On nematode behavior in an electric field

David R. VIGLIERCHIO and Pyung K. YU*

Division of Nematology, University of California, Davis, Ca 95616, USA

SUMMARY

In an electric field established in a wet sand bed at 10 V/cm migrating *Heterodera schachtii* move preferentially to the cathode. Migrating *Meloidogyne javanica* move preferentially towards the anode. The pH of the migrating bed can modify the proportions of nematodes moving towards the electrodes. Treatment of nematode populations with cationic or anionic detergent, protein solutions and enzymes, lipase or papain, also can modify the behavior of these nematodes in an electric field. The nature of the modifications of responses of the various treatments suggest that the mechanism is electrophoretic, under the parameters of these experiments. The electric field induces a net charge on the sensitive nematode with the concurrent generation of a directional field force which becomes superimposed upon random movement of the nematode.

RÉSUMÉ

Comportement de nématodes dans un champ électrique

Dans un champ électrique établi dans un lit de sable humide à 10 V/cm, les juvéniles d'*Heterodera schachtii* se déplacent préférentiellement vers la cathode. Les juvéniles de *Meloidogyne javanica* se déplacent préférentiellement vers l'anode. Le pH du sable peut modifier les proportions de nématodes se déplaçant vers telle ou telle électrode. Le traitement des populations de nématodes avec des détergents cationiques ou anioniques, des solutions de protéines et des enzymes, lipase ou papaïne, peuvent aussi modifier le comportement de ces nématodes dans un champ électrique. La nature des modifications apportées par ces divers traitements suggère que le mécanisme est électrophorétique, sous les paramètres de ces expériences. Le champ électrique induit la formation d'une charge nette sur le nématode sensible, ce qui provoque la formation d'un champ directionnel qui se superpose au mouvement aléatoire des nématodes.

There has been interest in the orientation of motile organisms in electric fields since the turn of the century. The interest in nematode behavior in electric fields has developed within the last two to three decades. If speculation is ignored, the essence of our knowledge can be summarized according to Croll (1970) "Nematodes are somehow 'aware' of currents and can orient with respect to them." It was of interest therefore to study some of the parameters of this phenomenon.

Materials and methods

The electric field migration apparatus was modified from horizontal paper electrophoresis equipment. The migration platform consisted of two glass plates cemented to spacers so that cooling water could be circulated between the glass plates to dissipate the heat generated by the electrical current. Heavy

* Present Address : Rhom and Haas Exp. Farm, Homestead, Fla., USA.

cloth wicks, the width of the migrating bed, were used to establish salt bridges from the electro baths to the migrating bed. The electric field was generated by a regulated DC power supply with a capacity of 500 V and 100 milliamps. The migration bed consisted of quartz sand, particle size less than 0.25 mm, 2 mm in thickness, 15 cm in width and 24 cm in length. The bed was saturated with the appropriate buffer from one edge to avoid bubbles and other forms of disruption of the homogeneity of the bed. Buffer solutions of less than pH 5 were phthalate-based, pH's 5 to 8 were phosphate-based and pH's greater than 8 were borate-based. A sand migration bed was selected because the nematodes employed migrated better in sand than in agar and there was less surface film desiccation and movement of water between the poles of the bed. After preliminary trials the electrical field characteristics were standardized with a potential gradient of 10 V/cm at current density of less than 3.3 mA/cm^2 for a 2-hours run. The sand bed support was marked longitudinally in 1 cm intervals so that strips of sand could be removed and analyzed for nematode content. Nematodes were placed along the central strip by pipette at the initiation of a trial. For nematode analysis the sand strips were removed from the support bed and placed on a Baermann funnel and the nematodes counted after 48 h. Nematode recovery was normally over 80 %. Nematode stocks of *Heterodera schachtii* and *Meloidogyne javanica* were obtained from greenhouse cultures and used within one week of separation. The results reported herein constitute the average of a minimum of three replications.

Two kinds of detergents were used in these experiments to modify the electric motility of the nematode. Cationic detergent (D+) consisted of a lauryl trimethyl quarternary ammonium salt; anionic detergent (D—) consisted of lauryl sulfate salt. Treatments with detergents and other non-enzymatic substances, for example 0.1 % egg albumin or 0.1 % gelatin, consisted of a 10-15 minutes incubation at 25° on a reciprocal shaker.

The papain enzymatic treatment followed the method of Stockell and Smith (1957) modified for use with nematodes. We used a lower reaction temperature, 25°, and a higher enzyme concentration, 10 %, because of a lower enrichment of the enzyme preparation and a reduced availability of substrate (nematode cuticle). Variable reaction times were also used. The porcine lipase enzyme treatment followed the method of Hemingway, Smith and Rook (1970) again modified for use with nematodes. Without the addition of taurocholate a reaction pH of 8.0 was used at a temperature of 25° for an enzyme concentration of 0.1 %.

The separation of nematodes from treatment or wash solutions was accomplished by centrifugation and decanting. Long term washings were accomplished by placing suspensions on a shaker at 25°.

Results

Heterodera schachtii

In a study of the response of *H. schachtii* to an electric field as a function of pH of the migrating medium, about half the population migrated away from the central inoculation zone at pH 4. As the pH was increased the proportion migrating decreased but rose again at slightly alkaline conditions then decreased under strongly alkaline conditions. Of those nematodes migrating the greater proportion migrated towards the cathode; a minimum appeared at neutrality (Fig. 1).

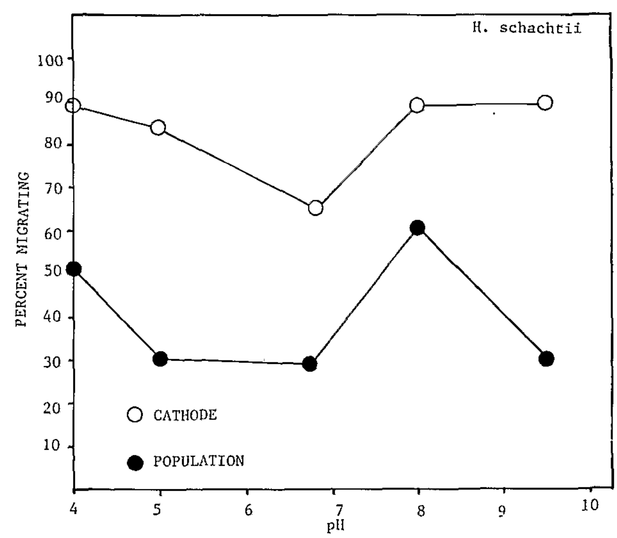

Fig. 1. Proportion of a healthy *Heterodera schachtii* population migrating in media of different pH. Open symbols indicate the percent of the migrating population moving towards the cathode in an electric field. Filled symbols indicate the proportion of the population migrating towards either electrode.

If *H. schachtii* larvae were treated with lipase, the proportion of the population that migrated changed very little with reaction time; however,

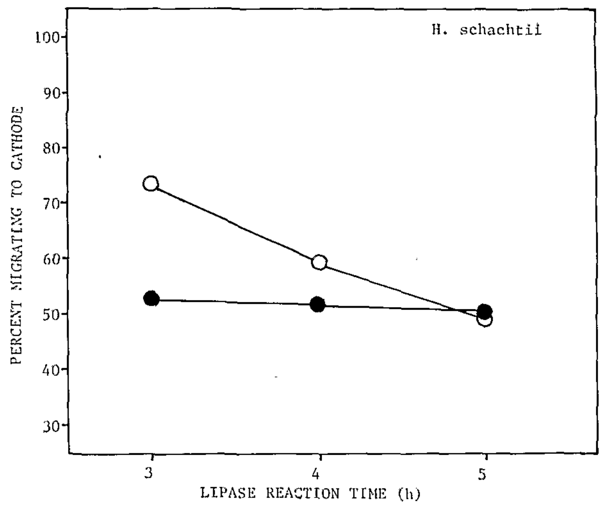

Fig. 2. Proportion of a *Heterodera schchtii* population migrating in an electric field at pH 6.8 pretreated for various reaction times with lipase. Open symbols indicate percent of the migrating population moving towards the cathode; filled symbols indicate the proportion of the population migrating towards either electrode.

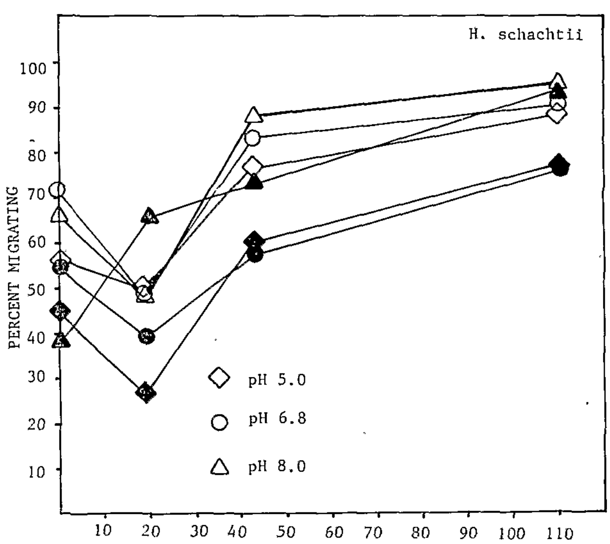

Fig. 3. Proportion of a *Heterodera schachtii* population migrating in an electric field at different pH's after pretreatment for 3 h with lipase followed by different recovery periods in buffer. Open symbols indicate percent of migrating population moving towards the cathode; filled symbols indicate the percent of the population migrating towards either electrode.

the proportion of the migrating population that moved towards the cathode decreased (Fig. 2).

When lipase-treated nematodes were allowed to recover in buffer the proportion of the migrating population that moved towards the cathode decreased from 0 to about 20 h then increased thereafter until 110 h. The proportion of the population migrating at pH's 5 and 6.8 followed a similar pattern but at a lower value; however at pH 8 the proportion continually increased from zero recovery time (Fig. 3). When lipase-treated *H. schachtii* larvae were allowed to recover for 110 h, retreated and then allowed to recover again, the porportion migrating to the cathode was reduced after about 20 h but increased thereafter to duplicate the pattern of the first treatment (Fig. 4). The third treatment with recovery showed a repetition of the pattern.

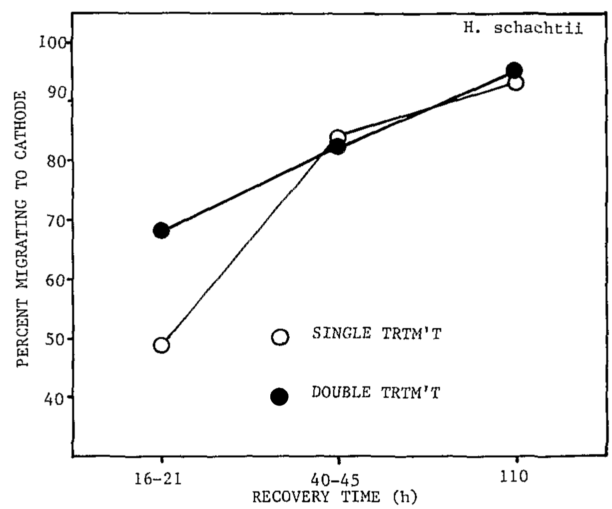

Fig. 4. Proportion of a *Heterodera schachtii* migrating population moving towards the cathode at pH 6.8 after different recovery periods following one 3 h lipase treatment, open circles, and a repeat 3 h lipase treatment followed by recovery of the population once lipase treated and recovered for 110 h, filled circles.

Treatment of *H. schachtii* larvae with a cationic or anionic detergent affected migration towards the cathode; cationic detergent increased and anionic detergent decreased the proportion migrating to the cathode as concentrations were increased. When lipase-pretreated larvae were treated with detergent, migration towards the cathode increased rapidly to a plateau with increase in cationic deter-

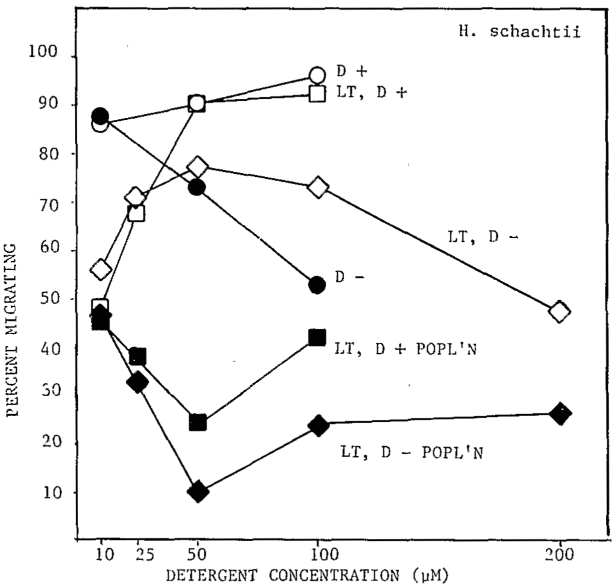

Fig. 5. Proportion of a *Heterodera schachtii* population migrating treated with different concentrations of detergent. Open symbols indicate percent of the migrating population moving towards the cathode; filled symbols indicate the percent of the population migrating towards either electrode. Anionic detergent is indicated D—; cationic is D+; LT, D+ indicates a lipase treatment followed by a cationic detergent treatment and LT, D— indicates a lipase treatment followed by an anionic detergent treatment.

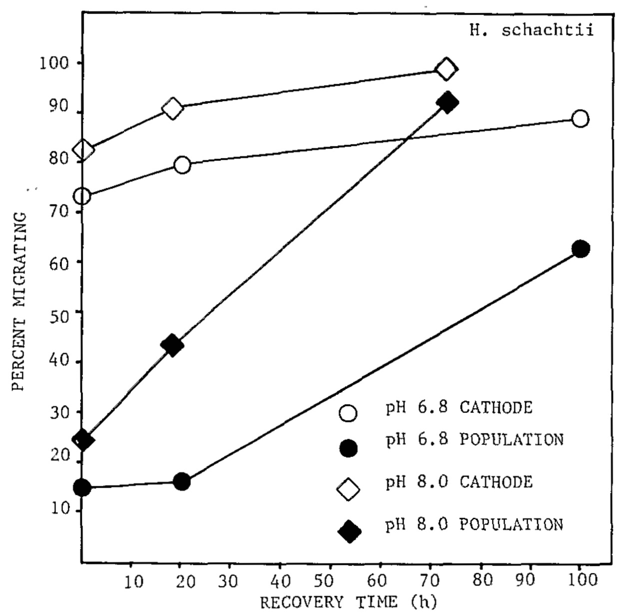

Fig. 6. Proportions of a *Heterodera schachtii* population migrating in an electric field at different pH's after a papain treatment followed by different recovery periods. Open symbols indicate percent of migrating population moving towards the cathode; filled symbols indicate percent of population migrating towards either electrode.

gent concentrations. With anionic detergent treatments the percent migration increased rapidly at low concentration and then fell with increasing concentration (Fig. 5). Above cationic concentrations of 50 μM there was little difference in percent migration to the cathode between untreated and lipase-treated larvae. With lipase-treated larvae a detergent concentration of about 50 μM appeared critical since the percent migrating towards the cathode maximized at this concentration while the percent of the population migrating minimized with either cationic or anionic detergent.

The response to an electric field of larvae treated with papain was modified in comparison to controls (Fig. 6). The percent of larvae migrating to the cathode was increased slightly and continued to rise slowly with additional recovery time. The proportion of the population migrating was depressed initially but rose dramatically with recovery time. The effect appeared to be greater when the migrating medium was slightly alkaline than when neutral.

Treating larvae with albumin solution reduced the percent migrating to the cathode modestly except at pH 8. Washing albumin-treated larvae 20 times with water reduced the percent migrating to the cathode dramatically at low pH but allowed a normal response in a high pH medium (Fig. 7). The response of larvae treated with gelatin solution was substantially depressed at low pH and slightly depressed at higher pH. Washing gelatin-treated larvae appeared to render the response pH independent, increasing migration towards the cathode at low pH, but decreasing at high pH in comparison to unwashed larvae.

Meloidogyne javanica

The proportion of a population of *M. javanica* larvae that responded to an electric field was low. Of those that did respond, a high proportion migrated towards the anode. The response appeared to be relatively pH independent (Fig. 8).

When *M. javanica* were treated with anionic

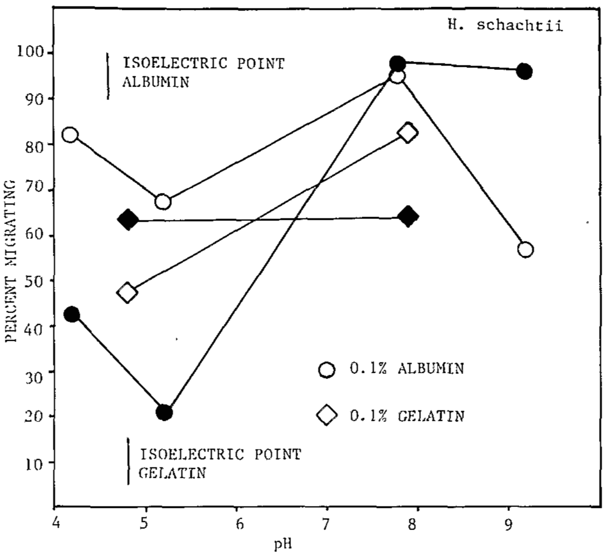

Fig. 7. Proportions of a *Heterodera schachtii* population migrating in an electric field at different pH's after albumin or gelatin treatment. Open symbols indicate percent of migrating population moving towards the cathode ; filled symbols indicate percent of population migrating towards either electrode.

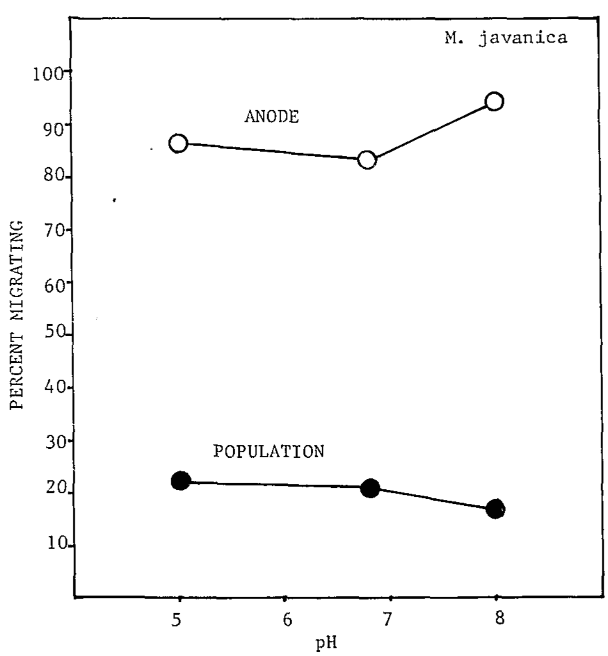

Fig. 8. Proportion of a *Meloidogyne javanica* population migrating in an electric field at different pH's. Open circles indicate percent of migrating population moving towards the anode ; filled circle, the percent of the population migrating to either electrode.

detergent the proportion of the population responding remained low. Of those responding the proportion migrating towards the anode remained very high. With cationic detergent-treated larvae the proportion of the population that responded was variable but remained low. Of those responding the proportion that migrated to the anode decreased dramatically with increasing concentration of detergent (Fig. 9).

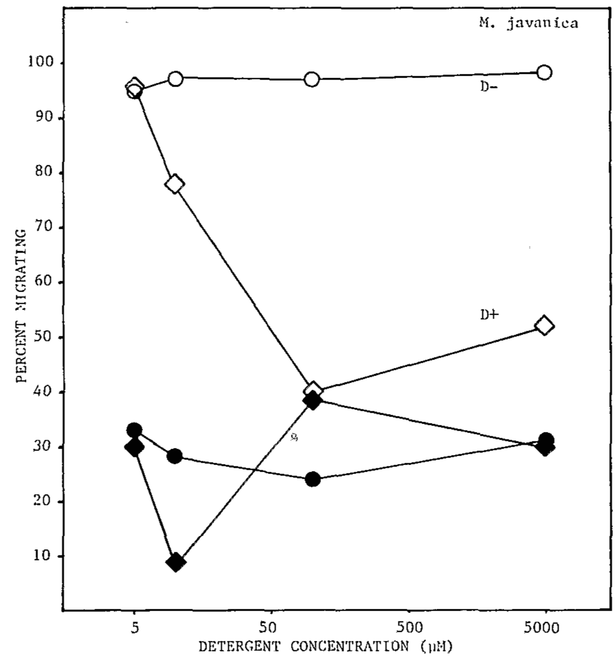

Fig. 9. Proportion of a *Meloidogyne javanica* population migrating in an electric field after treatment with different concentrations of anionic detergent, D— and cationic detergent, D+. Open symbols indicate percent of migrating population moving towards the anode ; filled symbols indicate percent of population migrating towards either electrode.

When *M. javanica* were treated with anionic detergent, the proportion of the population responding decreased with increase in pH. Of those responding the proportion migrating towards the anode remained very high and pH independent. With larvae treated with cationic detergent the population was virtually irresponsive at pH 5, but the proportion increased substantially near neutrality and decreased to very low value in a slightly alkaline migrating medium. Of the responding population the proportion migrat-

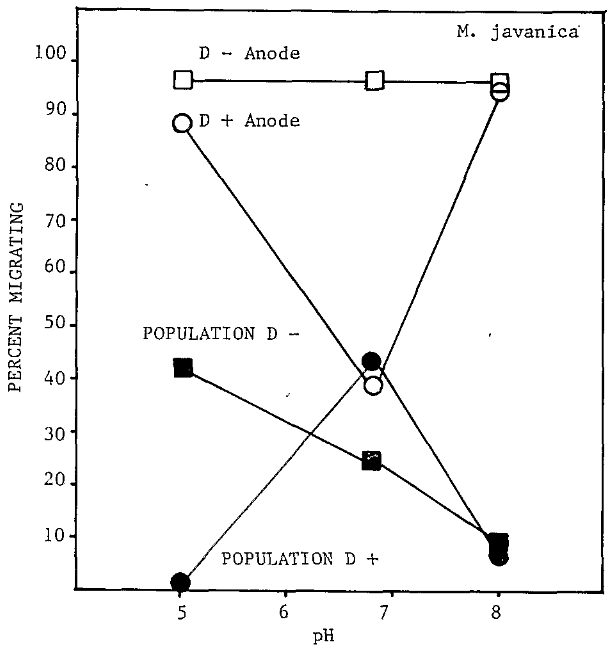

Fig. 10. Proportion of a *Meloidogyne javanica* population treated with anionic detergent (D—, 75 μM) or cationic detergent (D +, 75 μM) migrating in an electric field at different pH's. Open symbols indicate percent of migrating larvae moving towards the anode ; filled symbols indicate percent of the population migrating towards either electrode.

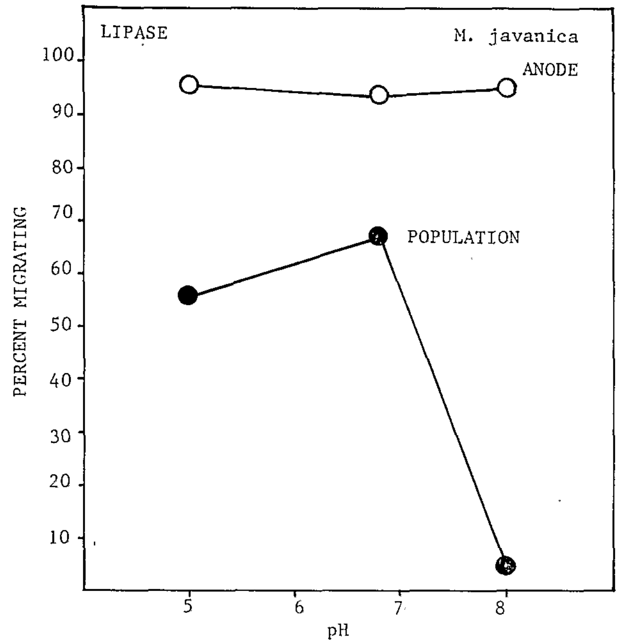

Fig. 11. Proportion of a *Meloidogyne javanica* population treated with lipase, migrating in an electric field at different pH's. Open circles indicate percent of migrating population moving towards the anode ; filled circles indicate percent of the population migrating towards either electrode.

ing toward the anode was very high at pH 5, decreased dramatically around neutrality, and increased again to very high values under slightly alkaline conditions (Fig. 10).

In *M. javanica* larvae treated with lipase the proportion of the population responding to the electric field rose substantially over controls at pH 5, increased somewhat at pH 6.8, and decreased to very low value at pH 8. Of those responding, the proportion migrating to the anode was very high and pH independent (Fig. 11).

Treatment of *M. incognita* larvae with papain modified the nematode behavior in an electric field depending upon the nature of the treatment (Fig. 12). A 0.5 h treatment doubled the proportion of the population responding but decreased the percent migrating to the anode to one-third. After a 2 h treatment the proportion of the population responding remained about the same but the percent of the nematodes migrating to the anode increased substantially. If a 2 h papain treatment was followed with a 3 h wash period the proportion of the population responding remained about the same, however the percent of nematodes migrating to the anode increased substantially over the unwashed condition. In a prolonged 4 h treatment followed by a 3 h wash the proportion of the population responding remained similar to shorter treatments, but the percent migrating to the anode was reduced to a low value. When a 4 h treatment was followed by a 40 h wash the proportion of the population responding was increased and the percent of the migrating population moving towards the anode increased to very high value. The behavior of larvae subjected to a 4-h papain treatment followed by a 3 h wash was similar to controls when migrating at pH 8.

Albumin treatment of *M. javanica* larvae modified nematode behavior in an electric field depending upon migration media pH (Fig. 13). At pH 5.2 the proportion of the population migrating was 0 and prolonged washing helped only slightly. At pH 6.8 the proportion of the population migrating was nearly normal and washing raised it slightly ; at pH 8 the proportion of the population responsive to an electric field was nearly 0. When there was a migrating population in an albumin treatment the

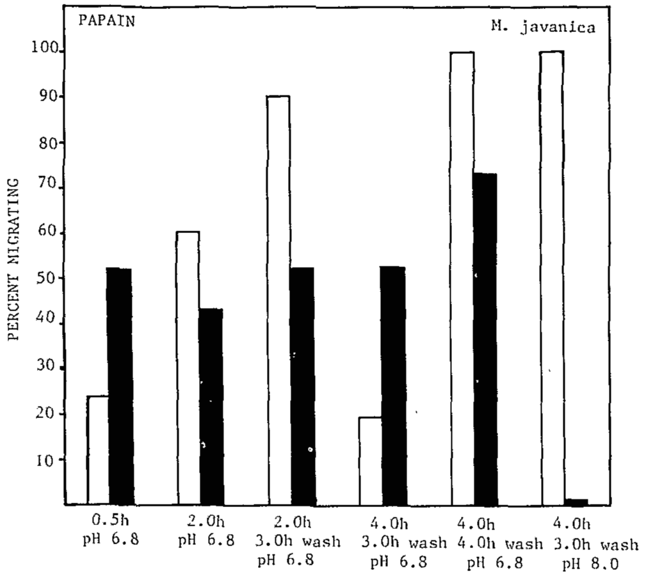

Fig. 12. Proportion of a *Meloidogyne javanica* population migrating in an electric field after treatment with papain for different reaction periods and different recovery periods and migrating in media at different pH's. Open bars indicate percent of migrating population moving towards the anode ; filled bars indicate percent of population migrating towards either electrode.

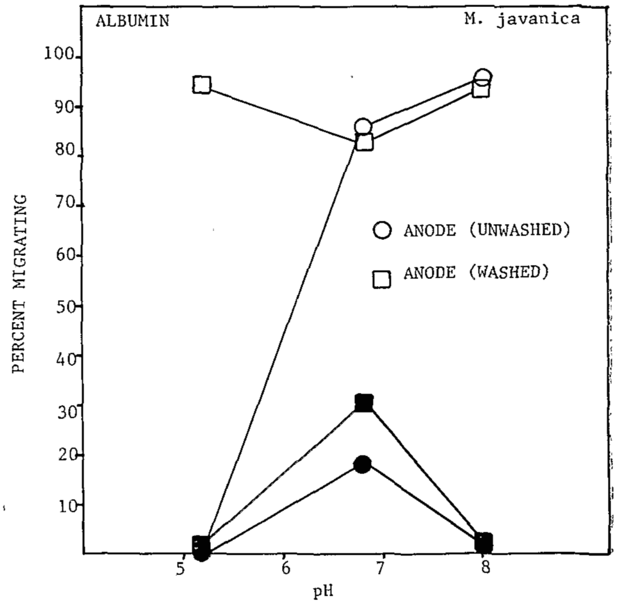

Fig. 13. Proportion of a *Meloidogyne javanica* population migrating in an electric field, after treatment with albumin followed by a wash or no wash, at different pH's of migrating media. Open symbols indicate percent of a migrating population moving towards the anode ; filled symbols indicate percent of the population migrating towards either electrode.

percent migrating to the anode was similar to controls.

Discussion

Heterodera schachtii

The behavior modifications caused by lipase treatment suggests biochemical modification of the body surface rather than a surface adsorption of enzyme. In studies where lipase treatments were followed by different recovery periods at which migration behavior was observed at different pH, the general shapes of the curves are consistent with the notion that the initial modification in behavior may have been due in part to enzyme adsorption diminishing with increase in recovery time to disappear at about 20 h ; the increase in sensitivity to the electric field with further increase in recovery time suggests a nematode response to biochemical modification of the body surface. When *H. schachtii* larvae were lipase-treated repeatedly after recovery periods they appeared able to recover their electrosensitivity in three to four days.

The behavioral responses of *H. schachtii* larvae treated with increasing concentrations of anionic and cationic detergents suggest a charge modification effect on the migrating population. With lipase pretreated animals the detergent effects predominated at high concentrations ; at low concentrations other interactions become important ; the nature of which is not understood.

The effect of papain treatment upon the proportion of the population migrating was dramatic ; initially it was reduced to 50 % or less of controls but with increased recovery time rose to 50 % or more of controls. It appears that the papain modified that portion of the body exposed to the environment and the animals reacted in a fashion making them more sensitive to the electric field. Treatment of *H. schachtii* larvae with simple proteins modified the response of *H. schachtii* to the electric field. The protein effects on the proportion of the population migrating were more striking in albumin-treated populations than gelatin-treated ones. It appeared that proteins were adsorbed on the nematode body to modify the nematode sensitivity to an electric field.

Meloidogyne javanica

The proportion of the population migrating was low and pH insensitive while the proportion of the migrating population migrating to the anode was very high and relatively pH insensitive. The proportion of *M. javanica* larvae migrating in an electric field was not particularly affected by treatment with the increasing concentrations of detergent. The proportion of the migrating population moving towards the anode was affected slightly by treatment with increasing concentrations of anionic detergent and greatly by treatment with increasing concentrations of cationic detergent.

Lipase treatment of *M. javanica* larvae had little effect on the proportion of the migrating population moving towards the anode ; however the treatment decreased the proportion of the population migrating at pH 8 to less than half of controls. At lower pH, lipase treatment increased the proportion migrating by a factor of two or more. Papain treatment increased the proportion of the population migrating in media at pH 8. There seemed to be an interaction between treatment time and subsequent washing of the nematodes ; long wash time appeared to allow *M. javanica* larvae to recover in a fashion rendering them more electro-negative. In a migrating medium of pH 8 papain-treated *M. javanica* larvae were rendered essentially insensitive to the electric field. Albumin-treated larvae responded similarly to controls at pH 6.8 but at migrating media pH's of 5.2 and 8 they were essentially insensitive to the electric field. A short wash of albumin-treated nematodes indicated little change from unwashed, suggesting that desorption of albumin was a slow process.

In summary the *H. schachtii* populations used under the conditions of these experiments migrated preferentially to the cathode while *M. javanica* populations migrated towards the anode. In the mild nondestructive treatments used in these experiments and others (Sukul, Das & Ghosh, 1975, 1977), the behavior of nematodes in electric fields under our conditions can best be explained as a simple electrophoretic response. Normally neutral, the nematode susceptible to an electric field acquires an induced net charge that is subject to electrostatic attraction. The constant force on the wriggling nematode causes it to drift preferentially in the sand as a protein would in an aqueous media. It is evident that detergents can bind to the nematodes to change the net charge as can proteins. Enzymatic proteins can modify the exposed surface of the nematode body by adsorption and biochemical action to make it more electro-sensitive. It appears that the surface properties of *H. schachtii* are substantially different from *M. javanica.* The fact that a small proportion of the population of either *H. schachtii* or *M. javanica* develops a net charge remains to be explained. The conclusion that the nematodes used under the conditions of these experiments migrated electro-phoretically as charged particles has been confirmed by the observation that these same nematodes subjected to combined electric and magnetic fields behave according to the laws of physics governing the motion of moving charged particles in magnetic fields.

Accepté pour publication le 23 août 1982.

References

CROLL, N. A. (1970). *The behavior of nematodes.* London, Arnold, 117 p.

HEMINGWAY, E. B., SMITH, G. H. & ROOK, J. A. F. (1970). The pattern of release of free fatty acids from milk fat under the action of intrinsic and added lipases. *J. Dairy Res.*, 37 : 83-96.

STOCKELL, A. & SMITH, E. L. (1957). Kinetics of papain action. I. Hydrolysis of benzoyl-l-argininamide. *J. biol. Chem.*, 227 : 1-26.

SUKUL, N. C., DAS, P. K. & GHOSH, S. K. (1975). Cationic mediated orientation of nematodes under electrical fields. *Nematologica*, 21 : 145-150.

SUKUL, N. C., DAS, P. K. & GHOSH, S. K. (1977). The influence of preincubation of *Lahonema digitatum* at different ionic concentrations to subsequent electrotaxis. *Nematologica*, 23 : 24-28.