THE INFLUENCE OF AERATION ON SURVIVAL AND HATCH OF MELOIDOGYNE JAVANICA

BY

H. R. WALLACE

Division of Horticultural Research, C.S.I.R.O., Glen Osmond, South Australia

There is a linear relationship between rate of hatch of eggs of _M. javanica_ and oxygen concentration over the range 5% O₂, 15% CO₂, 80% N₂ to 20% O₂, 80% N₂. No hatch occurred in the absence of oxygen. Increasing the time in an oxygen-free atmosphere reduced the subsequent hatch in aerated water. After 2 days without oxygen there was a marked decrease in the subsequent hatch due to the susceptibility of embryonated eggs to anaerobic conditions. It is concluded that low aeration due to waterlogging or soil depth may have a contrasting dual effect; it may kill embryos but it also maintains infectivity in larvae by inducing quiescence.

The partial pressures of oxygen and CO₂ in the soil atmosphere are determined by the rates of diffusion of these gases into and out of the soil. And of the factors that control diffusion, soil moisture is probably the most important. However, aeration is not only a function of macro-diffusion i.e. diffusion between soil crumbs, but also of diffusion through soil crumbs which at field capacity are filled with water (Currie, 1961). Because of their size, it seems likely that egg sacs and larvae occur in the intra- as well as in the inter-crumb spaces and are thus subject to wide fluctuations of aeration with change in soil moisture content. It is known that the hatch and mobility of _M. javanica_ is low when the pore spaces are filled with water (Wallace, 1966a, b). Van Gundy & Stolzy (1961) demonstrated that the lowest oxygen concentration that allowed development of the host and the nematode was 3.5% and that there was a linear relationship between movement of _M. javanica_ larvae and the rate of oxygen diffusion in a porous medium (Van Gundy & Stolzy, 1963). There appears to be a linear relationship between cumulative exposure to oxygen concentration and cumulative hatch from egg sacs which do not substantially interfere with the oxygen in the environment by their own respiration (Collis-George & Wallace, 1968). Low aeration, by inactivating larvae, prolongs infectivity (Van Gundy, Bird & Wallace, 1967) although absence of oxygen may reduce survival (Stolzy, Van Gundy & Letey, 1960).

The present work describes the effect of different oxygen-CO₂ ratios on hatch and the effect of absence of oxygen on the survival of eggs and larvae with the object of (1) determining the stages of development that are most resistant and susceptible to lack of aeration (2) the possible significance of aeration in the hatching curve in soil.
METHODS AND MATERIALS

The source of egg sacs of *M. javanica* and techniques for the preparation of egg suspensions have been described previously (Wallace, 1966a, b). Cylinders containing the required percentages of oxygen, CO$_2$ and nitrogen were used to study the influence of oxygen-CO$_2$ ratios on hatch. Five glass vials containing water which had previously been boiled and gassed with nitrogen were used in each of the six atmospheres (Fig. 1). A polystyrene hatching float (Wallace, 1966b) containing ten egg sacs was placed in each vial. By this means the egg sacs were maintained in a thin film of water. Six glass containers each containing five vials were gassed daily for 2 min at a flow rate of about 1 l per min, with the required gas mixture. The larvae that had hatched were counted at intervals by removing the vials and placing the floats in another vial containing de-oxygenated water before gassing.

To determine the effect of absence of oxygen on subsequent hatch, aliquots of 125 eggs were pipetted into each of 27 hatching floats. Two atmospheres with no oxygen were used: (1) 20% CO$_2$-80% N$_2$. (2) 100% N$_2$. Three replicate batches of 125 eggs were placed in normal atmospheric conditions as a control and the hatch determined at 2-day intervals over 10 days. Twelve tubes were placed in each of the oxygen deficient atmospheres and three tubes were removed from each atmosphere every 2 days so that the effect of exposure to anaerobic conditions for 2, 4, 6 and 8 days on subsequent hatch in aerated water could be measured. Hatching floats, vials and air tight containers were used as in the previous experiment.

To measure the effect of absence of oxygen on the survival of eggs and larvae, an atmosphere of 20% CO$_2$ and 80% N$_2$ was used. The eggs and larvae were placed in deoxygenated water in airtight vials within an airtight container without oxygen. Aliquots of eggs and larvae were removed at intervals and placed in aerated water. In the egg suspensions the numbers of eggs containing embryos or second stage larvae and hatched larvae were counted at weekly intervals over 4 weeks. In the larval suspensions the numbers of active larvae were counted until there was no further increase in numbers.

Capillary tubing was used to study hatch from single egg sacs in columns of water of the same diameter as the egg sac. Egg sacs were placed half way down a capillary tube 5 cm long and 1 mm bore. De-oxygenated water was introduced with a hypodermic needle to give a column of 0, 1, 2, 4, 6 or 10 mm long on each side of the egg sac. The tube was sealed at one end with plasticine and immersed in water. Water entered the capillary tube to a length of 2.5 mm but did not interfere with column of water at the centre. Four replicate tubes were used for each length of water column and the numbers of larvae that had hatched in 4 days were expressed as a percentage of the eggs remaining in the egg sac.

All experiments were kept in the dark in an incubator at 27°C. Where necessary data were analysed statistically by analysis of variance after transformation of percentage to angles of equal information.