BOF 76a: Daffodil physiological disorders (rust) project

Supplement to Final Report

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Background

As described in the final report of project BOF 76a, in 2014 and 2015 a *Stemphylium* species was consistently cultured from typical rust lesions on Cornish daffodil stems, but not from atypical lesions. This appeared to be the first report of *Stemphylium* on daffodils. At the time of report submission it had not been proven that the *Stemphylium* species was pathogenic to daffodils: the inoculation of fresh daffodil plants with *Stemphylium* isolates was still in progress. The results can now be reported.

Methods

In autumn 2015 bulbs of daffodil 'Golden Ducat' were planted and grown in polythene pots of 'Fertile Fibre' seed growing medium, six bulbs per 5L pot. Three inoculation experiments were done in spring 2016 once leaf and stem growth were sufficiently advanced.

In Experiment 1 (mid January 2016, flowering stems approx. 50% of final height, GS 2.3) inoculations were made in two ways: (a) with two–four mm^2 agar plugs containing mycelium and conidia taken from towards the edge of cornmeal agar (CMA) plates, and (b) with spore suspensions in sterile distilled water containing a wetting agent (0.05% Tween 20). Plugs of agar were placed, or spore suspension was applied with a pipette, at the base of the plants between the flower stem and leaves. Eight or nine isolates, each from a different field site, were inoculated, using one pot (six plants) for each isolate. Following inoculation each pot of plants was covered with a polythene bag for a few days to maintain a high humidity.

Experiment 2 (early February 2016, growth stage similar to Experiment 1, GS 2.3) and Experiment 3 (early March 2016, flower stems about 80% of final height, GS 2.4) were inoculated in a similar way but only agar plugs were used because of the relatively sparse sporulation obtained on CMA. (The isolates produced very few or no conidia on the more nutritious potato dextrose agar or 'V8' agar.) In Experiment 3 the leaves and stems were rubbed lightly with a nylon scouring pad to remove wax and damage the surface prior to inoculation.

Results

In Experiment 1, no convincing lesions were seen on the plants inoculated with spore suspensions, and only one out of the eight inoculated isolates gave limited, but convincing, typical rust lesions using the agar plug method. *Stemphylium* was successfully re-isolated from these surface-sterilised typical lesions. Brown streaks were observed following inoculation with two of the isolates, and a diffuse yellow spot with another, but in these cases *Stemphylium* could not be re-isolated; nor was *Stemphylium* re-isolated from the control.

In Experiment 2, one to four small but typical rust lesions were observed per plant for eight of the nine isolates, and *Stemphylium* was successfully re-isolated in each case. One of the isolates

produced some yellowing at the inoculation site, but *Stemphylium* was not re-isolated from it, nor was it re-isolated from the control.

In Experiment 3, brown necrotic streaks were seen in both the control and inoculated plants, with the stems subsequently drying-down quickly. Although the necrosis seemed to be more severe on inoculated plants, effective assessment was not possible.

Following re-isolation attempts, residual tissues were maintained at high humidity in polythene bags. Almost invariably this resulted in profuse sporulation of *Stemphylium* on the senescing tissues that had had typical rust lesions.

Discussion

The consistent isolation of a *Stemphylium* species from typical rust lesions from the field in two consecutive years, the successful reproduction of symptoms following inoculation, and the successful re-isolation of *Stemphylium* from all typical symptoms on inoculated plants, satisfied Koch's postulates of pathogenicity and could lead to the conclusion that *Stemphylium* sp. is the primary cause of typical rust symptoms in the daffodils tested. However, given the difficulties in consistently reproducing symptoms, and that the lesions do not appear to expand on living tissues, it is suggested that it is a relatively weak, opportunistic pathogen that may be infective only when tissues are at a particular stage and/or under particular environmental conditions. Further work is needed to confirm these results and investigate the relationship between daffodil and *Stemphylium*.

Conclusion

These additional findings do not alter the main conclusions and recommendations made in the final report. Although *Stemphylium* species are significant pathogens in several important crops (see Discussion in the final report), here *Stemphylium* appears as a weak pathogen only; to have a significant detrimental effect on daffodil quality, it may require the coincidence of crop damage (facilitating pathogen entry), soil conditions adversely affecting the crop (e.g. soil compaction and high SWC) and a sensitive crop growth stage (e.g. rapid stem elongation).