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INFLUENCE OF RELATIVE HUMIDITY ON THE DEVELOPMENT OF FUNGAL INFECTION CAUSED BY *BEAUVERIA BASSIANA* [*FUNGI IMPERFECTI, MONILIALES*] IN IMAGINES OF *ACANTHOSCELIDES OBTECTUS* [*COL. : BRUCHIDAE*]

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The incubation of imagines of *Acanthoscelides obtectus* SAY inoculated with conidia of *Beauveria bassiana* (BALS.) VUILLEMIN under different relative humidities, between 0 and 100 % R. H., shows that infection develops independantly of the humidity; the development of the fungus on the cadaver is only possible when the humidity values are near the saturation point.

Entomogenous fungi characteristically penetrate their insect host through the integument, in contrast to bacteria and viruses which enter only via the alimentary tract. A prime importance is attributed, therefore, to the relative humidity of the ambient atmosphere which may control the development of infective processes of *Fungi Imperfecti* such as *Beauveria*, *Metarhizium*, *Nomuraea* and *Paecilomyces* (MÜLLER-KÖGLER, 1965).

Analysis of recently published literature (MAC LEOD *et al.*, 1956; MOORE, 1973; PRIMAK, 1968; ROBINSON, 1966; WALSTAD *et al.*, 1970) shows that it is experimentally possible to infect insects with fungi at low relative humidities (45 to 70 %), whereas under natural conditions an epizootic only becomes apparent in very humid conditions (SCHAERFFENBERG, 1966).

On the basis of our own observations and from published data, we have been able to frame the hypothesis that a fungal infection progresses through the following 3 steps :

1. the initial infective phase is less dependant on the relative humidity conditions than previously generally understood due, perhaps, to a boundary layer of moist air which envelops the insect integument in an analogous way to the situation found in plants (WAGGONER, 1965),

2. the incubation phase of the disease, characterised by mycelial development and production of toxins in the haemolymph, progresses in conditions which are relatively independant of the ambient relative humidity,

3. saprophytic and reproductive phases of the fungus are characterised by mycelial invasion of all the tissues giving rise to a mummified cadaver of the insect and then development of conidiophores on the body surface associated with high relative humidity, nearing saturation point.

(1) Avec la collaboration technique de P. H. ROBERT et ANNICK GLANDARD.

MATERIAL AND METHODS

This hypothesis was tested by a demonstration and the results obtained follow below in the present note : imagines of *Acanthoscelides obtectus* SAY (*Col. : Bruchidae*) were inoculated indirectly by spraying 10 ml of an aqueous suspension of *Beauveria bassiana* (BALS.) VUILLEMIN (strain Bb n° 32 isolated from *Leptinotarsa decemlineata* SAY), containing 1×10^8 conidia/ml, on the inner surfaces of sterile plastic Petri dishes. Into each dish, 10 insects were introduced and left in contact with the inoculum for 24 hours at 25°C in continuous light (FERRON & ROBERT, 1975). The beetles were then placed in identical but clean Petri dishes perforated with a pin to allow gaseous exchanges between the dish and the environment inside a plastic dessicator. The relative humidities were controlled using the following = H_2O dist. (100 % R.H.), glycerol solution in distilled water (respectively 10 %, 20 % and 40 % per weight of glycerol to give 98 %, 95 % and 85 % of R.H.), saturated aqueous solutions of Na_2CO_3 (92 % R.H.), NaCl (76 % R.H.), Ca $(NO_3)_2$, $4H_2O$ (55 % R.H.), $CaCl_2$, 6 H_20 (35 % R.H.) and anhydrous $CaCl_2$ (0 % R.H.). The dessicators were placed at 20°C + 1°C with 16/24 hours of photoperiod.

RESULTS

In a preliminary series of experiments, tests were made at 7 relative humidities : 0 %, 35 %, 55 %, 76 %, 92 %, 98 % and 100 % R.H. Values were obtained for 2240 beetles in batches of 10; 320 insects per dessicator, 160 control insect and 160 test insects inoculated with the fungus. Each batch of 160 beetles was again divided into 2 sub-batches of 80, to compare the mycosis after death of cadavers in the humidity conditions of the corresponding dessicator with those in saturated humidity.

Under these conditions we obtained 100 % mortality in the infected batches 10 days after the beginning of incubation irrespective of the humidity under consideration. Acceleration of the infective process was noted in relation to increasing humidity (100 % mortality after 8 days at 100 % R.H.). For the same time periods in the control batches, mortality varied between 31 and 56 %, the highest values being obtained at the lowest relative humidities. No mycosis occurred in dead uninoculated insects placed in a saturated humidity, which shows that the observed mortality in the control batches cannot be attributed to the endemic presence of *B. bassiana* in the laboratory strain of *A. obtectus* or to accidental contamination during treatment.

Among the treated batches, the external development of mycelium on cadavers was only observed at relative humidity values higher than 92 % R.H., whereas, in all the batches, the fungus developed exteriorly when the cadavers were placed in a saturated humidity.

A 2nd series of experiments limited to 5 high values of relative humidity (85 %, 92 %, 95 %, 98 % and 100 % R.H.) carried out on 1600 beetles, confirmed the following results : 100 % mortality in the infected insects occurred after 7 days at humidities equal or superior to 95 % and after 9 days at 92 % and 85 % R.H. For the same periods, the mortality of control insects was less than 10 %. The development of mycelium at the surface of the cadavers occurred in all cases where insects were placed in saturated humidity, but only at humidities higher than 92 %, when insects had been kept at constant humidity levels from the start of the experiment. Moreover, it was found that the degree of mycelial development was more or less proportional to relative humidity values between 92 % and 98 %, suggesting that more detailed studies with improved techniques for measuring lower relative humidity values would be justified.

A 3rd experiment was carried out in order to confirm the previously obtained results. It seems, in fact, that there is a risk of erroneous interpretation of these results, given that the initial inoculation phase of insects, consisting of contact with the sprayed internal surface of Petri dishes, was not carried out under controlled relative humidity conditions in either of the previous experiments. An identical experiment was carried out, therefore (using 0 %, 35 %, 50 %, 76 %, 92 % and 100 % R.H.), taking care to place the insects in the above humidities as soon as they came into contact with the pathogen. For this purpose, spores were sprayed in Petri dishes previously perforated with pins to allow gaseous exchange with the ambient atmosphere as opposed to the unperforated dishes used previously. When the Petri dishes had been air dried the insects were introduced and the dishes were immediately placed in dessicators adjusted to the desired humidity. After 24 hours the insects were placed in clean, uncontaminated perforated Petri dishes and kept at the same relative humidity levels.

Under these conditions total mortality occurred 13 days after incubation irrespective of the humidity. As in previous cases, acceleration of the infection process was noted at a saturated humidity (LT 50 = 4 days at 100 % R.H. compared with LT 50 = 6 $\pm$ 0,5 days at other R.H. values). Mycelial development on cadaver surfaces was only observed at 100 % R.H. Furthemore, as in the other experiments, this always occurred when cadavers were placed in a saturated humidity, irrespective of the R.H. value at which the insects had been inoculated and incubated. We can conclude, therefore, that in the initial inoculation phase, as described above, the R.H. level has no influence of the insects and that the results of experiments I and II can be interpreted without ambiguity.

DISCUSSION

In conclusion, we have shown that *A. obtectus* can be infected with *B. bassiana*, by inoculation of the integument with conidia irrespective of the relative humidity; however, a mycelium does not develop on the surface of the cadavers except at high values of relative humidity above or equal to 92 %. The above results may help to explain why natural epizootics develop during moist weather, the inoculum on the cadavers only multiplying when climatic conditions allow the relative humidity in the microenvironment of the host to approach saturation point. The artificial dispersion of spores, such as considered in integrated control programmes against insect pests of crops, should allow infection of insects independent of humidity conditions. It is probable that the phenomenon of a boundary layer observed in plants intervenes equally in pathological processes in insects, since it has been already established that conidia of *B. bassiana* cannot germinate at less than 92 % relative humidity (SCHNEIDER, 1953).

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RÉSUMÉ

Influence de l'humidité relative sur le développement de l'infection à *Beauveria bassiana* [*Fungi Imperfecti*, Moniliales] des imagos d'*Acanthoscelides obtectus* [*Col., Bruchidae*]

L'infection des imagos d'*Acanthoscelides obtectus* SAY par des conidies de *Beauveria bassiana* (BALS.) VUILLEMIN a été étudiée, au laboratoire, en fonction des valeurs de l'humidité relative de l'atmosphère ambiante (de 0 à 100 % H. R.). Il a été ainsi démontré que l'infection est possible quelle que soit la valeur de l'humidite relative; par contre le développement végétatif du champignon et donc sa sporulation à l'extérieur des cadavres ne se produisent qu'à des humidités égales ou supérieures à 92 % H. R. On comprend, dans ces conditions, que le développement des épizooties naturelles, lié à la présence d'un inoculum pathogène, n'ait lieu que dans les biotopes où règne une humidité relative proche de la saturation; à l'inverse les résultats obtenus indiquent qu'il est possible d'infecter une population de ravageurs par dispersion artificielle d'un inoculum multiplié au laboratoire indépendamment des valeurs de l'humidité relative de l'atmosphère.

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