Two new species of *Wawelia* are described from rabbit and hare dung incubated under conditions of restricted water supply. A key to the five known species of *Wawelia* is provided.

MATERIALS AND METHODS

Species of *Wawelia* developed fruit bodies on droppings of rabbit (Oryctolagus cuniculus L.) and hare (Lepus capensis L.) after incubation for several weeks under conditions of restricted water supply. Samples, mostly of rabbit dung, collected from a wide range of habitats and locations, were incubated in the light either in Petri dishes lined by moist filter paper or in deeper dishes with transparent lids containing beach sand and supplied intermittently with small amounts of water. After 2–4 wk, thread-like stromata appeared on a few of the samples. These stromata formed conidia near their apices and, after 2–4 wk, scattered superficial perithecia along their length. After 2–4 wk, thread-like stromata appeared on a few of the samples. These stromata formed conidia near their apices and, after 2–4 wk, scattered superficial perithecia along their length.

In sterile forceps and wiped over the surface of TWA. The conidia germinated within 24–48 h and transfers were then made to fresh media. The ascospores of *Wawelia* are not violently discharged and accumulate at the ostioles of the peritheca. Clumps of such ascospores, or ascospores released by crushing a ripe perithecium, were suspended in a saturated solution of filter-sterilized pigs’ pancreatin (Sigma) at pH 9 in cavity microscope slides placed in moist chambers and incubated for 5 h at 37 °C. The treated ascospore suspension was streaked or spotted out onto dung extract agar (DEA, a filtered extract of 1% dried cow dung in 2% agar) or yeast extract sucrose agar (YESA, 4 g yeast extract, 20 g sucrose, 1 g KH₂PO₄, 0.5 g MgSO₄, 15 g agar l⁻¹ H₂O) and incubated at room temperature. Good germination (about 50%) occurred within 12 h. Untreated ascospores or spores treated by exposure to 60° for 1 h failed to germinate. Germinated ascospores were transferred to fresh media. Following isolation by all these methods, growth took place on a variety of laboratory media such as PDA, DEA, yeast extract agar (YEA, 1% yeast extract, 2% agar) and YESA. The production of stromata was stimulated by growing cultures of both fungi in slopes or Petri dishes on these media with the addition of a few sterilized (autoclaved) rabbit pellets. The cultures were incubated in diffuse light at room temperature. In some cases, small stromata developed on agar media without rabbit pellets.

*Wawelia argentea* J. Webster, sp. nov. (Figs 1–6)

*Stroma perithecia* filiforme, cum vel sine ramis, ad 30 mm longa et 0.1–0.5 mm diam. curvum et contortum, cylindracea vel leviter planum, argenteum-glauceum sed album ad acutum apicem, leve et lucidum prope basim, pruinosum supra. *Perithecia* superficialia singula aut corymbis per majorem partem longitudinis stromatis, late pyriformia cum lata plana basi et brevi collo, ad 400 μm diam. et 400 μm alta, primum argentea-glaucia, postea nigrigera-glaucia. Asci 4-sporati, late clavati, unitunicati, sine apicali apparatu, cum longa desinente basi et cylindrica superiore parte continenti dense
Figs 1–6. 

Fig. 1. Rabbit dung pellet with perithecial stroma (mm scale). The stroma bears superficial perithecia scattered along its base and conidia at the paler apex. Fig. 2. Ascus. Note the cut-off base, the absence of an apical apparatus and the longitudinal germ slit visible on the face of three of the ascospores. Fig. 3. Paraphyses and immature asci. Fig. 4. Conidiophores. Fig. 5. Conidia. Fig. 6. Culture grown in a 9 cm Petri dish on YEA with sterilized rabbit pellets. The stromata have developed conidia near their tips. Bar: Figs 2, 4 and 5 = 20 µm; Fig. 3 = 100 µm.


Perithecial stromata up to 30 mm long and 0.1–0.5 mm diam., thread-like, branched or unbranched, wavy and contorted, cylindrical to slightly flattened, silvery grey along most of their length, white at the pointed tip, smooth and lustrous near the base, pruinose in the upper portions due to the development of conidiophores and conidia (Fig. 1). The outer layer of the stroma is made up of textura porrecta hyphae, 4–5 µm diam. with grey walls 0.5–1.0 µm thick. Perithecia up to 400 µm diam. and 400 µm high, superficial, single or in clusters along much of the length of the stroma, broadly pyriform, with wide, flattened bases and short, cylindrical,
ostiolate necks. They are at first silvery grey and appear pruinose because of a covering of conidiophores, at maturity a darker grey. Asci 4-spored, cylindrical to broadly clavate, unitunicate, 87–93 µm × 10–12 µm, lacking an apical apparatus, with long tapering bases and cylindrical upper portions containing the closely packed ascospores (Fig. 2). Ascospores uniseriate, broadly elliptical in face view, slightly inequilateral when viewed from the side, smooth walled, dark grey to black, with a hyaline germ slit running the length of the spore, 15–18 × 9–12 µm (Fig. 2). Paraphyses thin-walled, tapering, septate, greatly exceeding the asci in length and composed of empty cells up to 12 µm wide (Fig. 3). Conidiophores developing directly from the outer cells of the stroma, branched or unbranched, variable in length, straight or contorted, sometimes zig-zag like, tapering to the tip, 2–3 µm diam. (Fig. 4). Conidiogenous cells integrated. Conidiogenesis holoblastic, conidiogenous cell proliferation sympodial. Conidial secession is indicated by a flattened, slightly thickened, scar. Conidia dry, ellipsoid-ovate, hyaline, smooth, with a slightly protruding tapering base and a flattened scar, 3–4 × 2–2.5 µm (Fig. 5).

Cultures. On YEA slow-growing, with a silky appressed mycelium on the agar until it made contact with the rabbit pellets. Within a few days of making contact with the pellets numerous positively phototropic stromata developed. They resembled those formed on incubated pellets, but were often longer (Fig. 6). Conidia were formed within a few days on the tips of the stromata. Perithecial rudiments were first observed near the bases of the stromata 14 d after inoculation. They did not mature over a period of observation of several weeks.

The type material was from a sample of about 60 pellets, collected by J. Webster at Cornworthy, near Totnes, S. Devon (Map ref. SK 847554) on 13 July 1995 and incubated on filter
paper in a Petri-dish on a window sill at a temperature of about 20–25°C. After 3 wk stromata were noted on eight of the pellets. When examined on 18 Sep. it was found that the perithecia contained 4-spored asci, a feature of *W. regia*, the type species of the genus. The shape of the stromata, the dimensions of the conidia, asci and ascospores differ, however, from those of *W. regia*. If a ripe peritheium is crushed in a drop of water, detached asci and separate ascospores escape, and examination of the bases of these asci shows that they are truncate. It is believed that the ascus walls deliquesce in the body of the perithecium.

Other material examined. Several other collections have been made by J.W. on rabbit dung:

From the same site near Cownhorne, 4 Aug. 1998, K(M)59521. Ripe perithecia were seen on 26 Sep. The asci and ascospores were somewhat smaller than in the earlier collection: 60–68 × 10–11 µm and 12.5–13 × 8–10 µm respectively. In culture, on PDA slopes with sterilized rabbit pellets, perithecial stromata were formed freely.

High Land of Orcombe, near Exmouth, Devon (SY 023796), 14 Sep. and 12 Oct. 1998. Plentiful material of *W. argentea* was found among samples collected on the edge of sea cliffs. In both collections the stromata grew to up to 50 mm tall. Cultures were established from excised stromatal tips on TWA which went on to develop ripe perithecia within 25 d. Asci were 50–67 × 10–11 µm and the ascospores 12–15 × 9.5–12.5 µm.

**Wawelia microspora** J. Webster, sp. nov. (Figs 7–11)

*Stromata* 20–30 mm longa, 0.1–0.3 mm lata, primum sine ramis, cylindrica, pallide fusca, cum roseis acutis apicibus, postea contorta et ramata, glauca vel fusca ad nigra, luculenta, plana, vel longis striae ad longitudinem. Rami distantes, desinentes in acutum album apicem, pulvinulenta. *Conidiophora* crescentes de superficie peritheciali hyalina, desinentes, ad 45 µm longae et 2.5–3 µm lateae. *Conidia* hyalina, elliptica ad cylindrica, cum basali plana cicatricae, 3–4 × 2 µm. *Perithecia* sparsa, late separata per longitudinem stromatis, primum levia et pallida flava-fusca, postea glauca-fusca ad nigra, pilosa, subglobosa ad late obpyriformia, cum lata plana basi et brevi papilliformi ostiolo: 230–360 µm diam. et circa 250 µm alta. *Asci* octospori, cylindricos, longo culmo, sine manifesto apicali apparatus, 75–88 × 5 µm. *Paraphyses* longiores quam asci, ramatae, compositae ex cellis parietibus tenuis, ad 8 µm latae. *Ascospores* uniseriatae, vel paulo superjacentes, inaequaliter vel ellipticae, glaucae vel nigrae cum longitudinali hyaline germi, primo biguttulate, terminis rotundis, 7.5–8 × 3–4 µm.


**Stromata** 20–30 mm longa, 0.1–0.3 mm wide, at first unbranched, cylindrical, pale brown, with pink, pointed tips which are phototopic, later becoming contorted and branched, greyish-brown to black, glistering, flattened or with longitudinal ridges, branches distant, tapering to a fine white point, powdery due to the production of conidia (Fig. 7). Outer layer of stroma made up of thick-walled *textura porrecta* hyphae up to 75 µm long and 2–4 µm, wide near the branch tips, widening to 10 µm in older parts. *Perithecia* sparse, widely separated along the length of the stroma, at first smooth and pale yellowish-brown, later greyish-brown to black and villose due to the development of conidiophores, sub-globose to broadly obpyriform with a wide flat base and a short papilla-like ostiole, or occasionally with two ostioles, 230–360 µm diam. and ca 250 µm high, (Fig. 8). The outer wall of the perithecium composed of *textura prismatica* of brown polygonal cells 10–20 µm across. *Asci* 8-spored, cylindrical, without obvious apical apparatus, I/KI negative, 75–88 × 5 µm (Figs 9, 10). *Ascospores* uniseriate or slightly overlapping, inaequaliter or elliptical in outline, grey to black with a longitudinal hyaline germ slit, at first biguttulate, ends rounded, 7.5–8 × 3–4 µm (Fig. 11). There is no evidence that the ascospores are discharged: they accumulate around the ostiole. *Paraphyses* exceeding the ascii, branched, composed of thin-walled sac-like cells up to 8 µm wide. *Conidiophores* developing from the surface cells of the stroma and the perithecial wall, hyaline, tapering, up to 45 µm long and 2.5–3 µm wide. The apex of the conidiophore is ’zig-zag’ like with a series of flattened scars. Conidogenesis holoblastic with a basal scar of secession. *Conidia* hyaline, elliptical to cylindrical with a basal flattened scar, 3–4 × 2 µm.

The type material was collected by J.W. at Cawsand Beacon, near Belstone, Dartmoor, Devon, SK 636915, on 3 Sep. 1995, following a period of prolonged dry weather. The dry pellets were incubated as described above. Within 3 wk some of the pellets produced thread-like, pointed, pink stromata which were strongly phototropic. Two months after collection scattered perithecia were noted along the length of some of the pellets and when one was crushed 10 wk after collection scattered perithecia were noted along the length of the stroma which were strongly phototropic, later becoming contorted and branched, greyish-brown to black, glistering, flattened or with longitudinal ridges, branches distant, tapering to a fine white point, powdery due to the production of conidia (Fig. 7). Outer layer of stroma made up of thick-walled *textura porrecta* hyphae up to 75 µm long and 2–4 µm, wide near the branch tips, widening to 10 µm in older parts. *Perithecia* sparse, widely separated along the length of the stroma, at first smooth and pale yellowish-brown, later greyish-brown to black and villose due to the development of conidiophores, sub-globose to broadly obpyriform with a wide flat base and a short papilla-like ostiole, or occasionally with two ostioles, 230–360 µm diam. and ca 250 µm high, (Fig. 8). The outer wall of the perithecium composed of *textura prismatica* of brown polygonal cells 10–20 µm across. *Asci* 8-spored, cylindrical, without obvious apical apparatus, I/KI negative, 75–88 × 5 µm (Figs 9, 10). *Ascospores* uniseriate or slightly overlapping, inaequaliter or elliptical in outline, grey to black with a longitudinal hyaline germ slit, at first biguttulate, ends rounded, 7.5–8 × 3–4 µm (Fig. 11). There is no evidence that the ascospores are discharged: they accumulate around the ostiole. *Paraphyses* exceeding the ascii, branched, composed of thin-walled sac-like cells up to 8 µm wide. *Conidiophores* developing from the surface cells of the stroma and the perithecial wall, hyaline, tapering, up to 45 µm long and 2.5–3 µm wide. The apex of the conidiophore is ’zig-zag’ like with a series of flattened scars. Conidogenesis holoblastic with a basal scar of secession. *Conidia* hyaline, elliptical to cylindrical with a basal flattened scar, 3–4 × 2 µm.

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Other material examined. Several other collections have been made by J.W. on rabbit dung:

Hay Tor, near Bovey Tracey, Devon (SX 755768), 14 July, 1998 K(M)59517. perithecial stromata observed 13 d later and ripe
perithecia on 8 Sep. Cultures derived from a single ascospore and from multi-ascospore inoculum formed stromata with numerous perithecia, preserved as K(M)59518.

Hound Tor, near Manaton, Devon (SX 741789), 14 July, 1998. Cultures prepared from stromatal tips formed stromata but without perithecia.


One collection on brown hare dung (M.J.R.): Exposed cliff-top heath at Hobbit, Orkney Mainland (HY 396064), 27 Sep. 1994. Immature perithecia present on incubated material on 15 Nov. 1994, which subsequently matured after being passed to A.J.S.W.

Cultures of the two species of Wawelia described here have been deposited in the following culture collections: CBS, Baarn, The Netherlands, CABI (IMI), Egham, and ATCC, U.S.A. CABl accession numbers are W. argentea IMI 380160 (1), W. microspora IMI 380161 (2).

**DISCUSSION**

Kuthubutheen & Webster (1986a) have pointed out that the conditions under which coprophilous fungi are usually studied in the laboratory, in which dung samples are supplied with plentiful water and are incubated at high humidity and at room temperature, differ greatly from the conditions to which such fungi are exposed in the field, where fluctuating and often low water contents of the substratum are the norm. When rabbit dung is deliberately incubated for long periods (several weeks) at r.h. values below saturation a number of fungi not normally observed on moist dung samples may develop. These include several species of Aspergillus and their Eurotium teleomorphs (A. candidus, A. repens), Penicillium claviforme, Stilbella erythracephala, Doratomyces stemonitis, D. namus and Isaria felina. Two species were originally discovered by incubating rabbit dung at low humidity, Onychophora coprophila (Gams, Fisher & Webster, 1984) and Wawelia octospora (Minter & Webster, 1983). It seems that there is a specialised group of xerophilous coprophilous fungi adapted to growth and fruiting on substrata with a relatively low water content. Kuthubutheen & Webster (1986b) have shown that some of these fungi, such as O. coprophila and I. felina, fruit more profusely when incubated at low r.h. The same is probably true of W. octospora and the two new species of Wawelia described here, but physiological studies are desirable. This is the most likely explanation for the apparent rarity of Wawelia as discussed by Lundqvist (1992). As far as we are aware there are no reports of field collections of Wawelia spp.: all the species known have been described from incubated dung samples. It is of interest that the three species reported from Britain seem to grow, although not exclusively, on leporid dung from exposed sites such as moorland, exposed sea cliffs, wind-swept heaths and sand dunes and, in view of the incubation conditions under which they occur, these are the kind of situations where fruiting might be expected. We have noted in material of all three species of Wawelia reported from Britain that, even under the relatively dry conditions of incubation, young stromata accumulate beads of liquid along their length. It is possible that these represent condensed water and we speculate that the stromata may be able to absorb water vapour as well as being able to absorb water in the liquid state.

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**REFERENCES**


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