Dactylorhiza 'Black Death'

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This apparently new and very aggressive disease of *Dactylorhiza* species first came to the attention of the Royal Horticultural Society (RHS) in 2014, when samples of affected plants were sent by Dr Roderick Woods to the RHS Plant Pathology department at Wisley. The samples were taken from Gibraltar Point National Nature Reserve, near to Skegness on the Lincolnshire coast. This internationally-important SSSI site covers an area of about 4.3km^2, and consists of sand dunes, salt marsh and other coastal features. In December 2013, parts of the reserve were affected by a tidal surge (the 'North Sea Flood') caused by storm Xaver, with the encroaching salt water damaging much of the vegetation over large areas, including extensive colonies of *Dactylorhiza* species and hybrids in the Freshwater Marsh area. Almost no *Dactylorhiza* plants appeared in this area in 2014. Since then, there has been a gradual recovery of the plants in this area so that a large, healthy population of *Dactylorhiza* was again present in the Freshwater Marsh by 2020.

Other areas, such as the East Dunes, were not inundated with the overtopping salt water in 2013, although they may have been flooded by rising water levels from below. *Dactylorhiza* plants in these areas also showed severe decline the following year (Figures 1 and 2). Large numbers of plants did not reappear at all in 2014, and those that did often showed symptoms that were more suggestive of a disease than simply the effects of flooding. In contrast to the Freshwater Marsh area, there has been no subsequent recovery of the orchid population in the East Dunes. A few orchids occasionally reappear, but they quickly develop symptoms as outlined below. The symptoms on plants that manage to grow back at all in the East Dunes consist of stunted growth, with dark brown lesions on the leaves. In many cases the lesions enlarge and merge together so that the leaf is killed. Where plants retain enough vigour to produce a flower spike, similar dark lesions often develop on the flower stalk, resulting in curvature, distortion and sometimes death of the spike (Figure 3). When the 2014 plant samples were received at Wisley, fungal spores were already visible on many of the lesions, and where this wasn't the case they soon developed after incubation of the material at high humidity for 24 hours or so. The spores didn't fit the description for those produced by *Cladosporium orchidis*, a known fungal pathogen of *Dactylorhiza* causing leaf spots and stem lesions (Wilson & Wilson, 2001). In fact it wasn't possible from the spore morphology to determine the precise identity of the fungus.

Fig. 1: Gibraltar Point, East Dunes area with healthy orchid population, photo taken June 2012.
Fig. 2: The same area in May 2014.

Photos by Roderick Woods.

The fungus was successfully isolated into aseptic agar culture from the material, producing slow-growing colonies that were initially colourless but eventually darkened as thick-walled hyphae developed (Figure 4). In addition to trying to identify the fungus based on morphological characteristics, DNA sequencing work was undertaken. It still proved impossible from its morphology (either in culture or on the plant material) for us to match the fungus to any known pathogen, and the sequencing results didn't provide a close match to any of the fungal DNA sequences deposited on Genbank, an open-access collection of nucleotide sequences encompassing more than 300,000 organisms.

Because we were having such difficulty with identification, cultures of the fungus were sent to the Mycology Department at the Royal Botanic Gardens, Kew, and to the Microbial Identification Service at CABI, both internationally-renowned for their expertise in identification of fungi. However, neither were able to conclusively identify the fungus. At this point it was decided to put the identification of the fungus on the back-burner, whilst attempts were made to prove that it was pathogenic and the actual cause of the symptoms exhibited by the affected plants. Whilst this seemed very likely, there was always a chance that it might instead be a secondary coloniser of the damaged plant tissues, or even an endophyte (a fungus usually residing harmlessly within a plant, that only reveals itself when the plant tissues die due to some other reason).

In order to prove pathogenicity to a level that is accepted by the scientific community, a set of procedures known as 'Koch's Postulates' must be completed. The potential pathogen (our fungus in this case) must firstly be recovered consistently into culture from the disease symptoms – as mentioned above this had already been done. Healthy plants of the same type must then be inoculated with the potential pathogen. If the inoculated plants develop the same symptoms as were seen on the original diseased plants, and the same organism can be subsequently re-isolated from the symptoms produced on those inoculated plants, Koch's Postulates is completed.

Initial attempts to complete Koch's Postulates were unsuccessful, due mainly to the difficulty in obtaining vigorous, healthy plants of a good size for use in the inoculation tests. The test plants are sprayed with a spore suspension of the fungus and then enclosed in plastic bags for 24-48 hours, which provides the very high humidity necessary for spore germination (and subsequent infection if the fungus is

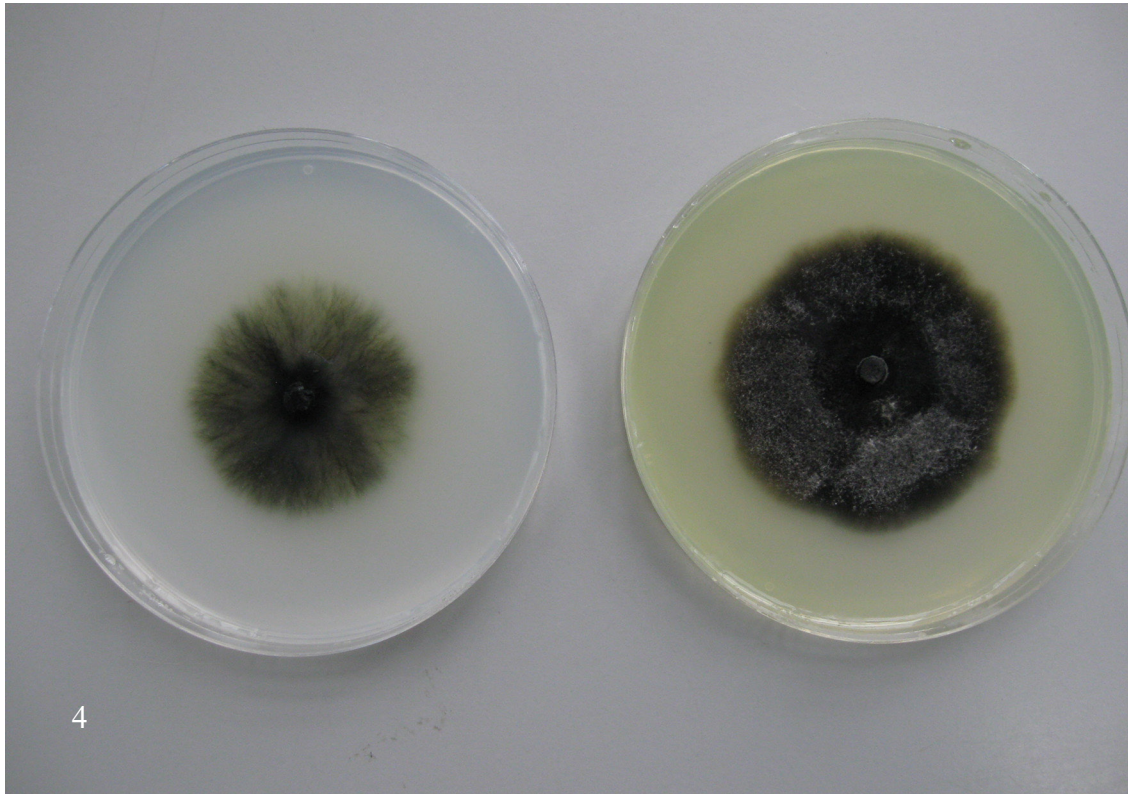

Fig. 3: Symptoms exhibited by surviving plants in the East Dunes area. These plants were sent to the RHS for examination.
Fig. 4: *Dactylorhiza* fungus in agar culture. Sucrose nutrient agar on left, potato carrot agar on right.

Photos by Roderick Woods (Fig. 3) & John Scrace (Fig. 4)

pathogenic). A second set of 'control' plants is put through the same procedure, except that these are simply sprayed with water rather than the fungal spore suspension. If the 'control' plants develop symptoms of the disease it indicates that something has gone wrong with the inoculation procedure, or that the apparently healthy plants used in the test were in fact already affected by the pathogen.

In the first few attempts a high percentage of the test plants, both inoculated and controls, died off not due to the disease but because they didn't have the necessary vigour to cope with the environmental conditions of the test. Eventually, however, some large tubers were kindly donated by Dave Trudgill, which when potted up grew into vigorous plants much more suitable for use in the tests. When these plants were inoculated with the fungal spore suspension, leaf spots began to appear within eight days. Within three weeks of inoculation the plants had developed typical and severe symptoms of the disease, and the fungus was successfully re-isolated from the lesions. 'Control' plants sprayed with water alone remained healthy (Figure 5). Koch's Postulates was thus completed and it can be said with certainty that our fungus is the cause of the symptoms, and also a very aggressive pathogen.

Most of the inoculated plants, having developed severe symptoms of the disease, failed to emerge the following spring, and no tubers were found when the contents of the pots were examined. As the fungus isn't normally found on the tubers of plants showing aerial symptoms of the disease it's likely that severely-affected plants simply lack the vigour to develop a replacement tuber for the following year, rather than the tuber itself being attacked and decayed by the fungus.

Now that the fungus has been proven to be the cause of the disease, attention can return to its identification. It is hoped to include it in the early stages of the Darwin Tree of Life project, an ambitious programme aiming to provide full genome sequences for all of the 70,000 eukaryotic (having genetic material contained within a cell nucleus) organisms in Britain and Ireland. This would provide extremely valuable information. In the meantime, preliminary work by Dr Brian Douglas, the Darwin Tree of Life co-ordinator at Kew, has shown that the fungus is possibly a novel species within the genus *Pyrenopeziza*.

Fig. 5: Host inoculation test. *D. purpurella*, three weeks post-inoculation. Control plant on right.
Fig. 6: Leaf symptoms, showing diffuse 'watersoaked' margin to active lesions and light brown spore production.

Photos by John Scrace (Fig. 5) & Roderick Woods (Fig. 6)

This aggressive pathogen poses a significant risk to *Dactylorhiza* populations. In addition to the extensive plant losses that still continue at Gibraltar Point, suspected cases have been found by Dave Trudgill (who has been battling the disease for several years at his own property in Scotland – Trudgill, 2015) at seven sites in Scotland (in Angus, Fife, Perthshire, South Uist and Stirlingshire) and one in Yorkshire. In most cases the disease at these sites has been confirmed by John Scrace from samples sent to him. In order to further determine the current distribution of the pathogen John would be grateful to receive samples from any other suspected cases. Microscopic examination of the material (and sometimes further testing) is required to confirm that the fungus is involved. Further details on sampling are given at the end of this article. So far the disease has only been found on *Dactylorhiza* species and hybrids, including *D. fuchsii, D. incarnata, D. maculata, D. praetermissa* and *D. purpurella*. However, if orchids of other genera growing close to affected *Dactylorhiza* plants show very similar symptoms it would also be worth sending these for examination.

The fungus produces large numbers of spores on the surface of the leaf and flower stalk lesions, and these are likely to be dispersed by rain splash. It's unclear whether the spores could also be wind-borne, in which case long-distance spread would be possible. However, even if this is not the case, heavy rain accompanied by strong winds could still lead to dispersal of spore-containing water droplets over many metres. Extended periods of leaf wetness are usually required by this type of pathogen in order for the spores to germinate and infect, so wet springs and summers are likely to lead to higher levels of disease than dry ones. It's also very likely that the fungus can survive on leaf debris, although precisely how long this could be for isn't known. It is recommended that strict hygiene should be practised if you have been on a site where the disease is known or suspected to be present. Hands should be washed thoroughly after handling plants that might be affected, and clothing should also be washed before visiting another site where *Dactylorhiza* might be present. Cleaning of footwear is particularly important. Ideally, footwear should be changed when leaving the affected site, brushed on-site to remove as much soil and plant debris as possible, and then washed thoroughly with hot, soapy water when back at home.

If you find plants in your garden that could be affected, then apart from sending samples for confirmation they should be destroyed as soon as possible. Burning is the best on-site option. Deep burial (well below normal cultivation depth) is also a possible option on level sites. Don't try and compost affected material, as home composting systems rarely reach the temperatures required to kill the propagules of many pathogens. For more details on disposal, type 'RHS – Disposing of Diseased Material' into your search engine.

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References

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Clues that the disease could be involved include a diffuse, 'watersoaked' margin to the brown lesion if the pathogen is active and spreading through the leaf tissues. Lesions caused by other factors such as physical damage are more likely to have an abrupt junction between brown and green leaf tissue. It is sometimes possible to see light brown spore production of the pathogen on the surface of disease lesions, although this is relatively uncommon. Both of these features are present on the leaf in Figure 6.