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Teaching techniques for mycology: 18. *Rhytisma acerinum*, cause of tar-spot disease of sycamore leaves

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Name of fungus

Teleomorph: *Rhytisma acerinum* (Pers.) Fr. (order Rhytismatales, family Rhytismataceae)
Anamorph: *Melasmia acerina* Lév.

Introduction: Features of interest

Tar-spot disease on leaves of sycamore (*Acer pseudoplatanus* L.) is one of the most easily recognised foliar plant diseases caused by a fungus (Figs 1 and 4). First described by Elias Fries in 1823, knowledge of it had become well-established by the latter half of the 19th century (e.g. Berkeley, 1860; Massee, 1915). The causal fungus, *Rhytisma acerinum*, occurs in Europe and North America on *A. pseudoplatanus* throughout its distribution range and also on other species of *Acer* (Sutton, 1980; Farr *et al.*, 1989), but it is less frequent in urban and industrial areas.

The black tar-spots visible in late summer and autumn are stromata containing many apothecial rudiments, but these only mature to form asci during the winter on fallen leaves (Jones, 1925; Duravetz & Morgan-Jones, 1971). Maturing apothecial stromata develop linear or convoluted ridge-like swellings which raise the thick black layer (clypeus) on the upper surface of the stroma (Fig 1). Eventually, the surface breaks along the ridges (Fig 2), exposing a greyish hymenium (Fig 3) which contains club-shaped asci and filamentous paraphyses with curved or recoiled tips (Figs 8 and 9). The ascospores are unicellular and needle-shaped, with an apical mucilage pad (Fig 10). In Britain and Europe, ascospore discharge from overwintered stromata takes place in March and April, just as the new sycamore leaves unfold. Because of the large size and elongated shape of the ascospores, their release can be observed by placing a ripe stroma on moist filter paper under a low-power binocular microscope where the spores are discharged in puffs and float in the air. In nature, they are carried even by slight air currents and probably become attached to fresh sycamore leaves by means of their mucilage pad, followed by their germination and penetration through stomata (Butler & Jones, 1949). Within a few weeks, an extensive intracellular mycelium develops and becomes visible to the unaided eye from mid-July onwards as brownish-black lesions surrounded by a yellow border (Fig 4). This is the anamorphic state, *Melasmia acerina* Lév. (Sutton, 1980). Each lesion contains several roughly circular raised areas less than 1 mm diam., the conidiomata (Fig 5), within which conidia are produced. In moist conditions, the conidia ooze out of one or several openings as milky droplets (Fig 6) whereas in dry weather, they stick together to form yellowish horn-like tendrils (Fig 7) which rapidly deliquesce when incubated in a water-saturated atmosphere. The tiny conidia, which are produced from phialides (Fig 11) lining the bottom of the conidiomata, are shaped like straight or slightly curved rods (Fig 12). There is no evidence that the conidia are capable of germination or re-infection of the host, and it is believed that they are in fact spermatia, fulfilling a sexual role.

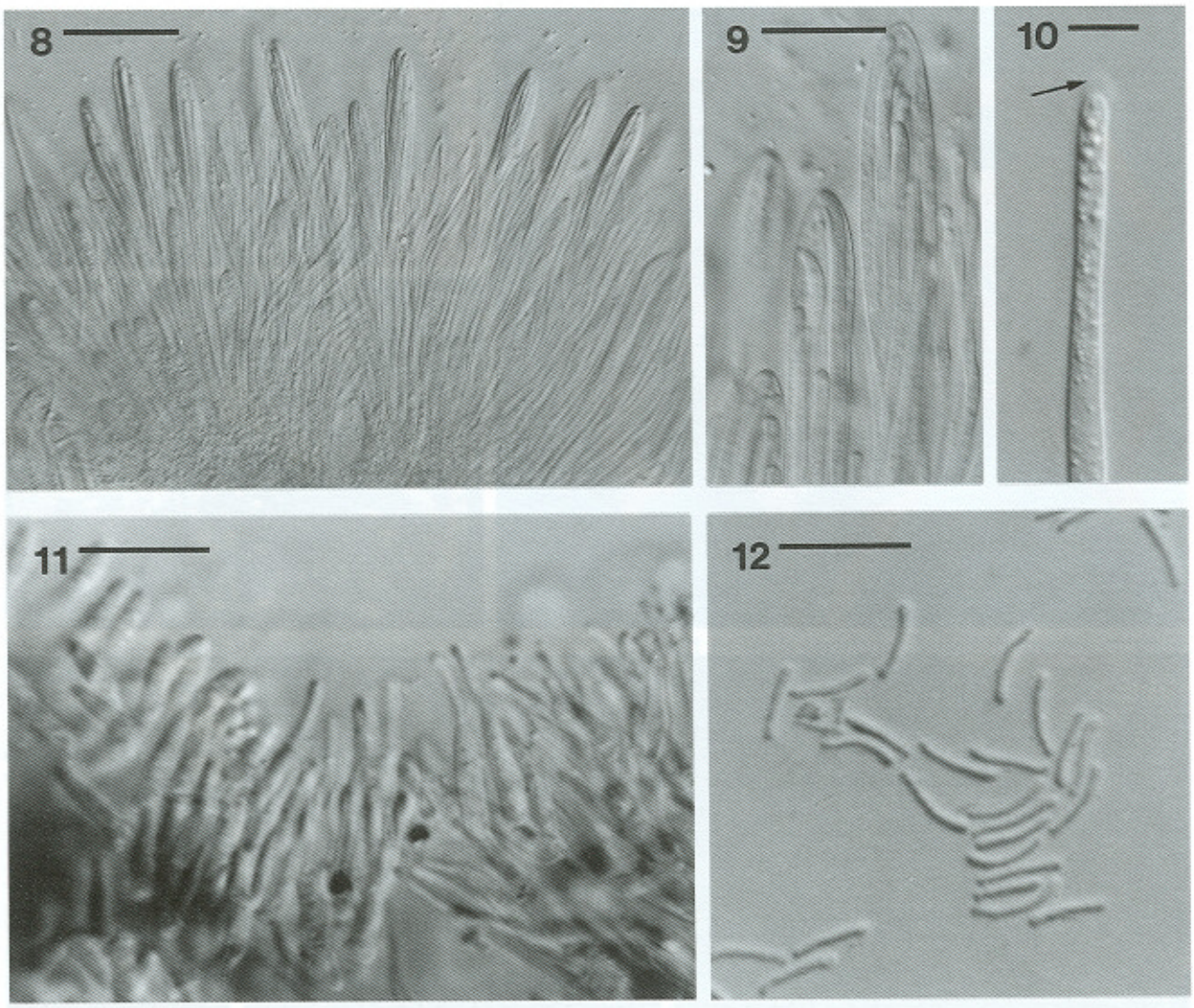
Rhytisma acerinum is particularly attractive for elementary mycology courses if both the anamorphic and teleomorphic states can be presented and the development of the former into the latter can be demonstrated. Further, the life-cycle of this biotrophic parasite is finely tuned towards that of its host as well as towards seasonality. Finally, the asci (Figs 8 and 9) as well as the release of ascospores by puffing are striking.

Source and storage of *R. acerinum*

Sycamore trees with tar-spot lesions should be located in early autumn before leaf fall, and the sites should be

Figs 1-3. *Rhytisma acerinum*. Fig 1 Overwintered leaf of sycamore (*Acer pseudoplatanus*) with several apothecial stromata. Collected in early April, the stromata are reaching maturity as revealed by their strongly corrugated surface. Fig 2 Mature overwintered stromata incubated on moist filter paper for 3 d. The clypeus has cracked open, exposing numerous apothecia with greyish hymenium. The central region of the stroma (bottom right) does not give rise to apothecia because this was the conidial region in the preceding growing season. Bar = 2 mm. Fig 3 Mature stroma with several apothecia as seen at higher magnification under a binocular microscope. With such material, puffing of ascospores can be readily seen. Bar = 1 mm.

Figs 4-6. *Melasmia acerina*. Fig 4 A whole leaf of *A. pseudoplatanus* in early August. Several black lesions of *M. acerina*, each producing a number of conidiomata, are visible. Each lesion is surrounded by a yellowing region. Fig 5 A single lesion of *M. acerina*. Several circular conidiomata with irregular openings are visible. Bar = 1 mm. Fig 6 Close-up of a lesion incubated in a water-saturated atmosphere over moist filter paper. The conidia have formed a milky exudate around the openings of the conidiomata. Bar = 1 mm. Fig 7 As Fig 6, but incubated in a dry atmosphere. The conidia have formed a yellowish horn-like tendril. Bar = 250 µm.



Figs 8-10. *Rhytisma acerinum*. Fig 8 Squash preparation of the hymenium from an apothecium. Asci are interspersed by thin elongated paraphyses. Bar = 50 µm. Fig 9 A single ascus containing a cluster of 8 needle-shaped ascospores. Bar = 20 µm. Fig 10 An ascospore discharged onto a microscope slide and mounted in water. Note the presence of the apical mucilage pad (arrow) which aids in the attachment of the spore to a sycamore leaf. Bar = 5 µm.
Figs 11-12. *Melasmia acerina*. Fig 11 Squash preparation of the hymenium from a conidioma. Phialides arise from short branches and produce elongated conidia. Bar = 10 µm. Fig 12 Mature conidia from an exudate. Bar = 10 µm.

revisited in March or April to collect fallen, overwintered infected leaves. The apothecial stromata are almost ripe when ridge-like swellings are prominent on their upper surface (Fig 1). Suitable material should be allowed to dry slowly at room temperature (r.t.) and can then be stored dry at 4 °C or -20° for several months until required for the practical class. Alternatively, damp fallen leaves collected in autumn or early spring should retain a high viability if stored in a deep-freezer (Butler & Jones, 1949). Leaves bearing the *Melasmia* state should be collected in late July or early August and stored air-dried or fresh at -20°.

Preparation of material

Day -3. Retrieve stored apothecial material, cut into suitably-sized pieces bearing one or a few lesions each, and place these with the upper epidermis facing upwards in a Petri dish with moist filter paper or tissue paper. Incubate at r.t.

Day -1. Treat *Melasmia* material in a similar way.

Day 0. Apothecial and conidial material should be presented to the students in the moist-chamber Petri dishes. When the Petri dish lids are removed and ripe apothecia are illuminated in bright light under a binocular microscope, ascospore discharge can be observed, the spores scintillating as they float in the air above discharging apothecia. Students should be asked to dig out a piece of the hymenium with a fine dissecting needle or scalpel tip, and mount it in a drop of water under a coverslip. Such preparations show discharged and undischarged inoperculate asci (Figs 8 and 9) as well as paraphyses which are often recurved. The mucilage

pads at the ascospore apex can be rendered more obvious by phase-contrast or Nomarski interference contrast optics (Fig 10) or by drawing dilute India ink under the coverslip.

Revived conidial material will be exuding mucilaginous milky blobs of conidia. Students should lift off the black covering of a conidioma and remove the conidiogenous layer from the floor of the conidioma. This material should be transferred to a small watch-glass with lactic acid to remove some of the spores, prior to mounting the remaining light-brown cores in lactic acid on a microscope slide, tapping the coverslip with a needle to flatten the preparation. With oil-immersion objectives, the very small phialides (Fig 11) and bacilliform conidia (Fig 12) should be visible.

Useful hints

The absence of *R. acerinum* from large cities has been explained by the sensitivity of its ascospores to aerial pollution (especially sulphur dioxide) during germination, and the abundance of tar-spots on sycamore leaves has been used as a visual indicator of air quality (Bevan & Greenhalgh, 1976; Greenhalgh & Bevan, 1978). However, care is needed when interpreting such data, e.g. Leith & Fowler (1988) have shown that other factors such as the removal of fallen leaves from city parks in autumn or the degree of exposure to drying winds can adversely affect the presence of *R. acerinum* in city centres.

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Putting mushrooms on the map - surveying the UK mycota

The annual BMS Autumn Taxonomy Meeting, held on 17 November 2001 at the Royal Botanic Gardens, Kew, attracted over 90 participants including regulars from among the mycological community and others with a broader interest in biodiversity and conservation issues. Many of those present were keen to update on the results of some of the ongoing survey work taking place in the UK. Shelley Evans and Derek Schafer organised and chaired the meeting.

The scene was set by Roy Watling with a talk on 'Encapsulating Field Mycology'. He outlined some of the continuing mycological studies at the 300-year old garden at Dawyck, which have been going on for the last five years and encompass an interpretive centre supported by the BMS. Naomi Ewald from the Hampshire Wildlife Trust then gave a powerpoint overview of the stipitate hydnum survey recently completed in the New Forest. This ambitious but highly successful partnership between a wildlife trust and the local fungus recording group produced over 200 new records for many sites. Liz Holden gave a summary of current waxcap-grassland surveys, with emphasis on recent work funded by Scottish Natural Heritage. Bruce Ing closed the morning session with a summary of his survey of corticolous myxomycetes in central London.

The afternoon started with David Mitchel explaining the potential and functionality of the NBN Gateway website concept. Alan Feest described 'A different approach to surveying' using simple repetitive and objective field techniques interpreted by statistical analysis. The meeting concluded with a stimulating debate between the speaker panel and the audience, focussing largely on the different approaches to survey work.

Shelley Evans