Practical Plant Pathology for Beginners

DAVID S. INGRAM & ROSEMARY WINNALL

This paper, based on Ingram & Robertson (1999), as a supplement to the 2021 Survey Strategy proposed by Winnall, Taylor & Ingram (this volume) provides advice on some of the practical aspects of identifying, storing and naming fungal plant pathogens collected in the field. Such advice may to some appear at first reading to be complicated and therefore daunting. In our experience it is not so in reality, and once put into practice in the real world of Greater Wyre, will become routine, almost instinctive, in a very short time.

Recognizing the Presence of Disease

The presence of plant diseases in a field or vegetable-garden crop is usually relatively easy to see, for most, once established, spread very rapidly through the entire stand. This is because modern cultivars of, for example, cereals or potatoes, and most garden vegetables, are normally genetically uniform with regard to disease resistance or susceptibility, and once one plant succumbs the rest follow. Recognizing the presence of disease in complex habitats such as may be found in the Wyre Forest, however, is more difficult, for a mix of host species will usually be present, and even where large stands of a particular species do occur, these will usually be genetically very diverse with respect to resistance and susceptibility to pathogens.

Spotting plant diseases, especially in the early stages of infection, may at times, therefore, feel like searching for a needle in a haystack. So what should be looked for, initially? The simple answer is 'something that does not seem quite right'. The cause may not be apparent immediately, but if one is familiar with the plants in a particular habitat, the eye and brain nevertheless record anything that does not fit the expected pattern, as occurs when, for example, someone has moved a vase in a familiar room. This is the signal to look at the scene more closely until the problem is identified: a small group of plants with discoloured petals or flecked leaves, perhaps, or an abnormally tall plant that stands out among those around it, or a group of plants looking off-colour. One soon learns to move very quickly from the 'something wrong' feeling to 'ah, yes, now I see it'.

Diagnosing the Cause of the Disease: signs and symptoms

Since plants cannot walk or speak, the diagnosis of their infections requires a different approach from that used in human medicine. Instead of first hearing about the 'signs' of infection from the patient – a headache or painful tooth, for example - the field plant pathologist must first use his or her eyes to look for and recognize the 'symptoms of infection', the host's physiological responses, such as wilting, yellowing, leaf spotting, petal discoloration, tissue distortion or tissue rotting.

Next, the possibility that external, physical, factors may be causing the symptoms, such as drought, flooding, or herbicide drift from a nearby cultivated plot must be eliminated. Finally, the plant pathologist must look for the physical 'signs' of infection: the presence of a specific organism – usually hyphae or spores - its form (morphology) and its identity. Sometimes the signs are obvious to the naked eye – masses of rusty-coloured spores, for example - but frequently they may only be revealed by the use of a hand lens or microscope, or the isolation of the pathogen by placing a piece of infected tissue on a selective culture medium in a laboratory, or by the use of an electron microscope or a biochemical or molecular test. And as in human diagnosis, if the plant pathologist is unable to reach a satisfactory conclusion quickly, the advice of someone better equipped and with greater knowledge or experience should be sought, for there is little to be gained and much to be lost by guessing or bluffing to save face.

Diagnosis in the Real World of the Forest

Let us now suppose that out in the forest that 'something wrong' feeling has been pinpointed as slightly stunted growth and black smudges on the petals of a small group of White Campion plants, followed, after examining the flowers more closely, with a realization that the discoloration is due to dusty black spores issuing from infected anthers (Fig. 1). In this case, identification of the pathogen to the genus and species level is straightforward, since only one fungus with black spores normally infects the anthers of *Silene latifolia* ssp. *alba*: the anther smut *Microbotryum lychnidis-dioicae* (Ustilaginales). If nearby there are plants of the closely related Red Campion, *S. dioica*, with similarly infected anthers, the pathogen is again likely to be *M. lychnidis-dioicae*, for like most Smuts, this species is relatively host specific, although in this case not completely so.

Fig. 1. White Campion, *Silene latifolia* ssp. *alba*, infected with Anther Smut, *Microbotryum lychnidis-dioicae* (Ustilaginales).

It is important not to jump to such a conclusion too quickly, however, for if the white flowered *Silene* has been misidentified and is in fact *Silene uniflora*, the Sea Campion, the Smut will probably be a different species, *M. silenes-inflatae*, since this Smut is normally highly specific to a single *Silene* species.

In many cases, as with Downy Mildews (Peronosporaceae, Oomycota), White Blisters (Albuginaceae, Oomycota), Powdery Mildews (Erysiphales, Ascomycota), many Rusts (Uredinales, Basidiomycota), and many ascomycete hemi-biotrophs (Ascomycota), identification is similarly straightforward, but in some cases, as with Rusts on grasses, identification may be more difficult.

In this example, if the signs and symptoms include rusty-brown or -black spore-producing lesions on the green leaves of the host, while one can be fairly confident that the pathogen is likely to be a Rust (Uredinales), both the genus and the species will require further investigation. First the host will have to be identified, so let us suppose that this has been done and the species is Cock's-foot (*Dactylis glomerata*) (Fig. 2). Now, there are four Rusts to choose from: *Puccinia coronata* (Crown Rust), *Puccinia graminis* (Black Stem Rust), *Uromyces dactylidis* (Cocksfoot Rust) and *Puccinia striiformis* var. *dactylidis* (Stripe Rust) so more work needs to be done.

Clearly the situation is complex, so first the shape and

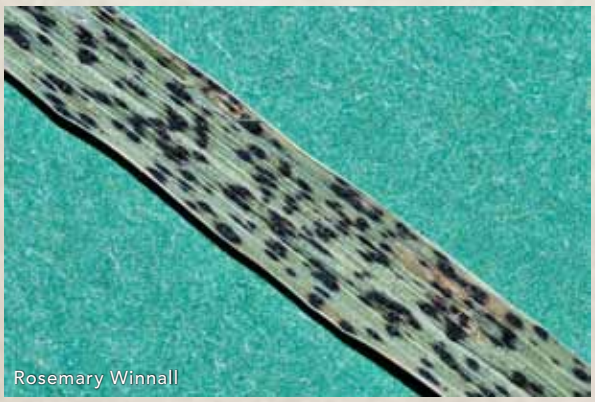

Fig. 2. Leaf of Cock's-foot, *Dactylis glomerata*, infected with Black Stem Rust, *Puccinia graminis* (Basidiomycota). Specimen collected by Ian Wright.

form of the lesions will need to be noted, followed by microscopic examination to determine what spores are present, and their morphology, colour and approximate size. Finally, consultation of books or on-line sources will be needed to reach a final diagnosis. With experience one soon gets a feel for the likely identity of specific Rusts on specific grasses, usually based on the macroscopic form and colour of the lesions, but even so, for survey purposes, it is essential to carry out the microscopic examination to be certain that the name one has applied to the specimen by instinct is indeed the correct one.

All this requires that in the field the infected host be identified accurately, photographed and the lesions described by eye, usually with the aid of a hand lens, and in many cases that specimens of infected leaves, shoots or other organs be collected and labelled, to be carried home for further examination. Once there, the specimens from the day's collecting will need to be stored safely, ready for later close examination with a microscope. Details of such procedures are described below.

If the collector does not possess a microscope, it is nevertheless important to record that a Rust has been noted on a particular species, to record other information relating to it and if appropriate to collect a specimen, for it is certain that a colleague in the WFSG will later be able to help with the microscopic examination.

Collecting Information and Diseased Specimens

As everyone in the WFSG knows, the bedrock of natural history research is recording. This needs to start in the field, so when studying plant pathogens, a paper or electronic notebook and writing materials are essential equipment, as is a high-resolution digital camera.

First, having noted the presence of a disease, the plant pathologist must try to identify the host from her or his own knowledge, or if necessary by consulting a Flora or a colleague. If the identity of the host remains obscure, as many photographs as practicable will be needed, especially of the flowers, for identification later. Next, as many photographs need to be taken as may be necessary to demonstrate the details of all the different types of pathogen lesions and spore stages present on the host, recording also the distribution of the pathogen on the whole plant, and recording the distribution of infected plants among the general population of plants around it. Also, using both the naked eye and a hand lens, dated notes will be needed to accompany the photographic record.

When all this has been done, it will be necessary to consider collecting a specimen of the infected tissue, but before making a decision, it is important to ask oneself whether the host is rare or threatened? If it is, the pathogen will probably be equally vulnerable too, and infected tissue specimens should not be collected. In this case it is still worth considering the possibility of collecting some host-free spores of the pathogen. A simple way of doing this, if the weather is dry, is to apply a short length of transparent adhesive tape, gently, to the surface of a lesion, so that spores become attached to it, and then to stick the tape, sticky-side down, to the surface of a dry glass microscope slide and place this

in a labelled envelope for examination later. If there are different types of lesions on a host, it is important to collect from each type, for more than one spore stage or even pathogen species may be present on a single host.

If the host is not rare, and the weather dry, it is well worth collecting a specimen of infected tissue, for this, along with field and laboratory notes, drawings and photographs will form part of the permanent record of the species. However, it is important only to collect as much of the specimen as will be needed to demonstrate the diversity of lesions, spore types and pathogens present, plus a little extra for dissection, microscopic examination of spores and so on in order to identify the pathogen unequivocally. It is good practice not to collect flowers, fruits or seeds, unless these are themselves infected.

As soon as the specimen has been collected, it should be placed in a container. Up to and even beyond the 1960s in some cases, a bulky and often heavy metal vasculum (Fig. 3) was usually used for this purpose. Specimens were often kept fresh while collecting by putting wet moss in the vasculum or lining it with a damp cloth or paper. The eighteenth century Birmingham botanist William Withering (Withering, 1776), an early proponent of vascula, suggested that 'If any thing happens to prevent the immediate use of the specimens … collected, they will keep fresh two or three days in the box, much better than putting them in water'. This is still good advice, whether the container is a vasculum, or as we now recommend, albeit environmentally less friendly, much lighter and less bulky, polythene bags or lidded clear plastic containers (Fig. 4), or both.

Containers have the advantage of preventing crushing, but if several specimens need to be collected in a day, may prove too bulky for convenience. In this case, it may be possible to bag a number of specimens individually and then to place them in groups in a smaller number of containers.

Fig. 3. Three metal vascula: the two black-painted ones are probably late nineteenth century, and the green one, twentieth century. Courtesy of the Royal Botanic Garden Edinburgh.

Fig. 4. Lidded clear plastic containers (13 cm x 9 cm x 8cm deep) currently used for collecting diseased specimens by RW.

It is also possible to buy portable plant presses for use in the field and to place the specimens in one of these (for further details, see below).

Immediately after each specimen has been collected, it should be assigned a reference number and this, the time, the place of collection and any other details should be written in pencil or with an indelible, quick-drying ink pen, on a piece of paper and placed in the bag, container or press with the specimen, for it is amazing how rapidly the memory fades. And finally, it is in our opinion worth writing the information about the specimen, including its reference number, in a notebook as a belt and braces strategy.

Preserving Specimens

After returning home it is first important to put some infected material aside for immediate examination (see below).

Next the specimens to be preserved should be 'pressed' to ensure that they dry out as rapidly as possible since this promotes the retention of the original colour and inhibits the development of mould. If the intention is to press only a small number of specimens, the 'brick and board method' is the simplest way of proceeding. First the specimens, having been allowed to become 'limber' (supple), as William Withering would say, should be arranged carefully to dry between several sheets of newspaper or other absorbent paper (William Withering advised the acquisition of 'half a dozen quires [then 24 sheets] of large soft spongy paper; such as stationers call Blossom blotting paper'). A note should be added to each specimen, giving its likely identity and the place and date of its collection. Next, the specimens should be stacked on a flat board, placing sheets of corrugated card to promote airflow at intervals between the sheets of absorbent paper.

When all the specimens have been dealt with, a sheet of flat wooden board should be placed on top of the pile, and a heavy weight, such as a work by Dickens or a brick placed on top. Finally, the stack should be left to dry in a warm but airy room, and the drying paper replaced from time to time, ideally without disturbing the specimens unnecessarily.

If specimens are to be pressed on a regular basis, however, it is probably best to obtain a press made specifically for that purpose. William Withering used


Fig. 5a. Graham Hill


Fig. 5b. Mark Watson

Fig. 5. Plant presses: a. screw down model, a design that has changed little since the days of the eighteenth century Birmingham botanist William Withering (photograph by Graham Hill); b. wooden slatted model being used in the field by scientists from the Royal Botanic Garden Edinburgh to press and transport dozens of specimens during an expedition to Nepal (photograph by Mark Watson, Editor in Chief, RBGE Flora of Nepal project).

a large screw-down version (Fig. 5a), but we advise a modern, lightweight model. Such presses are ideally approximately 45 x 60 cm in size, and are easily purchased from an on-line student laboratory supplier. We suggest the acquisition of a press made from two identical frames comprised of strips (laths) of wood set c. 15 cm apart at right angles to one another (Fig. 5b; or for images of a variety of types, simply put the words 'Botanical Plant Press' into your browser). When filled with drying specimens the two frames are then bound together with two webbing straps. Such presses have the advantage of being relatively cheap, are light to carry in the field if one wishes to place specimens in them while collecting, and are sufficiently permeable to water vapour to promote rapid drying when individual specimens are placed between layers of absorbent paper, with intermittent layers of corrugated card to promote air flow.

It will, of course, already have occurred to DIY enthusiasts that it is also possible and may well be cheaper to make a perfectly serviceable wooden frame press at home rather than buying a commercial model.

Finally, specimens of wood or bark should not, of course, be pressed, but simply placed on a few layers of absorbent paper, covered with a clean, dry cloth, and left to dry in a warm, airy room.

Storing Specimens

Large specimens of wood or bark may be stored in a cardboard box and large herbaceous specimens may be mounted on herbarium sheets of heavy mounting paper and held down with strips of adhesive paper or mounting paste, all available from most commercial plant press sellers, and then stored in folders. Most diseased specimens, however, are likely to be relatively small and are best stored in paper envelopes (Fig. 6a) folded to a standard size from sheets of A4 paper. These store easily in shoeboxes or home made equivalents (Fig. 6b).

However stored, all relevant information relating to a specimen must be recorded on the box, sheet or envelope, including the names of pathogen and host, the accession number of the specimen, the date when the specimen was collected, the grid reference (GR) and name of the place where it was collected, and the name of the collector. Any additional information, such as the address and other contact details of the collector, may be recorded on a separate piece of paper placed with the specimen in the envelope.

Classification of the specimens may, for example, be by accession number, place of collection, or host or


Fig. 6a. Rosemary Winnall

Fig. 6. An appropriately labelled specimen envelope (a) and storage box (b), as currently used and here photographed by Rosemary Winnall. The envelopes in this case were made from old, headed notepaper, folded so that the name and address were visible on the flap.

pathogen name, depending on the use to which they are to be put. Each specimen should, of course, be cross-referenced to the appropriate field collecting notes and photographs, and vice versa.

Specimens do sometimes deteriorate in storage from attacks by insects that feed on paper, plant tissues or fungal hyphae and spores. To limit the possibility of such damage, or to deal with it when identified, we suggest placing specimens, sealed in a polythene bag or box, in a freezer at – 25° C for a few days.

Examining Specimens

Initial examination with naked eye and hand lens

The first observations of diseased material in the field will have been by eye, and then with the aid of a hand lens to gain further information. Folding hand lenses may be purchased from most optical or photographic shops, or from on-line laboratory suppliers. The most useful magnifications are about x10, x15 and x20 and the quality should be sufficient to minimize distortion of shape and colour. The higher the magnification, the more detail will be revealed, but at the cost for some of increased difficulty in positioning the eye, the lens and the specimen to achieve the best focus. The lens is also best kept on a long string or leather loop around the neck so that it is always easily to hand.

Hand lenses fitted with an internal LED light may be helpful during darker weather or to those with poorer sight. A disadvantage with these, however, is that they can be rather large, rarely have a lug to attach a loop, and so must be carried in a pocket.

Spectacles are usually a problem when examining specimens with hand lenses, so ideally these should have a halter so that they can be removed and held hands free whenever necessary.

Examination with a microscope

Most plant diseases found in the field will be caused by fungi or fungus-like organism, and their spores and surface mycelium may usually be seen with the naked eye or hand lens, although the detail of spore shape and ornamentation will usually require the higher magnification of a microscope (Figs. 7a, 7b and 7c).

Many people in the WFSG will already be familiar with the techniques of microscopy and perhaps already possess appropriate equipment. The brief introduction that follows is, therefore, intended only to provide a broad picture as it relates to the plant pathogen survey for those without such experience. The monetary and time cost of investment in microscopic equipment can be high, so if there is sufficient interest, the possibility of holding a practical workshop(s), when Covid-19 regulations and vaccinations allow during 2021, will be explored so that more detailed advice may be provided.

Far greater detail of a specimen than can be seen with a hand lens will be revealed by use of a binocular stereoscopic dissecting optical microscope (Fig. 7a). This will be of low power – usually x20 to x200 magnification, and will have a considerable depth of focus and be easy to use with fresh, ideally un-pressed specimens. Such a relatively uncomplicated instruments, together with a light source (often built-in), are both now relatively cheap to obtain from student laboratory suppliers. They are especially useful to gain a good feel for: the disposition of a pathogen and its

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Fig. 7b. Rosemary Winnall


Fig. 7c. Ann Hill

Fig. 7. Light microscopes: a. The WFSG stereoscopic microscope fitted with a camera; b. Rosemary Winnall's compound, high power microscope fitted with an internal light source and a central attachment for a camera; c. Ann Hill's digital microscope.

spores within a lesion, as with Rust fungi (Uredinales) and Smut fungi (Ustilaginales); or the ways in which the asexual spores of Powdery Mildews (Erysiphales), Downy Mildews (Peronosporaceae) and White Blister-rusts (Albuginaceae) are held above the surface of the host as an aid to dispersal by wind or rainwater; or the overall form and external ornamentation of the saucer-shaped, spherical or flask-shaped fruiting bodies (ascocarps) that hold the sexual structures (asci and ascospores) of Powdery Mildews (Erisyphales).

A high power magnification, compound optical binocular microscope (Fig. 7b), either with a built-in or external light source, is needed for precise identification of fungal and fungus-like pathogens. Such microscopes are usually fitted with x10 magnification eyepiece lenses and x 10, x40 and x100 [oil immersion] nosepiece lenses, giving a range of magnifications from x 100 to x1000. Usually, special preparation of the specimen is required for observation with such an instrument (see below).

A range of qualities of high power microscopes is available, at prices to match. For survey purposes a basic model fitted with only x10 eyepiece lenses and x10 and x40 nosepiece lenses is all that will be needed, but if the reader thinks that he or she is also likely to carry out deeper research to understand better the biological relationship between hosts and pathogens, it will probably be best and cheaper in the long run to buy something more sophisticated at the outset.

The precise identification of fungal plant pathogen species often requires measurement of the spores. This is done by use of a graduated eyepiece micrometer fitted to the microscope and calibrated with the aid of a micrometer slide. These essential pieces of equipment for the serious amateur plant pathologist may be purchased with the microscope.

It should be added that digital high power (and stereoscopic) microscopes are also available for amateur use (Fig. 7c). These are variations on the traditional optical microscopes that use a digital camera to transfer an image of the specimen to a monitor, sometimes assisted by software installed on a computer.

Also, many optical and digital high power microscopes are capable of being fitted with appropriate light sources and cameras to allow standard photographic records of specimens to be kept.

Preparation of specimens for microscopic examination

For many purposes, simple tap water mounts of spores or infected host tissue teased apart on a glass microscope slide and covered with a glass cover slip is the only preparation required for simple microscopic identification of a pathogen.

For more detailed observation, however, it may be

necessary to fix (kill and preserve) and stain squashed, teased-apart host material in a chemical fixative-stain-mounting fluid such as lactophenol-cotton blue. This comprises a mixture of phenol crystals, glycerol, lactic acid and distilled water, supplemented with c. 0.1 % methyl blue. The methyl blue stains the chitin in the cell walls of pathogens belonging to the kingdom Fungi a bright cerulean blue. Thus it is especially valuable when dealing with host material infected with Rusts, Smuts and other pathogenic members of the Basidiomycota, and Powdery Mildews and other pathogenic members of the Ascomycota. While it does not stain as brightly the cellulosic, non-chitinous walls of Fungus-like pathogens, such as the Downy Mildews, White Blister-rusts and other members of the Oomycota, it is nevertheless valuable to use it with them since it helps to highlight the contrast between host and pathogen cells.

Note: lactophenol-cotton blue is **poisonous**, so it should *always* be obtained from a *reputable* laboratory supplier and the instructions on the bottle always followed to the letter.

Other fungal stains are available for the more adventurous fungal microscopist, and details of some of these may be found in Chapter 11 of Tronsmo *et al* (2020).

Teasing apart infected fresh or preserved infected host material prior to mounting or staining is best done with a pair of dissecting needles fitted with wooden handles. These may be purchased relatively cheaply from laboratory suppliers, but may also be made easily at home by cutting off and trimming straight, 10-15 cm, pencil-thickness portions of hazel or sycamore twigs and carefully pushing the eye end of a large sewing needle into one end of each twig with a pair of pliers. The grip of the wood on steel tightens as the twig sections dry.

Fresh host material needs little preparation before teasing apart in water or lactophenol-cotton blue. Dried and pressed tissues taken from a collection for examination, however, require special treatment. They will be full of air sucked into the material as it dried. They will also be precious, for it is probable that it will not be possible to collect an exact replacement again. Therefore, only very small portions of each pressed specimen should be removed for examination at any one time, and then placed on a glass microscope slide in a little water or glycerine and allowed to swell. The swelling and dispersal of air bubbles in water-mounted specimens may sometimes be hastened by gentle and careful warming.

Glass slides and cover slips, whether for mounting spores or teased-out infected material, need to be purchased from a student laboratory supplier and chosen carefully. The thinner the slide the better if a high power microscope is to be used. The thickness of the cover slip is also significant: too thick and it may hamper examination using x40 and especially x100 nosepiece lenses, too thin and it will easily break while being handled. In our experience, size no. 1.5 (22 x 22 mm square) is a useful compromise.

Infecting living plants with pathogens and sectioning infected material, both needed if a better understanding of the relationships between host and pathogen cells and structures is required, are more specialized skills that will not be required for the identification of pathogens in our survey. They are, therefore, beyond the scope of this paper, although an introduction to them will be found in Chapter 9 of Tronsmo et al (2020) and in the Appendix to Ingram & Robertson (1999).

Books

Print and electronic books (a basic list)

Specialized sources for Greater Wyre fungal plant pathogen surveys.

See recommendations in Ingram & Winnall (2020) and the following.

Chater & Woods (2019); Chater *et al.* (2020); Woods *et al.* (2018); and Woods *et al.* (2015), see for details 'References', below, and the individual reference list for each volume. All available for download from: https://www.aber.ac.uk/waxcap/downloads.

Ellis, M.B. & Ellis, J. (2017) *Microfungi on Land Plants: An Identification Handbook*, 2nd edn. Richmond Publishing Co. Ltd., Slough.

A.J. Termorshuizen, A.J. & Swertz, C.A. *(Roesten van Nederland (Dutch Rust Fungi)*, Termorshuizen contact: aadtermorshuizen@planet.nl (available in print or free on-line versions).

Ingram, D.S. & Robertson, N.F. (1999) *Plant Disease: A Natural History*. Harper Collins, London.

General Plant Pathology

Tronsmo, A.M., *et al.* (2020) for details see 'References', below.

Agrios, G. (2005) *Plant Pathology*, fifth edition. Elsevier, Academic Press, Burlington, USA & London, UK.

These books, available in print and electronic versions, provide a valuable introduction to the pathology of crop plants. The information provided also serves as an

introduction to the main groups of plant pathogens and their life-cycles, whether on wild or cultivated plants.

Websites

These are too numerous to name individually here, except to note that we find the one entitled Plant Parasites of Europe to be especially useful.

References

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Withering, W. (1776) *An Arrangement of British Plants: according to the Latest improvements of the Linnaean System, to which is prefixed, an Easy Introduction to the Study of Botany; Illustrated with Copper Plates*; printed for the author, by M. Swinney, Birmingham.

Woods, R.G., Stringer, R.N., Evans, D.A. & Chater, A.O. (2015) *Rust Fungus Red Data List and Census Catalogue for Wales*. A.O. Chater, Aberystwyth.

Lesions of the Rust fungus *Gymnosporangium sabinae* on the upper surface of a leaf of Pear *(Pyrus communis)* Graham Hill

Aecia of the Rust fungus *Gymnosporangium sabinae* on the lower surface of a leaf of Pear (*Pyrus communis*) Graham Hill

Aecial cups of the Rust fungus *Puccinia pulverulenta* on a leaf of Greater Willowherb *(Epilobium hirsutum)*
Rosemary Winnal

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Black spores of the Smut fungus *Ustilago avenae* being released from the infected ovaries of False Oat-grass *(Arrhenatherum elatius)*
Cherry Greenway

Lesions of the Smut fungus *Entyloma ficariae* on the lower surface of a leaf of Lesser Celandine *(Ficaria verna)*
Jean Young

Sporangiophores bearing sporangia of the Downy Mildew pathogen *Plasmoverna pygmaea* on a leaf of Wood Anemone (*Anemone nemorosa*)
Rosemary Winnall

A lesion of the Smut fungus *Urocystis violae* on the lower surface of a leaf of Common Dog-violet *(Viola riviniana)*
Rosemary Winnall