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Plant Pathology Plots

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Abstract. – Techniques are described for cultivating necrotrophic and biotrophic plant pathogens so that they can be available in the living state for use as class material at different times of the year other than the normal growing season.

The provision of living material for practical classes in plant pathology can present problems, one of which is that the academic year usually does not coincide with the growing seasons in which plant diseases are most obvious. Teachers in Universities may not have easy access to diseased material at the time their classes are held. Fortunately by judicious choice of examples, by the use of small scale plots in which the hosts and their pathogens are deliberately cultivated, with the aid of glasshouses, refrigerators and a little ingenuity, many pathogens can be induced to grow out of season. Experience gained at the University of Exeter in providing class material for undergraduate classes in Mycology and Plant Pathology, and to an M.Sc. course in Plant Pathology may be useful to others who face the problems of providing such material.

The first difficulty to overcome is the instinctive reluctance of trained gardening staff actually to cultivate diseased pants. All their training goes against this. The essential point for them to understand is that most plant pathogens are host-specific, and will not spread to unrelated crops. The choice of pathogens which grow on non-crop plants or even on garden weeds such as *Capsella* may help here. At Exeter we have a small garden some 100 m^2 in area which is used for cultivating pathogens, mostly in tiny plots about 1 m^2. Some examples of the pathogens grown, how to obtain them, and how to cultivate them are given below.

Plasmodiophoromycetes

Plasmodiophora brassicae, the cause of club-root of brassicas can be obtained from almost any garden or allotment where brassicas have been under continuous cultivation. The characteristic symptoms in the growing season are the wilting and poor growth of infected plants, which bear the characteristic root galls. Refuse piles and compost heaps where old brassicas have been discarded are other useful sources. Dense suspensions of resting spores can be obtained by mincing such galls in a household mincer. *Plas-*

modiophora brassicae occurs in a number of physiologic races. A universally susceptible host is Chinese Cabbage (*Brassica campestris* s. sp. *pekinensis*) cultivar Granaat. If seedlings are planted into a slurry of resting spores the roots develop galls within a few weeks. During the winter infected seedlings can be grown under supplementary lighting in the glasshouse. If plants are grown outside in the same plot the decay of root galls releases resting spores into the soil, and these remain viable for several years. Spore suspensions maintained at low temperatures, e. g. 4° C in a refrigerator will also remain viable for several months, and this method of storage is preferable to the storage of frozen galls. The disease is favoured by acid soils so the use of ammonium sulphate as a fertiliser of the garden plots is recommended.

Chytridiomycetes

Olpidium brassicae. The roots of cultivated brassicas almost invariably contain thalli of this holocarpic pathogen in the epidermal cells and root hairs of young roots. If such material is washed free of soil the sporangia can be detected and within a few minutes they release zoospores into the water. Pregerminated *Brassica* seedlings bearing root hairs can be placed in the zoospore suspension. The zoospores rapidly encyst on the root hairs, and penetration follows. If the seedlings are potted into moist sand the development of fresh thin-walled thalli follows within 4–5 days. Such seedlings when planted out into the garden will continue to develop thin-walled sporangia and the thick-walled stellate resting sporangia. If the garden-grown plants are to be used for class it is best to cover them with a cloche for 2–3 days to ensure that the soil is reasonably dry. When such roots are washed out in water zoospores are quickly released.

Synchytrium. In many European countries legislation prevents work with *S. endobioticum,* the cause of potato wart disease, except under licence. If living *Synchytrium* is required it is therefore necessary to substitute a different species. *Synchytrium taraxaci* makes a good substitute. A small plot of dandelions *(Taraxacum officinale)* can be easily extablished by transferring rooted rosettes from elsewhere. When established the plot is infected by transferring whole infected plants or single infected leaves. Infected material is recognised in spring and early summer by the golden yellow hypertrophied lesions on leaves, scapes and involucral bracts. Place an infected leaf in the centre of an uninfected rosette and spray it liberally with distilled water, preferably in the evening. Cover with a plastic bag to minimise evaporation. Signs of infection will be visible as yellow blisters on young leaves in 7–10 days. Towards the

end of the season brown resting sporangia become obvious. The rosette will die back in winter, but this can be delayed by covering with a cloche. The new rosettes in spring become infected from overwintered resting spores. Since infection is spread by zoospores, it may be necessary to water the plots liberally in dry seasons.

Oomycetes

Many members of the Peronosporales are pathogenic to plants, and range in their behaviour from facultative necrotrophs to obligate biotrophs. *Phytophthora infestans,* the cause of late blight of potato, can be grown in agar culture, and these cultures can be used to inoculate foliage to produce blight symptoms out of season. However prolonged culture on agar seems to reduce pathogenicity and we prefer to isolate fresh cultures each year. Leaflets showing early symptoms of blight (brown lesions on the upper surface of the leaflet, and a halo of sporangiophores visible at the lesion margin on the lower surface) are collected in the field in late summer. Small discs about 5 mm diameter are cut from the margins of the lesions with a flamed cork borer. The discs are placed aseptically on to V8 juice agar (8 juice 20%, $CaCO_3$ 0.3%, Agar 2%) with 2000 ppm of the antibiotic pimafucin. An alternative isolation medium is Pea-sucrose agar (250 g dried peas, soaked and mashed, sucrose 20 g, Agar 15 g, Distilled water 1 l). Pimafucin inhibits the growth of contaminating fungi and bacteria, and permits selective isolation of *Phytophthora.* Several discs can be laid on the agar surface, and the plates are incubated at 20° C for 7 days. The characteristic sporangia of *P. infestans* can be seen under a dissecting microscope, and transfers can be made from the margins of sporulating colonies to other media. V8 juice agar or Lima bean agar are suitable media for obtaining profuse sporangia. Zoospores can be induced to develop by scraping the sporangia from the surface of a 10 day-old culture plate and placing them in chilled water (5–10° C) for 2 hours.

Depending on the season suitable plants for infection can be reared in plots out of doors, or in a heated glasshouse with supplementary illumination. "Seed" potatoes purchased in the spring can be stored in a refrigerator at 5° C for several months. A few weeks before they are needed the sprouted tubers are potted. For material needed in November we pot the tubers in early September, and plunge the pots in a trench in the garden so that the top of the pot is level with the soil. If frost threatens the pots are protected at night by glass "tent" cloches. Plants grown in this way are preferred to glasshouse grown plants, because the latter are often etiolated, yellowish and "leggy".

Infection can be brought about by spraying a zoospore suspension onto the foliage with an atomiser. If the concentration of zoospores is very high this method of infection can cause dramatic collapse and necrosis of the foliage, but such material is of little use for class because the tissues may be too heavily damaged to demonstrate the relationship between the sporangiophores and the stomata. It is therefore preferable to place drops of zoospores, using a Pasteur pipette, on to the surface of the leaflets. It is also a good idea to place drops in the leaf axils. The resulting lesions will spread along the petiole and the stem, and epidermal strips showing emergent sporangiophores are more easily made from such tissues than from the hairy leaf surface.

After inoculation which is best done in the late afternoon the shoots of the potted plants should be enclosed in a large transparent polythene bag to maintain a high humidity. A few small canes should be used to prevent the polythene bag making contact with the foliage. Under glasshouse conditions (temperature ranging from 15° C–20° C) blight symptoms develop within 3–5 days. If material is needed for a given time it is sensible to do trial inoculations to get the timing right, and to stagger the inoculations over about 3 days to ensure that some material at the right stage is available for the class.

When classes occur in late winter it may be necessary to break tuber dormancy to induce sprouting. Ware (i. e. non-seed) potatoes can be induced to sprout by placing them in a polythene bag together with a wad of cotton wool soaked in ethylene dichloride. They should be left at room temperature for 12 hours. The treated tubers should be potted and grown in a glasshouse.

Albugo candida (white blister rust of crucifers) is a biotrophic pathogen which can be maintained in gardens on a range of cultivated or wild hosts. It is host-specific, so it is not possible to transfer it from one cruciferous genus to another. The cultivated hosts on which it grows well at Exeter are honesty *(Lunaria annua)* and aubretia *(Aubretia deltoidea)* and various cultivars of Brussels Sprouts and Cauliflower. *Lunaria,* being an annual host should be allowed to drop seed *in situ* and the seedlings, if crowded, develop an epidemic with profuse white blisters in early summer without intervention. Zoospore production can be induced in chilled distilled water from scrapings containing sporangia, and zoospore suspensions can be used to spread the disease to fresh seedlings. Since the fungus overwinters as oospores in dead tissues, the dead plant material should be left on the soil surface to provide fresh infection foci in the spring. A common wild host is the annual weed shepherd's purse *(Capsella bursa-pastoris).* Dense growths can be encouraged by collecting and sprinkling seed on the soil. The seeds

are apparently stimulated to germinate by light, so they should not be buried deeply. Staggered sowings ensure a succession of young plants. The infection is usually acquired naturally, but can be encouraged by dripping sporangial or zoospore suspensions into the centre of the rosettes of young seedlings. Infected plant remains should be left in the garden. A light raking of the soil in spring will encourage the germination of overwintered seed. Towards the end of the season infected material should be protected by cloches. Young seedlings can be transferred with blocks of soil into the glasshouse and, if inoculated with sporangia, will develop symptoms within a few weeks.

Albugo tragopogonis-pratensis, although less common than *A. candida,* can be maintained using similar techniques on Oxford Ragwort *(Senecio squalidus)* and Goatbeard *(Tragopogon pratensis).* Goatbeard is a biennial plant, and fresh sowings of seed should be made each year, otherwise the host plant will be lost after flowering.

Peronospora parasitica often occurs as a dual infection along with *Albugo candida* on *Capsella,* so no special steps need be taken to encourage it. On cultivated cruciferous herbs it grows well on Wallflower *(Cheiranthus cheiri)* and yellow Alyssum *(Alyssum saxatile). A. saxatile* is perennial and the pathogen may develop systemically on this host. *Peronospora farinosa* can be encouraged by allowing seed of fat hen *(Chenopodium album)* to germinate. The lesions are yellowish on the upper surface, and the pale violet sporangia develop on the lower surface of the leaves.

Bremia lactucae, causing downy mildew of lettuce occurs naturally on a wide range of cultivars. It flourishes on autum-sown lettuce grown under cloches. For demonstration to students it is often convenient to use infected seedlings. Seedlings at the 2-leaf stage are surface sterilized in dilute hypochlorite (eg. 5% "Domestos") then rinsed in water. The seedlings, with roots still attached, are placed in Petri-dishes on moist filter paper and inoculated by pipetting a dense suspension of sporangia (about 100/ml) on to them. The dishes should be incubated in a cool cabinet, at a temperature below 15° C with fluorescent light. Fresh sporangia are visible 10–14 days later.

Taphrinales

Two species of *Taphrina* can be maintained without difficulty. *Taphrina deformans,* the cause of peach leaf curl occurs naturally on most nursery stock, and all that is needed to keep it is to avoid the usual fungicidal sprays before bud-break. The reddish hypertrophied lesions on the leaves become visible soon after leaf emergence. Pruning of the branches in early summer induces the elongation of lateral shoots and prolongs the availability of diseased

leaves. Potted plants allowed to acquire the infection in the previous season can be induced to form leaves early under glasshouse conditions. *Taphrina populina,* the cause of golden leaf spot of poplar *(Populus nigra)*, is easy to maintain. Diseased shoots can be induced to root, and the resulting plants show disease in the following season. As with *T. deformans* pruning induces lateral shoot and leaf development. By this means the availability of hypertrophied leaves can be extended into October.

Erysiphales

Several members of this group of biotrophic pathogens can be encouraged by cultivating their hosts. *Erysiphe graminis* f. sp. *hordei* (barley mildew) can be obtained by sowing seed of the highly susceptible variety Golden Promise. Potted plants which have acquired the infection in the field can be transferred to the glasshouse as winter approaches. Seedlings can be infected by shaking conidia over them. Fresh lesions bearing conidia develop within 10–14 days. In the field or garden cleistocarps may develop, but since *E. graminis* is heterothallic, their appearance cannot be relied on. We use *Erysiphe polygoni* on *Polygonum aviculare* (knotgrass) or *E. heraclei* on *Heracleum sphondylium* (hogweed) for cleistocarps. *Polygonum aviculare* is a common field weed and can be grown by transplanting seedlings or by sowing seed. The mildew infection is usually acquired naturally. Cleistocarps develop on the underside of the leaves. Pruning of the plants in late summer induces fresh leaf growth. Protection of plants by cloches may be necessary to ensure the availability of material until late October.

Heracleum sphondylium can be cultivated by transplanting seedlings, or by sowing seed. Late infection can be induced by cutting back the shoots, and numerous cleistocarps can be found late in the autumn. The plant is biennial, so that successive sowings may be necessary.

Various species of *Sphaerotheca* can be encouraged to grow on their respective hosts. *Sphaerotheca pannosa* grows on cultivated or wild roses *(Rosa canina)*. The cleistocarps nestle in a felted mycelium on the stems. *Sphaerotheca mors-uvae* forms cleistocarps on twigs and fruits of gooseberry *(Ribes uva-crispa)* whilst *S. macularis* fruits on leaves of dandelion. *Podosphaera leucotricha* is common on shoots of apple and *P. oxyacanthae* on hawthorn *(Crataegus monogyna)* especially if it is clipped frequently. Bushes of hazel *(Corylus avellana)* if cut back to encourage young growth, develop cleistocarps of *Phyllactinia guttata* on the lower sides of the leaves, especially later in the season.

Clavicipitales

Claviceps purpurea causing ergot of rye *(Secale cereale)* is easily maintained. Sclerotia from rye or from other hosts are surface sterilised in 5% "Domestos". After a rinse in sterile water the sclerotia are sliced aseptically and the slices placed on 2% malt extract agar. Pure cultures can be prepared from the mycelium which grows out. 2 week old cultures develop conidia. Suspensions of conidia in sterile water are injected into the uppermost leaf sheath at the time of heading. After heading the *Sphacelia* honeydew stage will be seen on the ears, followed by the development of sclerotia. Infection can be carried out in the field in the summer, or at other times of the year in the glasshouse in in pot-grown plants. Dried sclerotia retain viability for 3 years. If sclerotia are allowed to overwinter on the soil surface outdoors, perithecial stromata will develop and the resulting ascospores may bring about natural infection, It is advantageous to collect ripe sclerotia and place them on the surface of soil in a pot, buried to the rim in the garden,and protected by fine netting. In the spring the overwintered sclerotia can be induced to develop perithecial stromata by allowing them to imbibe water and incubating them on moist sand at 15–20° C under continuous illumination. Under such conditions perithecial stromata develop in 4 weeks.

Epichloe typhina (choke of grasses) is best maintained on a tillering grass such as cocksfoot *(Dactylis glomerata)*. Diseased plants are detected by the suppression of inflorescences and the golden perithecial stroma around the uppermost leaf sheaths. If such a plant is dug up it can be divided into separate tillers, which can be easily rooted. Since the fungus is systemic all the tillers will develop into tussocks with the disease. Tillers separated and rooted in pots can be grown in the glasshouse, and can be induced to form conidial and perithecial stromata by exposure to "long day" illumination. – Other grasses on which *Epichloe* can be maintained are *Holcus mollis* and *Agrostis tenuis*.

Helotiales

Sclerotinia fructigena, the cause of brown rot of apple, develops naturally on apple trees which are not sprayed and where diseased apples are left to mummify on the ground during the winter. If it is necessary to start off the infection artificially inoculum in the form of conidia should be introduced by stabbing an inoculating needle or scalpel into ripe fruits whilst still on the tree. Most apple varieties are susceptible. *Sclerotinia laxa* can also be encouraged to develop on plums by similar means.

Many smut fungi are systemic, and when they grow on perennial hosts they can easily be cultivated.

Ustilago segetum var *avenae* causes loose smut of false oat grass *(Arrhenatherum elatius)* and tillers from an infected plant can be rooted. Usually all the inflorescences are smutted, and the smutted heads appear around June. By cutting back the tussocks at flowering time late flowering can be induced so that fresh material is available up to October. Potted plants can also be induced to flower out of season in the glasshouses by extended illumination.

Ustilago hypodytes (stem smut) grows on several grass hosts, and we have cultivated it on sea lyme grass *Elymus arenarius* and on *Bromus erectus.* If a silty pond is available *U. longissima* will continue to grow on reed grass, *Glyceria maxima,* causing linear sori on the leaves, and inhibiting flowering. *Ustilago zeae* can be inoculated onto seedling maize. Maize cobs bearing sori of chlamydospores can be dried, and the spores will retain viability for over a year. If a suspension of chlamydospores is smeared onto 0.1% malt extract agar numerous sporidia will develop within 48 hours, and conjugation will result in dikaryotic mycelia. A suspension of such material can be inoculated into the rolled leaves of seedling maize. Within 7–10 days pale hypertrophied lesions develop on the leaves. When transplanted to the field a proportion of the infected seedlings give rise to smutted cobs. *Ustilago violacea,* another smut of Caryophyllaceae grows systemically on male and female plants of red Campion *(Silene dioica)* and can be transplanted to the disease garden. Late flowering can be induced by cutting back flowering shoots in the summer. *Dianthus caryophyllaeus,* a pink, is also infected with this fungus, and infected plants can be divided for propagation. Another striking anther smut is *U. succisae* on devil's bit scabious, *Succisa pratensis,* recognised by the white diseased anthers in place of the healthy purple ones. The systemic pathogen can be transplanted and propagated by dividing the host plant, and again, late flowering can be induced by cutting. Plants of goatbeard, *Tragopogon pratensis* containing the systemic smut *Ustilago tragopogonis -pratensis* have been cultivated by transplanting, but since the host is biennial the infection tends to die out unless a seedling population can be established.

Ustilago segetum var *tritici* (= *U. nuda*) causes loose smut of wheat and barley. Barley seed containing a high proportion of infected embryos can be obtained from Official Seed Testing Stations which when sown gives rise to some infected heads. Infected heads emerge a few days earlier than healthy ones and the spores from them infect adjacent plants, so that seed collections from them

usually contain a proportion of infected embryos. Spore suspensions can also be used to infect healthy ears by vacuum infiltration (BATTS, 1955). Smutted plants can be reared out of season from infected seed in a glasshouse, using supplementary lighting to induce flowering.

Tilletia tritici, causing bunt of wheat, is now a rare pathogen because it can be successfully controlled by fungicidal seed dusting. It is easily grown. Bunt balls retain viable spores for many years. Chlamydospores are dusted onto tap-water agar, and germinate within 4–5 days at 20° C. The primary sporidia conjugate to form dikaryotic secondary sporidia and it is these which initiate infection. A suspension containing secondary sporidia is injected into the coleoptile of wheat seedlings, which are then sown in the garden. At heading infected heads are recognisable by the wider angles of their glumes compared with healthy heads. An alternative way of obtaining infection is to dust chlamydospores over seed grain by shaking the grain together with broken bunt balls in a box. However it is important to obtain untreated seed grain, i. e. seed which has not been treated with a fungicide.

Another smut which has been successfully kept in cultivation is *Urocystis agropyri* on *Elymus repens (Agropyron repens)* and *E. pycnanthus (A. pungens).*

One general precaution that may be necessary when growing small plots of cereals is to protect them against birds by enclosing them in a frame covered by netting.

Uredinales

Several rust fungi can be maintained in a garden, especially those which are systemic. In the case of heteroecious forms both of the alternate hosts need to be cultivated to obtain all stages of the life cycle.

Puccinia graminis is maintained on two grass hosts at Exeter, *Elymus (Agropyron) repens* and rye *(Secale cereale).* A short row of plants of *Berberis vulgaris* and *B. thunbergii* serve as alternate hosts. The *Elymus* plants are allowed to grow among the *Berberis* and develop black linear telia which overwinter, giving rise to basidiospores from which haploid infections become visible on *Berberis* in alternate years. The straws are supported by bamboo canes to help keep them in place. The *Berberis* plants may be started from rooted cuttings or from seed, but the seed has a chilling requirement for germination. Potted *Berberis* seedlings can be used to obtain aecia for class at any time of the year, using straws bearing telia collected in March. These can be stored dry and the teliospores retain viability for over a year. Urediniospores which have been freeze-dried in evacuated ampoules provide an alternative source for inoculating the grass or cereal host.

Puccinia poarum is another heteroecious rust and grows on *Poa* spp. and coltsfoot *(Tussilago farfara)*. For teaching it has the advantage of producing several crops of aecia on the coltsfoot throughout spring until late autumn. Material protected by cloches may bear aecia in November. The aecial pustules are very attractive to slugs and snails, and it may be necessary to use slug pellets to control them. A small plot containing a few coltsfoot plants and species of *Poa* such as *P. trivialis* of *P. pratensis* is all that is needed to maintain the disease. It is best introduced by planting an infected coltsfoot plant into the plot. The coltsfoot may also become infected with another rust *Coleosporium tussilaginis* which forms diffuse lesions in contrast with the circular pustules of *P. poarum*.

Puccinia caricina which alternates on *Carex* spp. and *Urtica dioica* is a convenient heteroecious rust for demonstrating the macrocyclic life cycle. If a pond or submerged tank is available in the garden *Carex riparia* or *C. acutiformis* can be grown, or, if not, a terrestrial sedge such as *C. pendula* may be used. Nettle plants can be grown alongside. Nettle plants can be potted and grown at any season of the year in the glasshouse. Telia can be collected on *Carex* leaves during the late winter, and can be stored for over a year in a refrigerator. They can be used to initiate pycnial and aecial stages on the nettle plants.

Puccinia menthae, the cause of mint rust, is autoecious. It is best cultivated on *Mentha viridis*. In spring the reddish hypertrophied stems bearing a systemic infection with pycnia and aecia become obvious. Later, local leasions with uredinia and telia occur on the leaves. The disease probably overwinters as teliospores on fallen leaves, and from these basidiospores infect the emerging shoots.

Puccinia malvacearum, the cause of hollyhock rust, grows on cultivated *Althaea rosea* and on the wild host *Malva sylvestris* (mallow). Plants grown from seed usually acquire the infection naturally. This is a micro-form with only teliospores and basidiospores. The teliospores do not need a period of rest before germination so they are particularly useful for demonstrating promycelium and basidiospore development. The plum rust, caused by *Tranzschelia discolor,* has *Anemone* as its alternate host. Plum cuttings can easily be established and they should be interplanted with corms of St. Bridget Anemone, *A. coronaria*. The plum leaves usually become infected naturally. Telia from fallen plum leaves develop basidiospores which initiate a systemic infection on *Anemone*.

Gymnosporangium confusum has a perennial mycelium which develops golden yellow telial horns on spindle-shaped swellings on stems of *Juniperus communis*. The alternate host is *Crataegus monogyna* (hawthorn). *Crataegus* plants are best established from seed-

lings collected in spring. Juniper can be established by germinating seeds, or from rooted cuttings. The telial material should be collected in spring and thoroughly wetted to induce swelling of the mucilage which envelops the teliospores. Material in this state is placed over the hawthorn plants preferably in the evening. Successful infection is evidenced by the appearance of pycnia after about 10 days, and the brown cylindrical aecia later. The aeciospores infect the juniper but it may take 3–4 years before the characteristic hypertrophy and development of telia are obvious.

Endophyllum euphorbiae-sylvaticae forms a systemic mycelium in shoots of wood-spurge, *Euphorbia amygdaloides*. Infected plants can be detected in spring by their yellow spindly growth and sweet scent from pycnial nectar. Plants transplanted at this stage continue to produce infected shoots for several seasons. They can be used to demonstrate the unusual germination of the aecio-teliospores.

Phragmidium mucronatum causing rose rust grows on many host varieties. It is common in regions with low levels of aerial pollution. It demonstrates well the formation of green "islands" around the lesions, and the detachable teliospores which extrude mucilaginous appendages when placed in water.

Angiospermous parasites

We have cultivated two angiospermous parasites. *Cuscuta epithymum* (Common dodder) grows well on *Ulex europeus* (gorse). It is most easily established by placing potted seedlings of gorse (heat treatment stimulates seedling germination) in contact with dodder shoots until haustoria have developed. The infected seedlings can then be planted in the garden. In mild winters the parasite survives as buds close to the haustoria. Seed of dodder can also be collected and sown around seedling gorse. – *Viscum album* (mistletoe) is established on apple branches by making a slit in the bark and rubbing a mistletoe berry into the crack. Mature flowering plants develop within 3–4 years.

This account does not exhaust the range of possible pathogens which can be cultivated, but merely illustrates some that we have found useful. In other areas similar techniques could be used for different pathogens.

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