3

Protozoa: Plasmodiophoromycota

3.1 Introduction

The Plasmodiophoromycota are a group of obligate (i.e. biotrophic) parasites. The best-known examples attack higher plants, causing economically significant diseases such as club-root of brassicas (*Plasmodiophora brassicae*), powdery scab of potato (*Spongospora subterranea*; formerly *S. subterranea* f. sp. *subterranea*) and crook-root disease of watercress (*S. nasturtii*; formerly *S. subterranea* f. sp. *nasturtii*). In addition to damaging crops directly, some species (*S. subterranea*, *Polymyxa betae*, *P. graminis*) also act as vectors for important plant viruses (Adams, 1991; Campbell, 1996). Other species infect roots and shoots of non-cultivated plants, especially aquatic plants. Algae, diatoms and Oomycota are also attacked. If the nine species of *Haptoglossa*, which parasitize nematodes and rotifers, are included in the Plasmodiophoromycota, the phylum currently comprises 12 genera and 51 species (Dick, 2001a). Genera are separated from each other largely by the arrangement of resting spores in the host cell (Waterhouse, 1973). This feature has also been used for naming most genera; for instance, in *Polymyxa*, numerous resting spores are contained within each sorus, whereas in *Spongospora* the resting spores are grouped loosely in a sponge-like sorus (Fig. 3.6). Accounts of the Plasmodiophoromycota have been given by Sparrow (1960), Karling (1968), Dylewski (1990) and Braselton (1995, 2001).

3.1.1 Taxonomic considerations

Plasmodiophoromycota have traditionally been studied by mycologists and plant pathologists. Many general features of their biology and epidemiology are similar to those of certain members of the Chytridiomycota such as *Olpidium* (see p. 145). However, it is now clear from DNA sequence analysis and other criteria that *Plasmodiophora* is related neither to the Oomycota and other Straminipila (Chapters 4 and 5) nor to the true fungi (Eumycota). Instead, it is distantly related to the Myxomycota discussed in Chapter 2 but belongs to a different grouping within the Protozoa (Barr, 1992; Castlebury & Domier, 1998; Ward & Adams, 1998; Archibald & Keeling, 2004).

Some believe that *Haptoglossa* is related to the Oomycota rather than Protozoa, although no molecular data seem to be available as yet to support this claim. Since *Haptoglossa* strikingly resembles *Plasmodiophora* in its infection biology, we shall include it in this chapter. With the possible exception of *Haptoglossa*, the phylum Plasmodiophoromycota is monophyletic and contains a single class (Plasmodiophoromycetes). We consider two orders in this chapter, Plasmodiophorales and Haptoglossales.

3.2 Plasmodiophorales

The zoospore of the Plasmodiophorales is biflagellate. The flagella are inserted laterally and are

of unequal length, the anterior one being shorter. Both flagella are of the whiplash type (Fig. 1.17c). Zoospores of this type are said to be **anisokont**. Transmission electron microscopy (TEM) studies have shown that the tips of the flagella are tapered rather than blunt (Clay & Walsh, 1997). Like the zoospore, the main vegetative unit – the amoeba, which enlarges to become a plasmodium – is wall-less. It is present freely within host plant cells, its membrane being in direct contact with the host cytoplasm. The plasmodia possess amoeboid features because they can produce pseudopodia and engulf parts of the host cytoplasm by phagocytosis (Claxton *et al*., 1996; Clay & Walsh, 1997). This has been interpreted as a primitive trait perhaps betraying a free-living amoeboid ancestor with a phagocytotic mode of nutrition (Buczacki, 1983). Some Plasmodiophorales can now be grown away from their host on artificial media for prolonged periods if bacteria are present. These are phagocytosed by amoeboid growth forms (Arnold *et al*., 1996). In their hosts, amoeboid plasmodia can digest their way through plant cell walls, moving to adjacent uninfected cells and thus spreading the infection within an infected root (Mithen & Magrath, 1992; Claxton *et al*., 1996).

The walled stages of Plasmodiophorales are confined to the zoospore cysts on the plant surface, and the zoosporangia and resting sporangia inside host plant cells. The wall of resting spores is particularly thick and has been shown to contain chitin (Moxham & Buczacki, 1983).

3.2.1 Life cycle of Plasmodiophorales

Certain details of the life cycle of the Plasmodiophorales are still doubtful (Fig. 3.1). However, the known stages show very little variation between different species, indicating that the life cycle is conserved throughout the order. A resting spore germinates by releasing a single haploid zoospore (**primary zoospore**) which encysts on a suitable surface by secreting a cell wall. After a while, an amoeba is injected from the cyst into a host cell such as a root hair where it enlarges to form a plasmodium, accompanied by mitotic nuclear divisions. Nuclear divisions at this stage are **cruciform**; the nucleolus is prominently visible throughout the mitotic process, elongating in two directions to take up a cross-like shape when viewed in certain sections by transmission electron microscopy. This feature is unique to the Plasmodiophorales (Braselton, 2001). After a while, nuclei divide mitotically in a non-cruciform manner, and the contents of the plasmodium differentiate into zoospores. This type of plasmodium is termed the **primary plasmodium** or sporangial plasmodium because it produces zoospores. The zoospores are called **secondary zoospores** because they arise from a sporangium, not from a resting spore. Once released, secondary zoospores may re-infect the host to give rise to further primary plasmodia and zoosporangia. Eventually, however, a different type of plasmodium, the **secondary plasmodium** or sporogenic plasmodium, is formed which undergoes meiotic nuclear divisions and produces resting spores (Garber & Aist, 1979; Braselton, 1995). It is not known where plasmogamy and karyogamy occur in the life cycle of the Plasmodiophorales.

All developmental stages of *P. brassicae* can be produced readily in the laboratory. Clubbed roots should be collected from a field or garden and kept frozen at −20°C. Seedlings of brassicas, susceptible Chinese cabbage cultivars or *Arabidopsis thaliana* should be grown in a soil with a high peat content which must be kept well watered. Infections can be established by adding slices of infected root material or a resting spore suspension to the soil. Zoosporangia will be formed within a few days, and root galls should be visible within 3–7 weeks (Castlebury & Glawe, 1993). Potato or tomato plants can be infected with *Spongospora subterranea* using similar protocols. Cabbage callus cultures are occasionally used as a simplified experimental system for life cycle studies of *P. brassicae* (Tommerup & Ingram, 1971).

3.2.2 *Plasmodiophora brassicae*

Plasmodiophora brassicae is the causal organism of club root or finger-and-toe disease of brassicas (Fig. 3.2) and was first described by Woronin (1878). The disease is common in gardens where

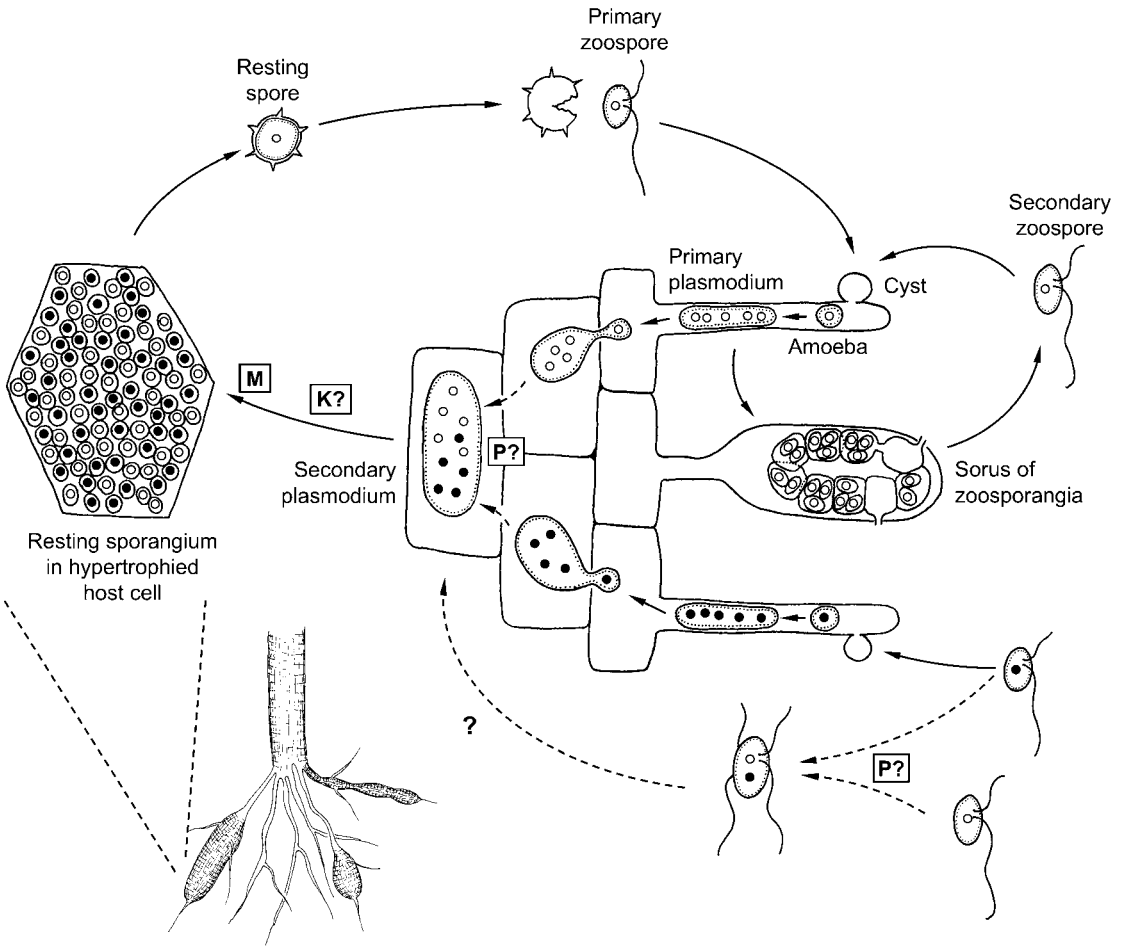

Fig 3.1 Probable life cycle of *Plasmodiophora brassicae*. A haploid resting spore forms a haploid primary zoospore giving rise to a multinucleate haploid primary plasmodium upon infection of a root hair. Secondary zoospores are also haploid, and the way in which they meet to form a secondary heterokaryotic plasmodium is not known for sure. Open and closed circles represent haploid nuclei of opposite mating type; the position of the diploid phase in the life cycle is unclear. Key events in the life cycle are plasmogamy (P), karyogamy (K) and meiosis (M). After Tommerup and Ingram (1971), Buczacki (1983) and Dylewski (1990).

brassicas are frequently grown, especially if the soil is acidic and poorly drained. A wide range of brassicaceous hosts is attacked, and root-hair infection of some non-brassicaceous hosts can also occur (Ludwig-Müller *et al*., 1999). The disease is widely distributed throughout the world.

Club root symptoms

Infected crucifers usually have greatly swollen roots. Both tap roots and lateral roots may be affected. Occasionally, infection results in the formation of adventitious root buds which give rise to swollen stunted shoots. Above ground, however, infected plants may be difficult to distinguish from healthy ones. The first symptom is wilting of the leaves in warm weather, although such wilted leaves often recover at night. Later the rate of growth of infected plants is retarded so that they appear yellow and stunted. Plants infected at the seedling stage may be killed, but if infection is delayed the effect is much less severe and well-developed heads of cabbage, cauliflower, etc., can form on plants with quite extensive root **hypertrophy** (swelling of cells) and **hyperplasia** (enhanced

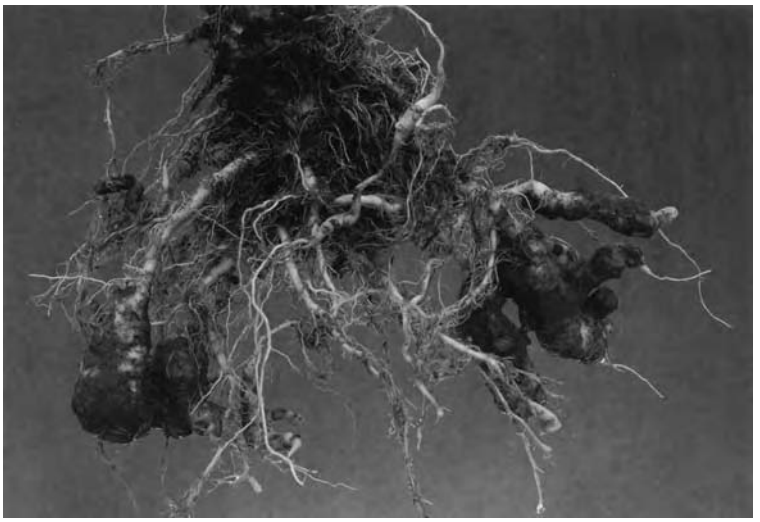

Fig 3.2 Club root of cabbage caused by *Plasmodiophora brassicae*.

division of cells). Microscopically, even infected root hairs are expanded at their tips to form club-shaped swellings which are sometimes lobed and branched (Fig. 3.3). Rausch *et al*. (1981) followed the growth of infected and uninfected seedlings of Chinese cabbage, a particularly susceptible host. Within the first 30 days, the growth rates of infected and control plants were almost identical, and clubs developed in proportion to shoot growth. Wilting of infected plants was observed beyond 30 days when the clubs developed at the expense of shoots. Plants growing in suboptimal conditions, e.g. in the shade, produced disproportionately smaller clubs. Generally, the root/shoot ratio is appreciably higher in infected plants, suggesting a diversion of photosynthetic product to the clubbed roots. The *P. brassicae* infection therefore acts as a new carbon sink.

The process of infection

Swollen roots contain a large number of small spherical resting spores, and when these roots decay the spores are released into the soil. Electron micrographs show that the resting spores have spiny walls (Yukawa & Tanaka, 1979). The resting spore germinates to produce a single zoospore with two flagella of unequal length, both of the whiplash type and with the usual 9 + 2 arrangement of microtubules (Aist & Williams, 1971). Germination of resting spores is stimulated by substances specific to Brassicaceae, possibly allyl isothiocyanates, which diffuse from the cabbage roots into the soil (Macfarlane, 1970).

The primary zoospore (i.e. the first motile stage released from the resting spore) swims by means of its flagella, the long flagellum trailing and the short one pointing forward. The process of root hair infection has been followed in a classical study by Aist and Williams (1971). Since the first such study, on penetration by *Polymyxa betae*, was written in German (Keskin & Fuchs, 1969), the German terminology is still in use today. Primary zoospores of *P. brassicae* are released some 26–30 h after placing a suspension of resting spores close to seedling roots of cabbage. The zoospores may collide several times with a root hair before becoming attached, and appear to be attached at a point opposite to the origin of the flagella.

The flagella coil around the zoospore body, which becomes flattened against the host wall, and pseudopodium-like extensions of the zoospore develop, being continuously extended and withdrawn. The flagella are then withdrawn, and the zoospore encysts, attached to the root hair (Fig. 3.4). The zoospore cyst contains lipid bodies and a vacuole which enlarges during cyst maturation, which takes a few hours. The most conspicuous ultrastructural feature of mature cysts is a long **Rohr** (tube), with its outer end pointing towards the root hair wall. This end of the tube is occluded by a plug. Within the tube

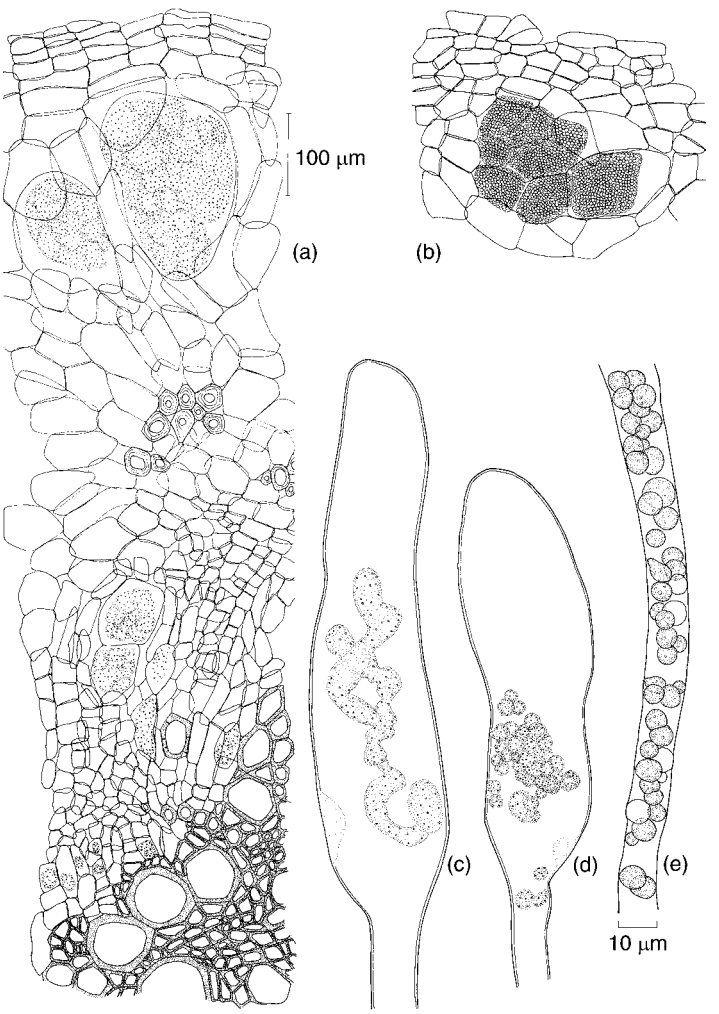

Fig 3.3 *Plasmodiophora brassicae*. (a) T.S. through young infected cabbage root showing secondary (sporogenic) plasmodia in the cortex. Note the hypertrophy of some of the host cells containing plasmodia, and the presence of young plasmodia in cells immediately outside the xylem. (b) T.S. cabbage root at a later stage of infection, showing the formation of resting spores. (c) Primary (zoosporangial) plasmodium in cabbage root hair 4 days after planting in a heavily contaminated soil. (d) Young primary zoosporangia in root hair. Note the club-shaped swelling of the infected root hair. (e) Mature and discharged primary zoosporangia. a and b to same scale; (c—e) to same scale.

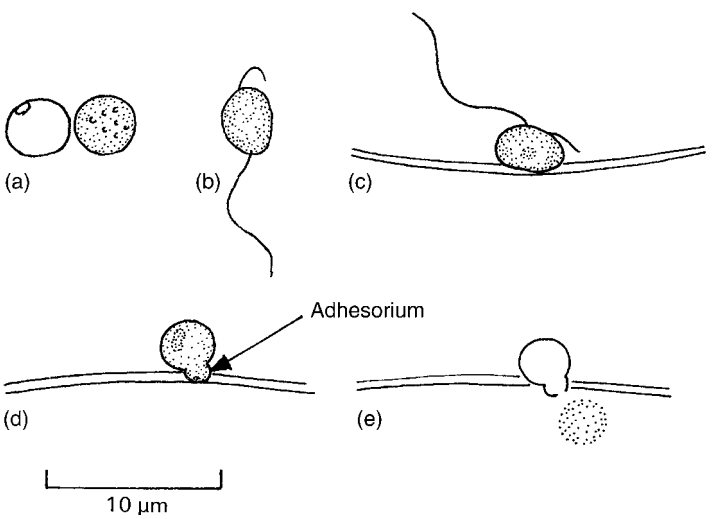

Fig 3.4 *Plasmodiophora brassicae*. (a) Resting spores, one full, one empty (showing a pore in the wall). (b) Zoospore. (c) Attachment of zoospore to root hair. (d) Zoospore cyst with adhesorium following withdrawal of flagellar axonemes. (e) Entry of amoeba into root hair. Based on Aist and Williams (1971).

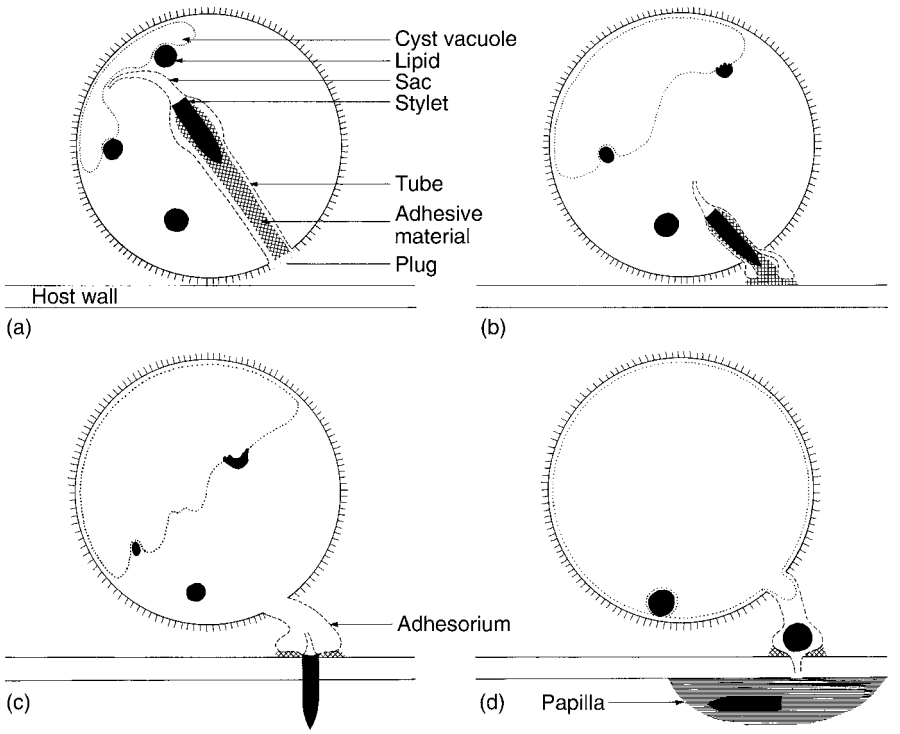

Fig 3.5 *Plasmodiophora brassicae*. Diagrammatic summary of penetration process (after Aist & Williams, 1971). The diagram shows a zoospore cyst attached to the wall of a root hair. (a) Cyst vacuole not yet enlarged. (b) About 3 h later, the cyst vacuole enlarges and a small adhesorium appears. (c) About 1 min later, the stylet punctures the host cell wall. (d) Penetration has occurred and the host protoplast has deposited a papilla at the penetration site.

is a bullet-shaped **Stachel** (stylet), the outer part of which is made up of parallel fibrils. Behind the blunt posterior end of the stylet, the tube narrows to form a **Schlauch** (sac).

Penetration of the root hair wall occurs about 3 h after encystment, as after this time the first empty vacuolated cysts are observed. The penetration process takes place rapidly, and an interpretation of it is shown in Fig. 3.5. Firm attachment of the tube to the root hair is brought about by the **adhesorium**, which may develop by partial evagination (i.e. turning inside out) of the tube (Fig. 3.5b). During evagination, an adhesive substance which has a fibrillar appearance in TEM micrographs is released onto the adhesorial surface from its storage site inside the tube. The enlargement of the vacuole is presumably the driving force which brings about complete evagination of the tube within 1 min, followed by thrusting the stylet through the host wall. The pathogen is injected into the host cell as a small, spherical, wall-less amoeba which becomes caught up by cytoplasmic streaming. After penetration (Fig. 3.5d), a papilla of callose is deposited around the penetration point beneath the adhesorium, possibly as a wound-healing response. Similar penetration mechanisms have been described for other Plasmodiophorales, including *Spongospora subterranea* (Merz, 1997), *S. nasturtii* (Claxton *et al*., 1996) and *Polymyxa betae* (Keskin & Fuchs, 1969). Details of the infection process by *P. betae* have been filmed (see Webster, 2006a). A yet more elaborate process of infection is found in *Haptoglossa*, which parasitizes nematodes and rotifers (see p. 65).

Development of zoosporangia

Within the infected root hair, the amoeba may divide into several uninucleate amoebae. Later the nuclei within each amoeba show cruciform divisions, giving rise to small multinucleate

primary plasmodia. Each plasmodium divides up to form a group (**sorus**) of roughly spherical thin-walled zoosporangia lying packed together in the host cell (Fig. 3.3). Separate protoplasts might coalesce at this stage. Each zoosporangium finally contains 4–8 uninucleate zoospores. These are morphologically identical to primary zoospores. Some mature zoosporangia become attached to the host cell wall and an exit pore develops at this point through which the zoospores escape. The zoospores of other sporangia are released into those with an exit pore. Occasionally, zoospores escape into the lumen of the host cell. Liberated zoospores can re-infect plant roots, thereby completing an asexual cycle (Fig. 3.1).

Sexual reproduction

In *P. brassicae*, resting sporangia are not formed in root hairs after the first cycle of infection, but are located mainly in older infections in strongly hypertrophied regions of the root cortex. There is evidence that resting sporangia are involved in sexual reproduction (Fig. 3.1) because meiotic nuclear divisions with synaptonemal complexes have been observed in maturing resting sporangia (Garber & Aist, 1979). Further, each resting spore normally contains one haploid nucleus (Narisawa *et al*., 1996). Thirdly, infection experiments have established that resting sporangia are formed only if two genetically dissimilar nuclei are present (Narisawa & Hashiba, 1998) which could be contributed either by two uninucleate zoospores or by a binucleate zoospore.

The positions of the preceding stages of sexual reproduction – plasmogamy and karyogamy – in the life cycle of *P. brassicae* are still a matter of doubt. One possibility is that secondary zoospores fuse to form a dikaryon, followed by karyogamy. Quadriflagellate binucleate swarmers have indeed been observed and can result from the fusion of zoospores (Tommerup & Ingram, 1971). However, it is not yet clear whether these quadriflagellate spores can infect plant cells from the outside. Quadriflagellate binucleate zoospores may also arise from incomplete cleavage of cytoplasm during zoospore formation.

Plasmodia of *P. brassicae* have been shown to break through plant cell walls, thereby spreading an infection from root hairs into deeper tissues of the root cortex (Mithen & Magrath, 1992). A conceivable alternative would be their migration through plasmodesmata. It is possible that two primary plasmodia or uninucleate amoebae arising from separate root hair infections fuse upon encountering each other deep inside the host plant. Such a fusion would produce a secondary plasmodium, and could be followed by karyogamy and meiosis, which would lead to the development of resting spores (Fig. 3.1).

Hypertrophy of infected host cells

As the plasmodia within a host cell enlarge, the host nucleus remains active and undergoes repeated divisions. Hypertrophy and an increased ploidy of the host nuclei result, at least in callus culture experiments, because the mechanism for host cell division is apparently blocked (Tommerup & Ingram, 1971).

Unsurprisingly, the grossly hypertrophied clubs contain enhanced levels of plant growth hormones. The concentration of auxins (especially indole-3-acetic acid, IAA) in clubbed roots was measured to be about 1.7 times as high as in uninfected roots (Ludwig-Müller *et al*., 1993), and that of cytokinins was 2–3 times elevated (Dekhuijzen, 1980). Isolated secondary plasmodia of *P. brassicae* have been demonstrated to synthesize the cytokinin zeatin (Müller & Hilgenberg, 1986), and the amount of zeatin produced would be sufficient to establish a new carbon sink. The situation is more complicated with respect to auxins which are not synthesized by plasmodia. Instead, the pathogen interferes with the host's auxin metabolism, which is complex (Normanly, 1997). The tissues of healthy crucifers contain relatively large amounts of indole glucosinolates such as glucobrassicin (= indole-3-methylglucosinolate) which is converted by the enzyme myrosinase to 3-indoleacetonitrile (IAN), a direct IAA precursor. Conversion of IAN to IAA is catalysed by nitrilase. Increased concentrations of indole glucosinolates, IAN and IAA have been measured in clubbed roots (Ludwig-Müller, 1999), and the expression of nitrilase and myrosinase was also enhanced. Further, nitrilase

protein was detectable by immunohistochemical methods only in cells containing sporulating plasmodia. The activities of the above enzymes might be regulated by the signalling molecule, jasmonic acid (Grsic *et al*., 1999). However, these metabolic changes were confined to a narrow window of time, and other sources of IAA, such as its release from IAA–alanine conjugates by the activity of amidohydrolase, are likely to contribute (Ludwig-Müller *et al*., 1996). The host–pathogen interactions leading to enhanced auxin levels in clubbed roots are therefore very intricate.

At first, only cortical cells of the young root are infected, but later small plasmodia can be found in the medullary ray cells and in the vascular cambium. Subsequently, tissues derived from the cambium are infected as they are formed. In large swollen roots, extensive wedge-shaped masses of hypertrophied medullary ray tissue may cause the xylem tissue to split. At this stage, the root tissue shows a distinctly mottled appearance. When the growth of the plasmodia is complete, they are transformed into masses of haploid resting spores. Only during the late stages of resting spore development do the host nuclei begin to degenerate. Eventually, the resting spores are released into the soil as the root tissues decay.

3.2.3 *Spongospora*

The life cycle of *S. subterranea*, the cause of powdery scab of potato, is similar to that of *P. brassicae* (Harrison *et al*., 1997; Hutchison & Kawchuk, 1998). Diseased tubers show powdery pustules at their surface, containing masses of resting spores clumped into hollow balls. The resting spores release anisokont zoospores which can infect the root hairs of potato or tomato plants. In the root hairs, plasmodia form which develop into zoosporangia. Zoospores from such zoosporangia are capable of infection, resulting in a further crop of zoosporangia. Zoospores released from the zoosporangia have also been observed to fuse in pairs or occasionally in groups of three to form quadri- or hexaflagellate swarmers, but whether these represent true sexual fusion stages is uncertain. *Spongospora nasturtii* causes a disease of watercress in which the most obvious symptom is a coiling or bending of the roots. Zoosporangia and resting spore balls are found in infected root cells (Fig. 3.6), and plasmodia can migrate through the root tissue by breaking through host cell walls (Claxton *et al*., 1996; Clay & Walsh, 1997). The encounter of two plasmodia might initiate sexual reproduction and thus complete the life cycle without any need for the parasite to leave the host (Heim, 1960).

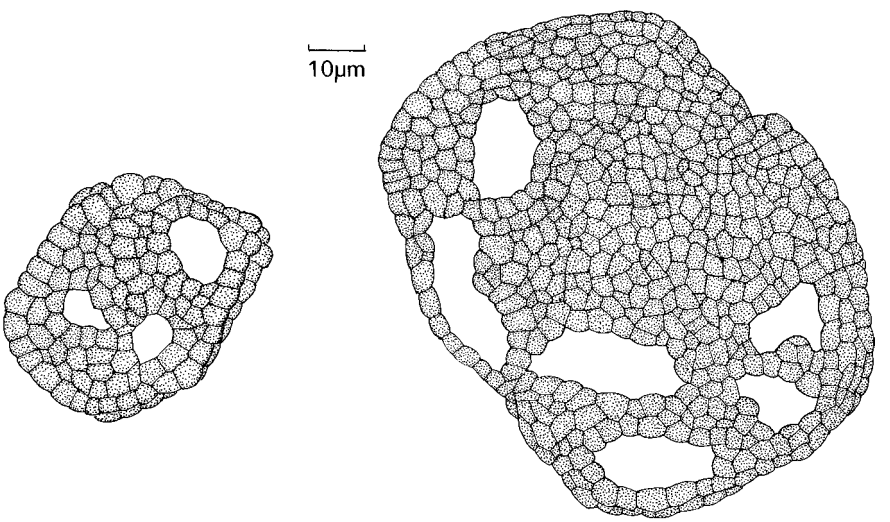

Fig 3.6 *Spongospora nasturtii*. Spore balls from watercress roots with crook root disease.

In addition to being the causal agent of powdery scab of potatoes, *S. subterranea* is also important as the vector of potato mop-top virus disease, which can reduce the yield of tubers by over 20% in some varieties (Campbell, 1996; Harrison *et al*., 1997). The virus is transmitted by the zoospores and can also persist for several years in spore balls in the soil. It seems to be located inside the resting spores (Merz, 1997). Zoospores of *S. subterranea* can cause zoosporangial infections in the root hairs of a wide range of host plants outside the family Solanaceae, and can transmit viruses to them. Thus *S. subterranea* and numerous wild plants can provide a reservoir of infection for the potato mop-top virus even if potatoes have not been grown in a field for many years. Other members of the Plasmodiophorales also act as vectors for plant viruses, notably *Polymyxa betae* which transmits the beet necrotic yellow vein virus, and *P. graminis* which transmits several mosaic viruses on most major cereal crops.

3.3 Control of diseases caused by Plasmodiophorales

3.3.1 Club root

The control of club root disease is difficult. Because resting spores retain their viability in the soil for up to 20 years, short-term crop rotation will not eradicate the disease. The fact that *Plasmodiophora brassicae* can infect brassicaceous weeds such as shepherd's purse (*Capsella bursa-pastoris*) or thalecress (*Arabidopsis thaliana*) suggests that the disease can be carried over on such hosts and that weed control is important. Moreover, it is known that root hair infection can also occur on many ubiquitous non-brassicaceous hosts such as *Papaver* and *Rumex*, or the grasses *Agrostis*, *Dactylis*, *Holcus* and *Lolium*. All infections of non-brassicaceous hosts are probably reduced to the zoosporangial cycle, and no root clubs are formed. Whether such infections play any part in maintaining the disease in the prolonged absence of a brassicaceous host is not known.

General measures aimed at mitigating the incidence of clubroot traditionally include improved drainage and the application of lime, which retards the primary infection of root hairs. Since the effect of liming does not persist, it is possible that it may simply delay the germination of resting spores and thus prolong their existence in the soil (Macfarlane, 1952). More recently, boron added at $10-20\,mg\,kg^{-1}$ soil in conjunction with a high soil pH has been shown to suppress primary as well as secondary infections (M.A. Webster & Dixon, 1991). Early infection of seedlings can result in particularly severe symptoms, so it is important to raise seedlings in non-infected or steam-sterilized soil. The young plants can then be transplanted to infested soil. Since it is known that some resting spores survive animal digestion, manure from animals fed with diseased material should not be used for growing brassicas.

Infection can be retarded by the application of mercury-containing compounds or benomyl, but these are now banned in many countries. At present, no economically and ecologically acceptable fungicide appears to be available, although research efforts continue (Mitani *et al*., 2003). Some attempts have been made to establish biological control methods for *P. brassicae* (Narisawa *et al*., 1998; Tilston *et al*., 2002), but it is doubtful whether such methods will gain full commercial viability in the near future.

In recent years, increasing emphasis has been placed on breeding club root resistant cultivars of crop plants. The weed *Arabidopsis thaliana*, which develops the full set of club root symptoms, has been used as a host for such studies because it is accessible by molecular biological methods. Natural resistance in *Arabidopsis* is based on a single gene and involves the **hypersensitive response**, in which infected plant cells die before the pathogen has had a chance to multiply. The resistance of susceptible cultivars can be enhanced by transformation with various resistance genes, e.g. a gene from mistletoe (*Viscum album*) encoding viscotoxin, a thionin-type cystein-rich polypeptide with antimicrobial activity (Holtorf *et al*., 1998). Further, mutant lines with reduced levels of IAA precursors show reduced club development (Ludwig-Müller, 1999).

In contrast to *Arabidopsis*, natural resistance in cabbage is multigenic, with no obvious hypersensitive response (Ludwig-Müller, 1999). Breeding for resistance is difficult (Bradshaw *et al*., 1997) and may not provide long-lasting success due to the development of new virulent races of *P. brassicae* on the resistant cultivars after a few years in the field. By 1975, 34 different physiological races of *P. brassicae* from Europe had already been differentiated based on infection experiments with *Brassica* cultivars varying in their degree of resistance (Buczacki *et al*., 1975). Further, *P. brassicae* can still infect root hairs and reproduce by zoosporangia even in resistant cultivars.

3.3.2 Powdery scab and crook root

Powdery scab of potatoes is normally of relatively slight economic importance and amelioration of the disease can be brought about by good drainage. Potato mop-top virus infections can be more serious, however. Transgenic plants containing the viral coat protein gene have been shown to be completely resistant against infections by the virus (Reavy *et al*., 1995), and it may be possible to produce transgenic crop plants in future.

Crook root of watercress can be controlled by application of zinc to the water supply. The zinc can be applied by dripping zinc sulphate into the irrigation water for watercress beds to give a final concentration of about 0.5 ppm, or by the

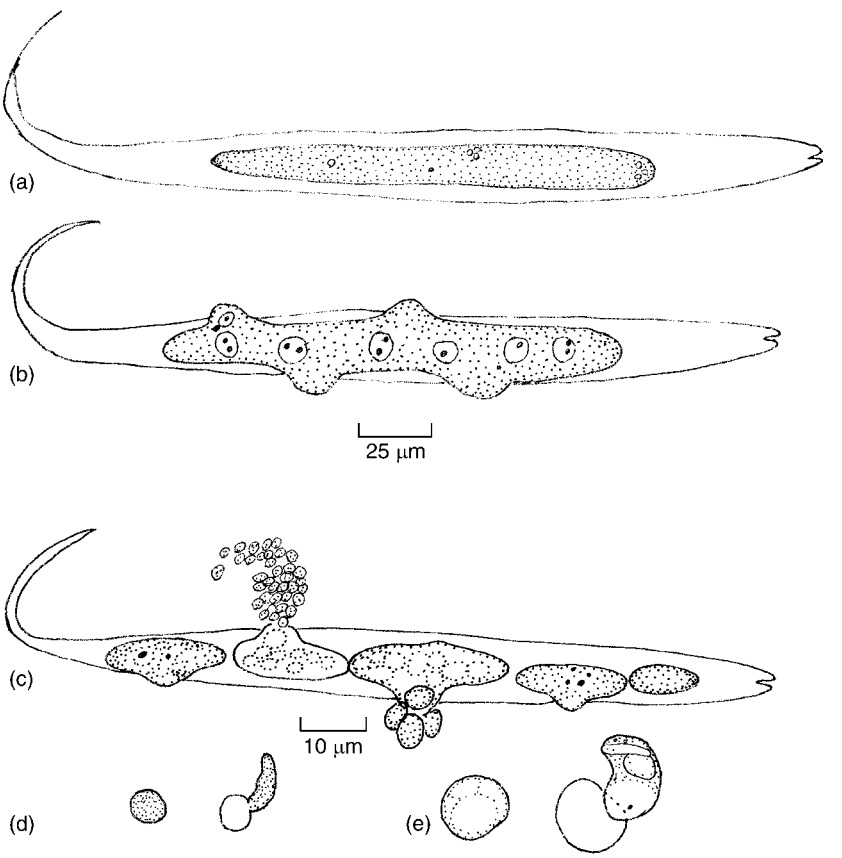

Fig 3.7 *Haptoglossa heteromorpha* parasitizing nematodes. (a) Single young thallus in a dead nematode. (b) Single maturing sporangium with developing dome-shaped exit papillae. (c) Nematode body containing several plasmodia and sporangia. One sporangium has released large aplanospores, and an adjacent one small ones. (d) Small aplanospores, one germinating to form a gun cell. (e) Large aplanospores, one germinating to form a gun cell. (a–c) to same scale; d,e to same scale. Redrawn from Glockling and Beakes (2000a).

addition of finely powdered glass containing zinc oxide (zinc frit) to the beds. The slow release of zinc from the frits maintains a sufficiently high concentration to inhibit infection (Tomlinson, 1958).

3.4 *Haptoglossa* (Haptoglossales)

3.4.1 General biological features of *Haptoglossa*

If a slurry of soil or herbivore dung is spread on a weak medium such as tap water agar or cornmeal agar, the nematodes or rotifers contained within these samples may become parasitized and killed by fungi producing thalli within the cadavers. Although superficially resembling the plasmodia of *Plasmodiophora*, this term cannot be applied to *Haptoglossa* because its thalli are surrounded by a wall at all stages of development. One or several thalli may fill almost the entire body cavity of a nematode and become converted into sporangia upon maturity (Fig. 3.7). Sporangia of some species of *Haptoglossa* release zoospores which are anisokont, with both flagella of the whiplash type. Zoospore release occurs through one or several exit papillae (Barron, 1977). Zoospores of *Haptoglossa* are weak swimmers and encyst within a few minutes in the vicinity of the host cadaver from which they were released. Other species of *Haptoglossa* do not release zoospores but produce non-motile spores (aplanospores) resembling cysts of the zoospore-forming species. Aplanospore release occurs by explosive rupture of the exit tube, followed by several further, progressively weaker bursts of discharge (Glockling & Beakes, 2000a). A few hours after their formation or release, cysts or aplanospores germinate to produce an elongated or glossoid (= tongue-shaped) cell, which is also often called a **gun cell** or an infection cell. This explosively injects a small amount of walled protoplasm (**sporidium**) containing a nucleus and a few organelles into a host passing by (see below). The sporidium enlarges to form a new thallus and, upon host death, a new sporangium. The mechanism of gun cell discharge is rather similar to that found in cysts of *Plasmodiophora* or *Polymyxa*. This, together with the occurrence of anisokont zoospores, has been taken as an indication that *Haptoglossa* should be included in the Plasmodiophoromycota (Beakes & Glockling, 1998; Dick, 2001a), whereas formerly the genus was thought to be related to the Oomycota.

The aplanosporic species of *Haptoglossa* produce spores of two distinctly different sizes,

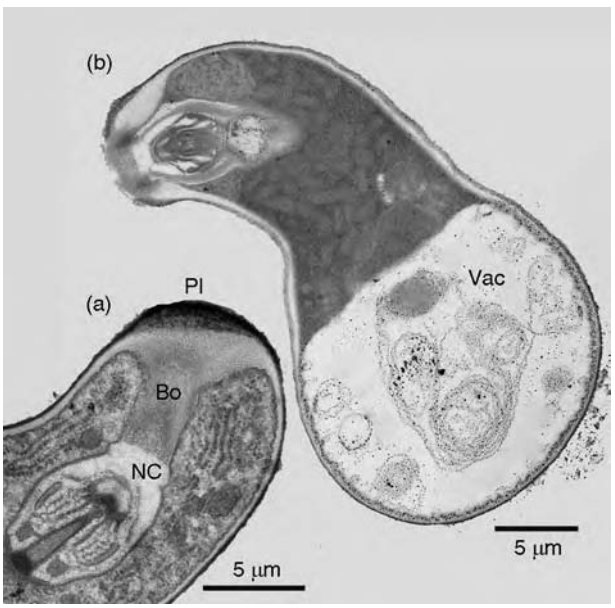

Fig 3.8 *Haptoglossa* sp. (a) Tip of a developing gun cell. The muzzle is still sealed by its plug (Pl). Bore (Bo) and needle chamber (NC) are visible. (b) Transmission electron micrograph of a mature gun cell. The basal part of the gun cell is entirely occupied by the enlarging posterior vacuole (Vac). Original prints kindly supplied by S. L. Glockling.

although any one sporangium produces propagules only of either size (Glockling & Beakes, 2000a; Fig. 3.7). In contrast to the Plasmodiophorales, sexual reproduction or resting stages have not yet been described for any species of *Haptoglossa*, and it is difficult at present to explain the occurrence of spores of different sizes. What appears clear is that each thallus is the result of a discrete infection event.

3.4.2 The gun cell of *Haptoglossa*

Germination of the spherical zoospore cyst or aplanospore of *Haptoglossa* occurs by means of a short germ tube which enlarges to form the elongated gun cell (Robb & Lee, 1986a). This remains attached to the cyst until maturity and is perched on top of it in many species. The mature gun cell (Figs. 3.8, 3.9a) shows strong ultrastructural similarities to the infection apparatus of *Plasmodiophora* (see Fig. 3.5) and is the object of considerable mycological curiosity. A tube leads into the pointed tip of the gun cell but its opening (**muzzle**) is separated from the exterior by a thin wall (**plug**) for most of its development (Fig. 3.8a). The formation of this internal tube from the tip of the gun cell backwards has been likened to inverted internal tip growth and is mediated by a scaffold of actin fibres against the turgor pressure of the gun cell (Beakes & Glockling, 1998). The inner (non-cytoplasmic) surface of the anterior part of the tube (**bore**) is lined with fibrillar material. A second wall separates the bore from a swollen section of the tube, the **needle chamber**. This contains a projectile (**needle**) resembling the bullet of *Plasmodiophora*, but terminating in a much finer tip, possibly reflecting the different properties of the host surface which it has to puncture. The needle is held in place by a complex set of cones and cylinders (Fig. 3.8a) which are thought to exercise a restraining function, fixing the needle against the high turgor pressure of the gun cell. The cones and cylinders may contain actin filaments. The shaft of the needle is much wider than its tip. The posterior (innermost) part of the tube (**tail**) coils around itself and the nucleus, almost touching the side of the needle chamber. The tail is walled,

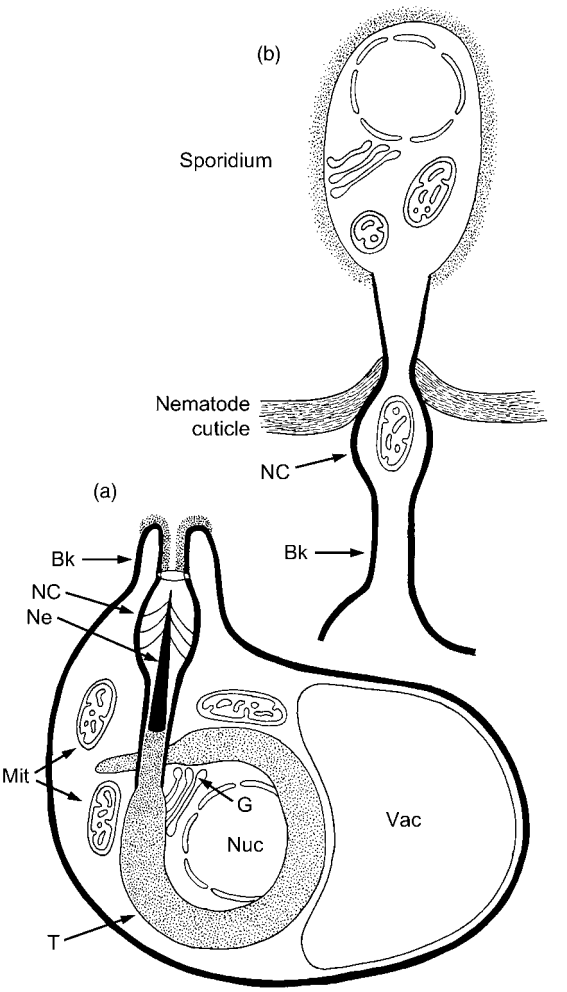

Fig 3.9 Schematic drawings of the nematode penetration mechanism in *Haptoglossa*. (a) Gun cell ready for discharge. The tube has already protruded to form a beak (Bk), the exterior of which is lined by a glue originating from the inside surface of the bore. This aids in the attachment of the gun cell to a passing nematode. The needle (Ne) is held in position by actin filaments inside the needle chamber (NC), which is separated from the outside by a wall. Behind the needle chamber is the coiled tail (T) which contains wall material in its lumen (dotted area). In fact, the tail is multi-layered, but this has not been illustrated here. The tail coils round the nucleus (Nuc) and a Golgi stack (G), and mitochondria (Mit) are also located in the vicinity. The posterior of the gun cell is filled by one large vacuole (Vac). (b) Tip of a fired gun cell showing the everted tail which has penetrated the nematode cuticle and has formed a sporidium inside the nematode body (above the cuticle). The wall material formerly located inside the tail has formed the sporidium wall. The detached needle is also visible inside the nematode body. For a more detailed description of the eversion process, see Glockling and Beakes (2000b).

and additional electron-dense cell wall precursor material is deposited within the lumen of the tail. Synthesis of the tube is mediated by one large Golgi stack which is always closely associated with the nucleus and faces the inward-growing tube tip, emitting vesicles towards it. As the tube extends and coils round the nucleus, the nucleus and Golgi stack turn like a dial by 360° (Beakes & Glockling, 1998, 2000). The turgor pressure of the gun cell is probably generated by a large posterior vacuole (Fig. 3.8b), similar to that found in cysts of *Plasmodiophora*. The osmotically active solutes required for turgor generation may originate from the degradation of lipid droplets within the enlarging vacuole.

Shortly before discharge, the increasing turgor pressure of the posterior vacuole is thought to push the tip of the gun cell forward; the wall sealing the muzzle is lost, and the bore shortens and extends a beak-like projection (Fig. 3.9a). The cell wall material from the interior of the bore now forms the external beak wall, and the needle is ready for injection. The nature of the discharge trigger probably varies between different species of *Haptoglossa* and may be chemical or mechanical. The beak wall is thought to act as an adhesive and immediately glues the gun cell to the cuticle of a passing nematode or rotifer. Firm attachment is necessary to provide resistance against the recoil of the needle attempting to penetrate the tough cuticle of the host, as it is for the penetrating bullet in adhesoria of *Plasmodiophora*.

Beakes and Glockling (1998) speculated that stretch-activated membrane channels (see p. 8) might be involved in triggering the launch of the needle. Following attachment, Ca^{2+} ions entering the needle chamber would cause the actin-rich cones and cylinders near the needle tip to contract and rupture. Once the constraints exercised by the cones and cylinders are broken, the high turgor pressure of the gun cell will immediately fire the needle, followed by explosive eversion of the entire tube which forms a syringe, conducting the nucleus, Golgi apparatus and mitochondria of the gun cell through the nematode cuticle (Fig. 3.9b). The infective propagule is called a sporidium because it is surrounded by a wall, the material for which is probably contributed by precursor material at the end of the tail section (Robb & Lee, 1986b; Glockling & Beakes, 2000b).