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PENETRATION OF OVICIDAL FUNGI INTO ALTERED EGGS OF ASCARIS LUMBRICOIDES

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Abstract. Eggs of *Ascaris lumbricoides* exposed to temperatures of 40–60 °C for 1 hour or irradiated by high dosed of UV light are more massively attacked by ovicidal fungi than the intact eggs. The authors assume that the more rapid and increased effect of ovicidal fungi in the soils of the tropics and subtropics is due to greater insolation of the surface layers and their higher temperature.

The ovicidal fungi are capable of attacking and destructing *Ascaris lumbricoides* eggs under experimental conditions during several days or weeks. The rapidity of the ovicidal effect is dependent particularly on the species of ovicidal fungus and type of ovicidity (Lýsek 1976), ovicidal activity (Lýsek and Chalupová 1976), growth and ovicidal rate (Chalupová et al. in press), but also on ambient climatic conditions causing different actual ovicidal activity of the fungus (Giboda et al. 1974). In preliminary experiments performed under tropical conditions (Lýsek, unpublished results) at the soil temperature of about 40 °C the fungus *Humicola fuscoatra* Traaen completely destroyed the egg inoculum of *Ascaris lumbricoides* and *Ascaridia galli* of the size of 0.1 ml during three days. In the experiments carried out under temperature conditions of the temperate zone in Czechoslovakia, the same fungus required two weeks for the destruction of the same number of eggs. The same strain of the ovicidal fungus thus exhibited a substantially different ovicidal activity under different conditions.

Giboda et al. (1974) demonstrated that the temperature influences considerably the ovicidal activity of the fungus. This phenomenon was therefore expressed in a double evaluation of ovicidal activity: actual ovicidal activity, limited by existing climatic conditions, and potential ovicidal activity exhibited under optimal laboratory conditions. The second value is at the same time the maximum value of the ovicidal effect of the fungus. However, this finding of the immediate effect of climatic conditions does not allow to demonstrate to what extent is the increased ovicidal effect due to an increased activity of fungi at higher temperatures or whether the climatic conditions produce such changes which facilitate the penetration of the ovicidal fungus into the eggs.

The aim of the present paper was to ascertain whether and to what extent the exposure of eggs to changed physical conditions may affect the ovicidal activity of the fungus if its original metabolic activity is preserved.

MATERIAL AND METHODS

Live eggs of *Ascaris lumbricoides* recovered from pigs at the slaughterhouse were used in the experiments. The egg surface was sterilized after Lýsek et al. (1976). This method ensures that the original material will be homogeneous at the stage of a single blastomere. The experimental ovicidal fungus was *Verticillium chlamydosporium*, strain 9 from the collection of ovicidal fungi in the Department of Biology, Faculty of Medicine, Palacký University, Olomouc. The fungus was isolated

from the soil of a low-land pasture in Czechoslovakia in 1966. The strain was subsequently cultured on Sabouraud glucose agar, Czapek-Dox agar and live eggs of *Ascaris lumbricoides* with sterile surface. It still keeps its ovicidal properties of the third type of ovicidity (Lýsek 1976). The level of its ovicidal activity tested by our method (Lýsek and Chalupová 1976) was 25 % before the experiment.

a) Experimental egg suspension in test-tubes was exposed to temperatures of 40, 50 and 60 °C for 1 hour. The control group of eggs was kept at the temperature of 25 °C.

b) In the second part of the experiment the experimental eggs were exposed to three different doses of UV light, 47,148 Jm^{-2}, 94,296 Jm^{-2} and 188,592 Jm^{-2}. These values were obtained by irradiating the eggs with Phillips fluorescent tube from the distance of 20 cm for 10, 20 and 40 minutes. The eggs were placed in Petri dishes in a monocellular layer with a minimum quantity of water to prevent the absorption of the irradiation by water.

After exposure the eggs were mixed with spores of the experimental fungus in the ratio of 40 : 1 and the ovicidal effect was tested. The stage of develcpment reached during the test, i. e., within a week, was evaluated with all experimental and control eggs. Each experiment was twice repeated. The resulting value of ovicidal activity was determined on the basis of a control of 200 eggs of each experiment.

RESULTS

The rate of egg alteration was determined indirectly on the basis of the development of experimental eggs compared to the control eggs (i. e., non-irradiated and cultured at 25 °C). The development of eggs exposed to three experimental temperatures is shown in Table 1. The development of irradiated eggs is summarized in Table 2. The eggs

Table 1. Development of *A. lumbricoides* eggs exposed to increased temperatures after one-week experiment

Stage of development	Control	Temperature		
	25 °C	40 °C	50 °C	60 °C
1 blastomere	14 %	13 %	100 %	100 %
2 blastomeres	4 %	1 %	—	—
4 blastomeres	7 %	13 %	—	—
blastula	61 %	49 %	—	—
gastrula	14 %	24 %	—	—

Table 2. Development of *A. lumbricoides* eggs exposed to UV irradiation after one-week experiment

Stage of development	Non-irradiated eggs	Length of irradiation		
		10 min.	20 min.	40 min.
1 blastomere	31 %	82 %	95 %	98 %
2 blastomeres	24 %	9 %	1 %	0 %
4 blastomeres	32 %	1 %	3 %	2 %
blastula	11 %	8 %	1 %	—
gastrula	2 %	—	—	—

exposed to increased experimental temperatures before infection with *Verticillium chlamydosporium* were more readily attacked by the fungus than the control eggs (Fig. 1). The same relationship was found also in the UV irradiated eggs (Fig. 2).

DISCUSSION

As it is shown in Table 1, one-hour exposure of eggs to the temperature of 40 °C slightly speeded up their development, but the appearance of the eggs remained intact. After one-hour exposure to 50 and 60 °C the development of embryos inside the eggs was stopped. In some of the eggs the embryos vacuolized which clearly indicates that they were damaged. This agrees with the assumption of Magrová (1967) that even a short-term exposure of eggs to the temperature of 50 °C results in a complete cessation of their development.

Proportionally with increasing temperature increased also the number of eggs attacked by the fungus. Since the ovicidal activity was tested at a temperature of 25 °C, the increased number of attacked eggs could not be due to a higher metabolic activity of the fungus. As this relationship is linear (see Fig. 1), it may be concluded that with increasing temperature increases also the alteration of eggs and their shells allowing the ovicidal fungi to penetrate into the eggs. This relationship was observed already in eggs exposed to 40 °C which produced a more rapid development as opposed to the control.

The UV light irradiation, even in the highest dose used, did not cause death of all exposed eggs (Table 2). No morphological changes either in egg shells or embryos were observed under light microscope. However, also in this case the relationship was proportional and with increasing rate of irradiation increased also the number of attacked eggs. The fact that even the highest dose of irradiaton did not kill the eggs suggests that they were relatively little dammaged. The produced alteration of egg shells,

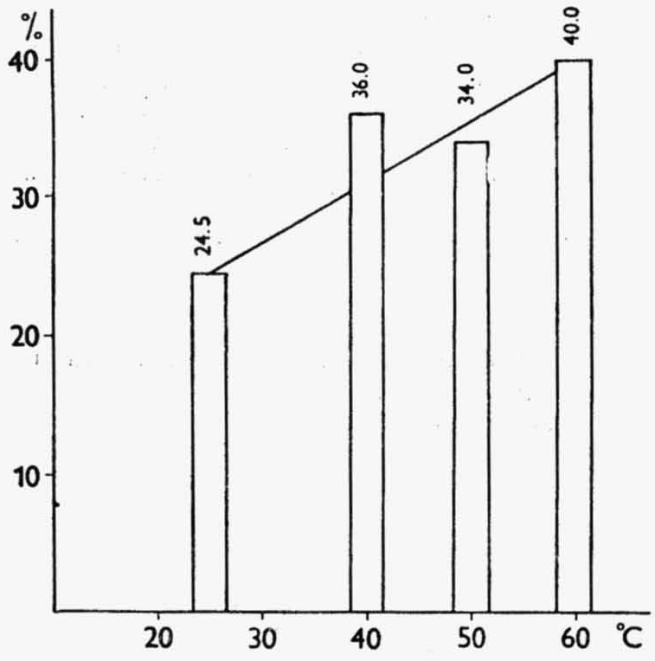

Fig. 1. Relationships between the number of attacked eggs and previous exposure to the temperatures of 40, 50 and 60 °C. Control temperature 25 °C.

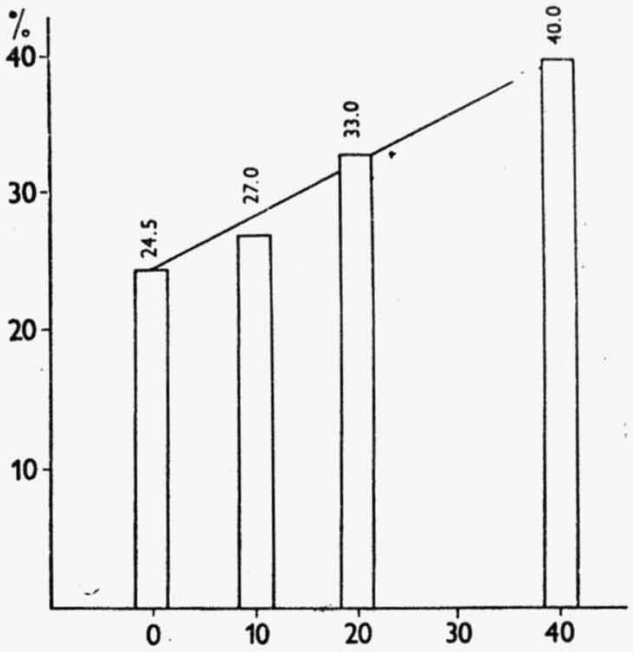

Fig. 2. Relationships between the number of attacked eggs and previous exposure to UV light. The doses of irradiation are expressed in minutes.

though not definable morphologically, enabled the fungi to penetrate into the eggs. Such an intensive UV irradiation does not occur in nature, but our results allow to conclude that even a feeble but long-term irradiation may produce similar alterations of egg shells. In free nature this may concern only the eggs exposed to direct insolation in the surface layers of soil.

The above facts correspond to our previous results of experiments performed directly under conditions of a tropical climate. According to these results, the heating of upper soil layers may result in a more rapid destruction of eggs by ovicidal fungi. This seems to be due to two independent factors: increase of the metabolic activity of ovicidal fungus and simultaneous alteration of egg shells of geohelminths which facilitates the penetration of the fungus.

ПРОНИКАНИЕ ОВИЦИДНЫХ ГРИБКОВ В ИЗМЕНЕННЫЕ ЯЙЦА *ASCARIS LUMBRICOIDES*

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Резюме. Яйца *Ascaris lumbricoides*, подвергнутые действию температуры 40—60 °С в течение 1 часа или облучению высокими дозами ультрафиолетового света, заражаются овицидными грибками более массивно, чем неповрежденные яйца. Авторы полагают, что скорейшее и повышенное действие овицидных грибков в почвах тропиков и субтропиков причинено повышенной инсоляцией поверхностных слоев и их повышенной температурой.

REFERENCES

GIBODA M., LÝSEK H., LEŠŤAN P., Effect of some ecological factors on the activity of ovicidal fungi. Čs. Hyg. 19: 20—23, 1974. (In Slovak.)

CHALUPOVÁ V., LENHART K., LÝSEK H., The study of the relation of some physiological and biochemical characters of mutants of ovicidal fungus *Botryotrichum piluliferum* to ovicidal activity. Acta Univ. Olomouc. Fac. Med., in press.

LÝSEK H., Classification of ovicide fungi according to type of ovicidity. Acta Univ. Olomouc. Fac. Med. 76: 9—13, 1966.

—, BAŤOVÁ D., GIBODA M., Sterilization of the surface of live *Asaris* eggs. Acta Univ. Olomouc Fac. Med. 76] 15— 19, 1976.

—, CHALUPOVÁ V., Quantitative determination of activity of ovicide fungi. Acta Univ. Olomouc. Fac. Med. 79: 59—63, 1976.

MAGROVÁ E., Effect of some physical factors on exogenous development of eggs of *Trichocephalus trichiurus* and *Ascaris lumbricoides* under laboratory conditions. Biológia (Bratislava) 22: 364—374, 1967. (In Slovak.)

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