potentialities of *M. anisopliae* should not be neglected (Nirula et al., 1955; Rhada et al., 1956; Marschall, 1968; Diomandé, 1969). Recently, the specific action of certain strains of entomopathogenic fungi on *Oryctes* larvae has been emphasized (Ferron and Diomandé, 1969; Ferron et al., 1972). In this study we have examined the susceptibility of *Oryctes rhinoceros* adults to several entomopathogenic fungi.

MATERIAL AND TECHNIQUES

Culture of insects. The adults were obtained from an insectary stock that has been maintained at La Minière for several years (Hurpin and Fresneau, 1967). The stock of *Oryctes* was reared in groups of ca. 100 larvae in 1 cu. meter containers. However, these experiments were made with lots of 10 or 20 adults individually reared in 250-ml cylindrical plastic boxes two-thirds filled with moistened peat. The tests were conducted at 28°C and examined weekly for 4–5 mo.

Contamination by fungi. In addition to *M. anisopliae,* the *Oryctes* were subjected to contamination by other fungi: *Beauveria bassiana, B. tenella, Paecilomyces fumoso-roseus,* and *Spicaria rileyi.* However, the largest number of experiments was carried out with different strains of *M. anisopliae,* classified as either the minor or the major types according to the separation established by Johnston (1915) and revived by Veen (1968). The characteristics of the strains used are given in Table 1.

Multiplication of the conidiospores occurred on agar nutritive medium, in Roux flasks, at the optimum growing temperature for each species. The inocula were prepared as homogenized aqueous suspensions (supplemented with Tween 80 in a concentration of 0.2% in the case of *Metarrhizium*), ti-

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TABLE 1
Characteristics of the Strains of Entomopathogenic Fungi used in the Experiments

Species	No.	Original host
Metarrhizium anisopliae		
minor form	41	*Phyllophaga pleei*
	48	*Bombyx mori*
	52	*Melolontha melolontha*
	59	*Hoplia* sp.
	60	Noctuidae
	61	*Thaumetopoea pityocampa*
	70	*Anagyrus* sp.
	71	*Leptinotarsa decemlineata*
major form	5[a]	*Strataegus aloeus*
	32	*Cetonia aurata*
	51	*Oryctes rhinoceros*
	54	*Oryctes monoceros*
	55	*Oryctes elegans*
	56	*Oryctes boas*
	66	*Oryctes nasicornis*
Paecilomyces fumoso-roseus	4	*Mamestra brassicae*
	6	*Cirphis unipuncta*
	7	*Carpocapsa pomonella*
	8	*Melolontha melolontha*
	9	*Mamestra brassicae*
Spicaria rileyi	3	*Mamestra brassicae*
Beauveria bassiana	32	*Leptinotarsa decemlineata*
Beauveria tenella	6[b]	*Melolontha melolontha*

[a]No. 5 = American Type Culture Collection No. 26468.
[b]No. 6 = A.T.C.C. 26156.

trated by Malassez hematimetric cell, and adjusted to the desired concentrations (from 1.10^8 to 1.10^2 conidia/ml).

The adults were infected by spraying with 10 ml of these suspensions using the apparatus described by Burgerjon (1956) for the biological titration of *Bacillus thuringiensis* preparations. After the tegument of the insects was air dried, they were held in culture under the conditions previously described.

Pathological controls. During each weekly examination, the cadavers were set in a humid chamber to promote the external development of the mycelium. The diagnosis was made according to the morphology of the conidiophores and spores formed. A more detailed microscopical examination was essential in the specific cases of infection by strains of *M. anisopliae* in order to differentiate the strains of the major type or of the minor type according to their respective sizes.

RESULTS AND DISCUSSION

Age influence of the imagos on susceptibility to the green muscardine disease. Due to the longevity of the *Oryctes* imagos (up to 6 mo or sometimes more under our rearing conditions), two preliminary experiments were made to determine the influence of the host age on susceptibility to the mycosis. These experiments were carried out with two strains of *M. anisopliae* (M.a. No. 51 and M.a. No. 66). Lots of insects 1, 2–4, 7–9, 16–18 wk old, respectively, were infected under identical conditions by suspensions of spores titrated to 1.10^2 to 1.10^7 spores/ml.

The values presented in Table 2 show that, in general, we obtained a mortality due to mycosis with about 50% of insects sprayed with 10 ml of a suspension containing 1.10^5 conidia/ml. However, practically all the lots were attacked by the fungus in higher concentrations. A graphic calculation of the 50% lethal time (LT_{50}) does not reveal differences in susceptibility to infection according to the age of the insect at low doses: with a concentration of 1.10^5 spores/ml, the LT_{50} was 95, 85, and 100 days for insects 1, 4, and 6 wk old, respectively, when infected with strain M.a. No. 51; and 65, 75, and 60 days for the lots of insects 1, 2–4, 7–9 wk old when infected with strain M.a. No. 66. This uniformity was not observed when the infection involved higher doses. Thus with strain M.a. No. 66 at a concentration of 1.10^7/ml, we obtained a LT_{50} of 45, 35, and 15 days for adults 2–4, 7–9, and 16–18 wk old, respectively.

Because of the age of insects used in the experiments can cause a marked variation in the responses obtained, this variable was avoided in later experiments by using only lots of insects of the same age (4 wk old).

Susceptibility of Oryctes rhinoceros *imagos to* Beauveria bassiana, B. tenella, Paecilomyces fumoso-roseus, *and* Spicaria rileyi. The contamination of insects was achieved by spraying with titrated suspensions of 1.10^4 to 1.10^8 conidia/ml. Under these conditions, no case of mycoses was observed regardless of the species or the strain of fungi used. With the *Beauveria bassiana* No. 32 strain, it was necessary to utilize a more concentrated pathogenic inoculum, 1.10^9 conidia/ml, in order to obtain four cases of white muscardine disease from the ten infected *Oryctes*.

Comparative susceptibility of adults of Oryctes rhinoceros *to the major and minor forms of* Metarrhizium anisopliae. Because it was demonstrated in former studies (Ferron and Diomandé, 1969; Ferron et al., 1972) that the larvae of *Oryctes* sp. are selectively susceptible to strains of the major form isolated from insects of the genus *Oryctes*, the development of the green muscardine fungus in adults was studied in the light of these observations.

Virulence of minor form strains. Eight strains of the minor form, isolated from insects that belong to very different taxonomic groups, Coleoptera and Lepidoptera, were tested. The green muscardine fungi of the minor type which were identified during the 15 wk of this study are given in Table 2. In general, the susceptibility of imagos to infections seems to be very slight with any of the strains utilized. It is only when a large quantity of spores (1.10^8 conidia/ml) obtained from only two strains (M.a. No. 41 and M.a. No. 59) was employed that a definite development of mycosis was noted (five and four cases of ten individuals tested). These results, therefore, are analogous to those obtained with *Beauveria bassiana*.

Virulence of major form strains. The great majority of the strains studied in this series of experiments differ from the previous ones in that they come from hosts that belong to only one taxonomic group. The rearing of different species of *Oryctes* (*O. rhinoceros, O. monoceros, O. nasicornis, O. elegans,* and *O. boas*) at the insectary at La Minière during recent years has resulted in the isolation of various strains of *Metarrhizium*. Only two strains of the major type, isolated from another genus of the Scarabaeidae, were used. These were biotypes that came from *Cetonia aurata* (Cetonidae) and *Strataegus aloeus* (Dynastidae).

The results (Table 3) indicate a clear variation in the responses of the adults, depending upon the origin of the *Metarrhizium* strains. The strains isolated from different species of *Oryctes* generally cause a mortality due to mycosis in more than half of insects infected by a pathogenic inoculum with a concentration equal to or higher than 1.10^6 spores/ml. However, the strain isolated from *Strataegus* was pathogenic only in concentrations 10 or 100 times greater. The strain isolated from *Cetonia* was not lethal to *Oryctes rhinoceros*.

The particular case of strain No. 69 of M. anisopliae. In July 1972, we received from

TABLE 2
Susceptibility of Adults of *Oryctes rhinoceros* to Mycosis Caused by *Metarrhizium anisopliae* in Relation to their Age

	Age of the adults at the time of the treatment (wk)	50% Lethal time (LT_{50}) and mortality by mycosis (m) in relation to the dose of spores after 5 mo of breeding													
		1.10^7/ml		1.10^6/ml		1.10^5/ml		1.10^4/ml		1.10^3/ml		1.10^2/ml		Control	
		LT_{50}	m	LT_{50}	m	LT_{50}	m	LT_{50}	m	LT_{50}	m	LT_{50}	m	LT_{50}	m
M. anisopliae Strain No. 51	1	55	10/10	65	10/10	95	5/10	105	1/10	115	5/10	115	0/10	110	0/10
	4	55	10/10	65	8/10	85	6/10	110	1/10	110	0/10	105	1/10	110	0/10
	6	35	9/10	60	8/10	100	2/10	100	0/10	100	0/10	100	0/10	105	0/10
M. anisopliae Strain No. 66	1			45	9/10	65	6/10							100	0/10
	2- 4	45	12/12	60	9/12	75	6/12	90	0/12					100	0/12
	7- 9	35	10/10	45	10/10	60	5/10							85	0/10
	16-18	15	10/10	20	8/10									40	0/10

TABLE 3
Green Muscardine Disease Caused by *Metarrhizium anisopliae* After Infection of the Adults of *Oryctes rhinoceros* by Spores of Minor Type (Lots of 10 Insects)

Strains of *M. anisopliae*	Original host	Mortality by mycosis in relation to the concentration of the inoculum					
		1.10^8/ml	1.10^7/ml	1.10^6/ml	1.10^5/ml	1.10^4/ml	Control
M.a. No. 41	*Phyllophaga pleii*	5	1	0	0	0	0
M.a. No. 48	*Bombyx mori*	1	0	0	0	0	0
M.a. No. 52	*Melolontha melolontha*	1	0	0	0	0	0
M.a. No. 59	*Hoplia* sp.	4	3	0	0	0	0
M.a. No. 60	Noctuidae	1	0	1	1	1	0
M.a. No. 61	*Thaumetopoea pityocampa*	1	1	2	0	1	0
M.a. No. 70	*Anagyrus* sp.	2	1	0	0	0	0
M.a. No. 71	*Leptinotarsa decemlineata*	0	0	0	0	0	0

Wallis Island, in the South Pacific, a sample of *Oryctes* sp. affected with green muscardine fungus. Isolation of the sample yielded a mixed infection of a minor and major type. These were separated and designated M.a. No. 69A (minor form) and M.a. No. 69B (major form). The virulence of these two strains was studied in larvae as well as in adults of *Oryctes rhinoceros* (Table 4). The results support the conclusion that the strains of the major type are more pathogenic to *Oryctes*. A simultaneous infection by the minor form and the major form, on the larvae as well on adults, with concentrations of inocula varying from 2.10^5 to 2.10^7 spores/ml (at the following mixtures: 1.10^5 spores/ml of M.a. No. 69A + 1.10^5 spores/ml of M.a. No. 69B, 1.10^6 spores/ml of M.a. No. 69A + 1.10^6 spores/ml of M.a. No. 69B, 1.10^7 spores/ml of M.a. No. 69A + 1.10^7 spores/ml of M.a. No. 69B), did not show a mixed infection by the two strains as was observed with the original sample.

In the absence of supporting data, which would only be possible to obtain as a result of systematic examination of a large number of field-collected samples, it is difficult to interpret this particular case. A separate analysis of the specificity of the two strains, M.a. No. 69A and M.a. No. 69B, agrees with the observations made with other biotypes of these two forms. However, the development of a minor form on an adult initiated investigation into the possibility that a saprophytic development could occur on a cadaver following an accidental infection. It was determined that it was

TABLE 4
Case of Green Muscardine Disease Caused by *Metarrhizium anisopliae* After Infection of the Adults of *Oryctes rhinoceros* by Spores of Major Type (Lots of 10 Insects)
Co = Control

Strains of *M. anisopliae*	Original host	Mortality by mycosis in relation to the concentration of the inoculum						
		1.10^8/ml	5.10^7/ml	1.10^7/ml	1.10^6/ml	1.10^5/ml	1.10^4/ml	Control
M.a. No. 5	*Strataegus aloeus*	4		3	0	1	1	1
M.a. No. 32	*Cetonia aurata*	0		0	0	0	0	0
M.a. No. 51	*Oryctes rhinoceros*			10	8	6	0	0
M.a. No. 54	*Oryctes monoceros*		10	5	5	0	0	0
M.a. No. 55	*Oryctes elegans*	10		8	4	2	2	0
M.a. No. 56	*Oryctes boas*			10	6	2	3	0
M.a. No. 66	*Oryctes nasicornis*	10		10	6	4	0	0

TABLE 5
Comparative Development of the Green Muscardine Disease on Larvae and on Adults of *Oryctes rhinoceros* After Infection by the Strains M.a. No. 69A (Minor Form) and M.a. No. 69B (Major Form)[a]

Strains of *Metarrhizium*	Development of the mycosis on larvae				Development of the mycosis on adults			
	1.10^7/ml	1.10^6/ml	1.10^5/ml	Control	1.10^7/ml	1.10^6/ml	1.10^5/ml	Control
M.a. No. 69A	0	0	0	0	1	1	1	0
M.a. No. 69B	7	6	0	0	7	7	2	0

[a]Note: Each experiment involved 10 insects, third-instar larvae or adults. In both cases the infection was obtained by spraying titrated suspensions of spores.

possible to have a partial development of a minor strain on a cadaver if a very concentrated inoculum of 1.10^8 spores/ml was sprayed on an insect that has been dead for just a few hours. The same infection has no effect when occurring on old cadavers which have probably been invaded by bacterial flora. This is why we consider the sample studied as an individual case does not contradict the results of our laboratory experiments.

CONCLUSION

The experiments on the infection of *Oryctes rhinoceros* by different species of entomopathogenic fungi (Fungi Imperfecti) confirm their particular susceptibility to the green muscardine disease caused by *Metarrhizium anisopliae*. As we have already pointed out in a similar study with larvae (Ferron et al., 1972), only the strains of the major type isolated from insects belonging to the genus *Oryctes* seems to be pathogenic. In the laboratory, about half of the infected insects died by mycosis when the inoculum used reached a concentration of about 1.10^5 to 1.10^6 spores/ml. The time necessary to obtain this mortality was 75–80 days. The total mortality caused by mycosis was obtained only with concentrations of about 1.10^7 spores/ml. The LT_{50} could be decreased under optimum conditions to approximatively 35 days.

As we have established in the case of the mycosis caused by *Beauveria tenella* in *Melolontha melolontha* (Ferron, 1972), the adults of *Oryctes* most likely play an important role in the propagation of the disease. Nevertheless, their susceptibility to the mycosis, which is lesser than that of *M. melolontha*, probably gives a very limited effect to this phenomenon, which could be determined only by the collection of large samples in their natural biotopes and holding them for a prolonged quarantine period.

Also, it has been observed that the saprophytic development of strains of *Metarrhizium* of the minor type was possible on cadavers if they had been infected by a very concentrated inoculum of spores and if the cadavers had not already undergone bacterial decomposition.

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