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SHORT COMMUNICATION

Protoplasts in the *in vivo* Life Cycle of *Erynia neoaphidis*

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The entomogenous fungus *Erynia neoaphidis* formed protoplasts within the body of its aphid host, *Acyrtosiphon pisum*. The protoplasts were filamentous, irregular, oval or amoeboid. Their ultrastructure differed from that of walled cells of the fungus: protoplasts had smaller mitochondria and more stacked, rough endoplasmic reticulum, their plasma membrane had a thin, fibrous coat on the exoplasmic surface, and they lacked the electron-dense bodies present in walled cells. Invaginations of the protoplast plasma membrane suggest that the protoplasts feed by pinocytosis.

INTRODUCTION

Studies on the induction of fungal protoplast formation and the regeneration of the cell wall state *in vitro* have been reviewed by Villanueva (1966) and in Villanueva *et al.* (1973). However, very little is known about naturally occurring fungal protoplasts. Fungi known to exist as protoplasts in their natural life cycle include *Coelomomyces punctatus* Couch (Powell, 1976) and *Entomophthora egressa* MacLeod & Tyrrell (Tyrrell, 1977), both pathogens of insects.

This paper reports the occurrence of protoplasts in the natural life cycle of the aphid pathogen *Erynia neoaphidis* Remaudière & Hennebert (formerly known as *Entomophthora aphidis* *sensu* Nowak.). This is the first record of this fungus forming protoplasts and the first description of the ultrastructure of protoplasts of any member of the Entomophthorales developing *in vivo*.

METHODS

Healthy adult pea aphids (*Acyrtosiphon pisum* Harris) were inoculated with *E. neoaphidis* using the methods described by Wilding (1969). At intervals after inoculation, infected aphids were fixed in a mixture of glutaraldehyde and formaldehyde in sodium cacodylate buffer. Following postfixation in osmium tetroxide, they were dehydrated through a graded ethanol series culminating in anhydrous acetone, embedded in Transmit Resin (TAAB Laboratories Equipment) and sectioned with a diamond knife on an LKB Ultratome III. Sections were stained with lead citrate (Reynolds, 1963) and viewed with either an AEI EM 6G or a Philips 300 transmission electron microscope.

RESULTS AND DISCUSSION

From the invasion of the host until shortly before its death, *E. neoaphidis* exists as protoplasts (Figs 1-5). In thin sections, these protoplasts vary in size and shape, some being filamentous (Fig. 1) others irregular, oval or amoeboid (Figs 3-5). Light microscope

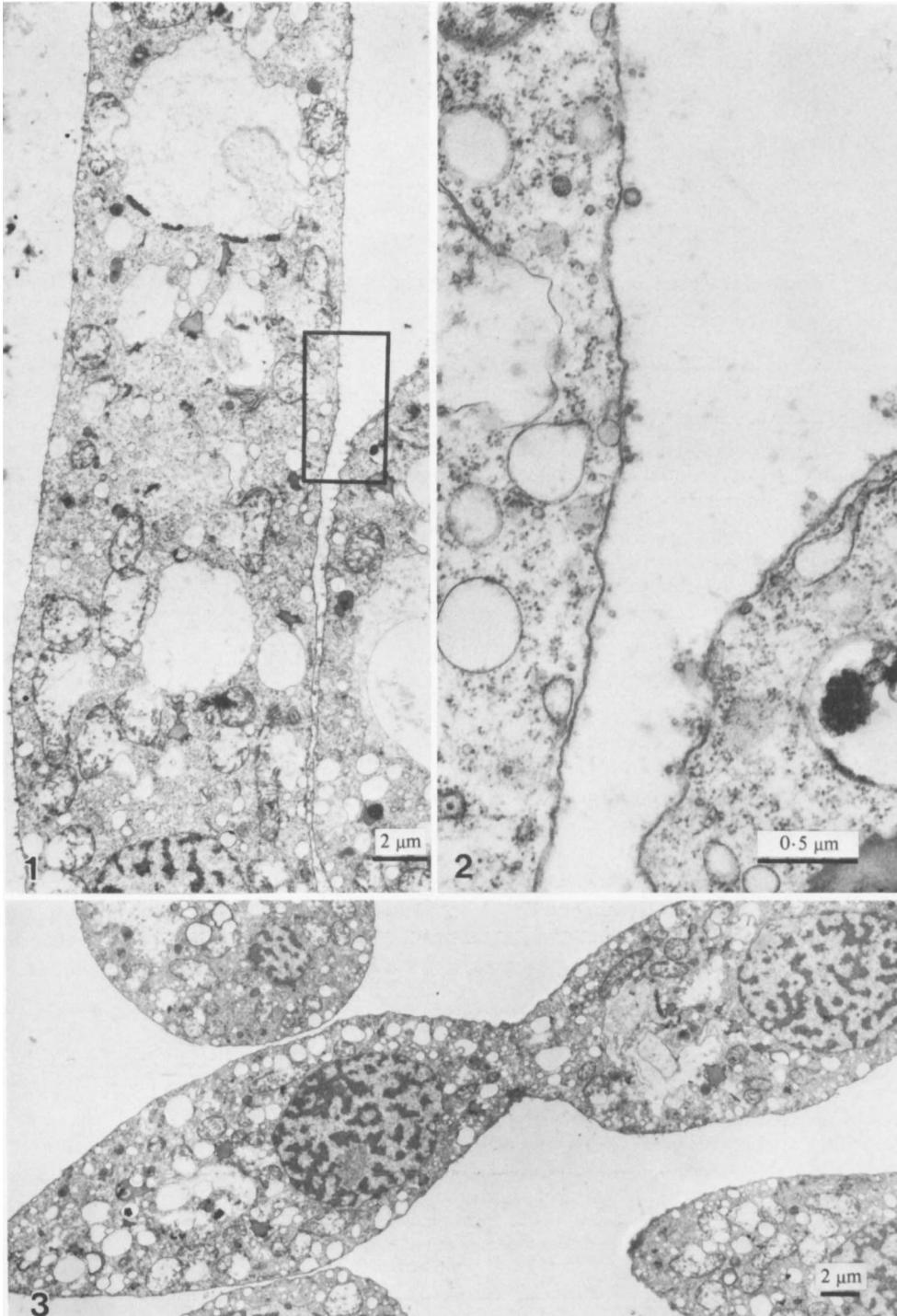


Fig. 1. Part of a filamentous protoplast of *E. neoaphidis*.

Fig. 2. Details of area outlined in Fig. 1. The protoplast plasma membrane appears to be coated with fibrous material.

Fig. 3. A protoplast constricted in the middle, possibly in the process of budding.

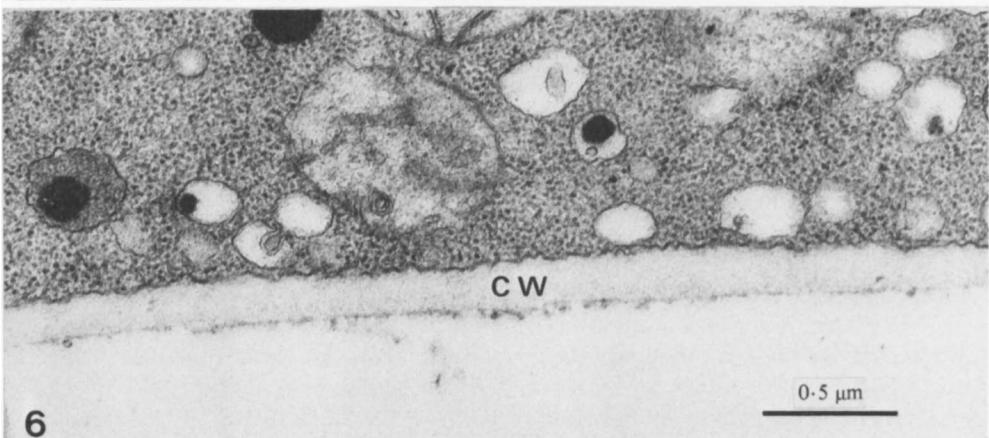
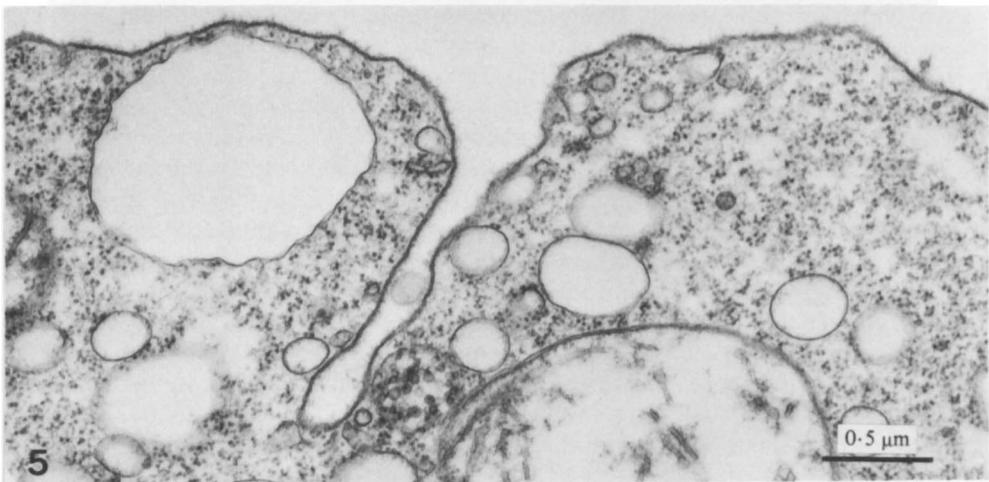
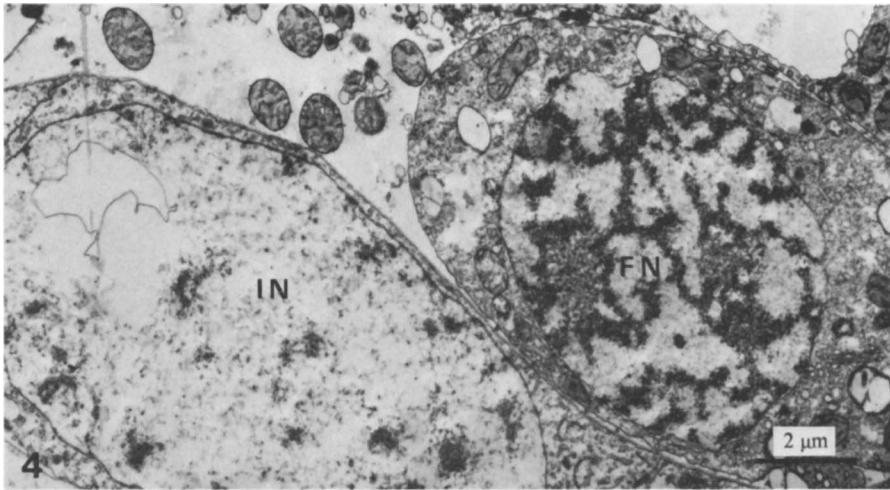


Fig. 4. Protoplast of *E. neoaphidis* lying between insect cells. Note differences between the insect cell nucleus (IN) and the fungal nucleus (FN).

Fig. 5. Invagination of protoplast plasma membrane. Note fibrous exoplasmic coat.

Fig. 6. Part of a cell bounded by a cell wall (CW). Compare with Figs 1–5. Stained with barium permanganate and lead citrate.

observations suggest that protoplasts multiply by budding and are able to colonize the solid tissues of the host, initially the fat body.

The ultrastructure of the fungal cells differs from that of insect cells in a number of ways. For example, the fungal nuclei contain conspicuous aggregations of heterochromatin (Figs 3 and 4) which are absent from nuclei in host cells (Fig. 4). Also, during mitosis, extranuclear, annular or tubular spindle pole bodies polarize the intranuclear spindle of *E. neoaphidis*, whereas mitotic nuclei of *A. pisum* are associated with centrioles.

The ultrastructure of *E. neoaphidis* protoplasts (Figs 1–5) also differs in several ways from that of the walled cells (Fig. 6). (a) The plasma membrane of the protoplast is often invaginated (Fig. 5) and these invaginations may be surrounded by endoplasmic reticulum, mitochondria and microbodies. Such invaginations, which are absent from the walled cell, suggest that the protoplast is feeding by pinocytosis. (b) The plasma membrane of the protoplast possesses a thin, fibrous coat on the exoplasmic surface, apparent as a fuzzy layer in Figs 2 and 5, which is not readily visible in the walled cell. (c) The protoplast contains more stacked, rough endoplasmic reticulum and multivesicular bodies than the walled cell. (d) Mitochondria in walled cells are usually elongated, often cup or 'doughnut' shaped and contain numerous cristae, typical of zygomycetous fungi. Protoplast mitochondria, however, are smaller, oval and with fewer cristae. (e) Protoplasts lack the numerous electron-dense bodies which are found in the cells with walls. However, all of these differences vary in degree depending on the developmental stage (i.e. with time after inoculation) and possibly also on the position of the protoplast within the host body.

It is not clear why entomogenous fungi produce protoplasts. Possibly, through pinocytosis, they enable the fungus to feed more efficiently. Also, the fungus appears to multiply as protoplasts, which would be advantageous because energy would not be expended in cell wall synthesis. The fungus might then be enabled to multiply rapidly, and to colonize and kill its host before the host defence system could have any impact.

The protoplast state may be more common in the life cycle of entomogenous fungi and particularly, as Tyrrell (1977) suggested, in members of the Entomophthorales, than is presently realized. A number of workers studying insects infected with entomophthoraceous fungi have described or illustrated protoplasts apparently without realizing it. Humber (1975) showed an electron micrograph of protoplasts in his thesis on *Strongwellsea magna* Humber (= *Erynia magna* Remaudière & Keller). Some of his light micrographs also appear to illustrate protoplasts. However, little comment is made on these findings in the text. Balazy (1978) observed that *Entomophthora batkoi* Balazy (= *Conidiobolus batkoi* Remaudière & Hennebert) produced amoeboid-like hyphal bodies, suggesting that either the fungus has an extremely plastic cell wall or, more probably, that the bodies were protoplasts. Sorokin (1883) also reported amoeba-like hyphal bodies in the haemolymph of a cricket infected with *Entomophthora colorata* Sorokin, and Thaxter (1888) noted that the conjugating hyphal bodies of *Empusa fresenii* Nowakowski (= *Neozygites fresenii* Remaudière & Keller) from which zygospores form, have no proper wall.

There are two main reasons why the protoplasts have been overlooked in the past. One is that with a light microscope, walled cells are not easily distinguished from protoplasts. The other arises because a filamentous form suggests that the fungus is enclosed in a cell wall. However, as we have now established, *E. neoaphidis*, at least, produces filamentous protoplasts.

In addition, our unpublished observations have shown that, in their respective aphid hosts, *Entomophthora planchoniana* Cornu and *Neozygites fresenii* form protoplasts but *Conidiobolus obscurus* Remaudière & Keller (= *Entomophthora obscurus* Hall & Dunn) and *Zoophthora radicans* Remaudière & Keller (= *Empusa radicans* Brefeld) do not.

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