Grower Summary

BOF 076a

Understanding physiological disorders in narcissus – project extension to study the three-year-down crop

Final 2016

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Headlines

- High soil water content in the months before flowering favoured the development of daffodil rust
- The fungus *Stemphylium*, not previously recorded from daffodils, was consistently isolated from typical rust lesions
- Narcissus Late Season Yellows Virus and Arabis Mosaic Virus were among viruses identified in daffodil leaves, but were not specifically linked to rust lesions
- There were no associations between soil or leaf levels of nutrients and rust

Background

The physiological disorder known as 'daffodil rust' spoils daffodil quality and can make the cut-flowers unmarketable. The popular cultivar 'Golden Ducat' is prone to rust. In mild cases it results in only a few inconspicuous, rusty lesions on the stem, but in more severe cases the lesions are conspicuous, numerous and lead to brittleness, cracking or bending of the stem, sufficiently disfiguring to down-grade the product or make it unmarketable. Commercial daffodil production in the UK is dependent on cut-flowers sales, so it is important to avoid any harm to customer's perception of product quality.

To gauge the extent and economic cost of rust, AHDB Horticulture, (formerly HDC) organised surveys of daffodil growers in 2002, 2003 and 2011–2013. The findings confirmed that rust was causing ongoing and commercially significant losses. A pathological or nutritional cause for rust had been tentatively, though not entirely, ruled out. Some physiological disorders are characterised by the appearance of brown or black spotting and have been linked with adverse environmental conditions. Based largely on the observation of crops, environmental factors, such as temperature and water availability, were suggested as responsible for daffodil rust. In an earlier project, daffodil cultivars had been grown in a temperature-gradient with the water content of their growing medium regulated at a range of levels. None of the temperature/watering regimes used resulted in the appearance of rust, however, and it was concluded that the range of experimental conditions used may simply have been insufficiently extreme to elicit the disorder. Another option was to observe the responses of daffodils growing in the field in uncontrolled, but real, conditions. Consequently this project was set up in 2012 to test the hypothesis that the soil-water environment (soil structure, water availability, soil temperature, nutritional status, etc.) is involved in the onset of rust.

Summary

In 2012, ten, 1,000-bulb plots of daffodil ‘Golden Ducat’ were commercially planted amongst daffodil crops at a range of locations across west Cornwall. ‘Golden Ducat’ is regarded as perhaps the most rust-prone of commercial cultivars, and South-West England was considered probably the most rust-prone bulb-growing area. Growing the crop for three years on sites with a diversity of soil, aspect, shelter, management systems, etc., was expected to capture a wide range of environmental conditions with which to work. The plants were closely monitored and soil water content (SWC), soil and air temperature, relative humidity and precipitation were logged at each site. The incidence and severity of rust was assessed regularly through each growing season, recording all characteristically rust-coloured lesions but excluding any darker, larger ‘chocolate spot’ lesions when these were encountered. Fertiliser applications and the levels of nutrients in the soil were collated, and in the second and third crop-years, soils and leaves were analysed for nutrient and trace element concentrations and stems and rust lesions were tested for the presence of fungal and bacterial pathogens and viruses.

The ‘Golden Ducat’ plots grew normally at all sites. In spring 2013 some plants at each site developed rust lesions, though with a very mild level of severity and a low incidence (between <1 and 14% of stems affected across the ten sites). Rust was more severe in the second and third years. Across the plots, severity varied between very mild and, in a few cases, serious to the extent that cracking developed on a few heavily affected stems, approaching a level that would make them unmarketable. Rust incidence varied, with between 5 and almost 100% of stems affected across the sites. Half the sites had a high rust incidence (>50% of stems affected) in both 2014 and 2015, whereas rust levels varied between years at the other sites; this suggested that specific sites were not tied to high rust incidence, but that external factors were also involved.

Year-by-year examination of the environmental data showed there were large differences in SWC between sites. Considering SWC in the four to five months before flowering, plots with higher SWC always developed higher rust levels, and although higher rust levels sometimes developed at sites with lower SWC, the reverse was not true. In winter 2012–spring 2013 three of the four sites with higher rust levels (>100 stems with rust per plot), St Buryan, Tregiffian and Rosevidney, also had higher SWC (>25mm/100mm), but at Penventon there was a similar higher rust level despite a lower SWC (*ca* 21mm/100mm).

In winter 2013–spring 2014 the highest rust levels (>900 stems with rust per plot) were found at St Buryan, Tregiffian, Roseworthy and Bodilly, which also had high SWC (*ca* 27–

30mm/100mm). However, Kelynack and Mawla had rust levels only slightly less severe (>700 stems/plot) despite their SWC being lower (*ca* 20–25mm/100mm, about the same as at the remaining sites which had relatively low rust levels). Ignoring the SWC for March 2014, SWC over the previous few months appeared reasonably 'predictive' for the highest levels of rust in April. Over winter 2014–spring 2015 a similar pattern was observed. The highest rust levels (>900 stems with rust per plot) were found at St Buryan, Roseworthy and Bodilly, which also had higher SWC (*ca* 27–28mm/100mm). Fourburrow had the same high rust levels, though its SWC was lower (*ca* 23mm/100mm). The other sites had lower (<800 stems/plot) or very low (<100 stems/plot) rust levels and a similar SWC to Fourburrow (20–25mm/100mm). When SWC was expressed as the accumulated daily SWC over the five-month period November–March, regression analysis showed that there were some statistically significant associations between SWC and rust levels the following spring.

These findings suggest that a high SWC during the few months before flowering results in higher rust levels. Regression analysis confirmed the statistical significance of associations between SWC and rust levels. Further analysis is needed to define the period over which a high SWC can be 'predictive' of impending high rust levels, but the effect appears to be the result of a longer period of high SWC affecting the plant, rather than a short pulse that 'triggers' a prompt response. Interestingly, putative early rust lesions were sometimes found on the elongating subterranean part of the stem, suggesting an earlier onset than the familiar rusty lesions on green stems would indicate. However, some other factor, currently unknown, must interact with SWC because higher rust levels were sometimes found at sites with a lower SWC (at Penventon in 2013, Kelynack and Mawla in 2014 and Fourburrow in 2015).

It is proposed that the onset of rust lesions in daffodils after prolonged periods of high SWC is a type of oedema. Oedema occurs in many plants when environmental conditions are such that the uptake of water by the roots exceeds that being transpired by the leaves. The increased internal water pressure results in the swelling of groups of mesophyll cells, leading to the distortion of the surrounding tissues with surface blistering and eventual bursting and the development of necrotic, often rusty-coloured patches on the leaves, stems or other organs - oedema. In contrast to the large site-to-site differences in SWC found in the study, other environmental factors (such as temperature and relative humidity) varied little between the ten sites. So it did not appear that they were directly involved in the mechanism controlling the development of rust/oedema, though this does not rule out an indirect involvement of, say, temperature.

To further assess the possible involvement of nutritional factors in daffodil rust, the major nutrients and trace elements were analysed in the soil from each site. Across the ten locations

the measured nutrient and trace element concentrations covered a wide range, and by the second or third years in some cases approached levels where top-ups might be applied had another year's growth been contemplated. However, it was considered unlikely that plants in the ten plots were becoming deficient, all plots remaining vigorous and with no signs of nutrient deficiency. Despite the wide range of nutrients involved, regression analysis found no evidence for any statistically significant association between soil nutrient concentrations and rust levels. The major nutrients and trace elements were also analysed in leaves from the ten sites. Although little is published of the 'typical' or 'normal' levels of nutrients in daffodil tissues, the key finding was that there was no evidence for any statistically significant association between leaf nutrient concentrations and rust levels. Hence, while it may be impossible to prove a negative, these findings argue against a nutrient-based theory of rust.

Stem samples were taken from each site in the second and third years to investigate any bacterial or fungal pathogens associated with rust lesions. No evidence for any bacterial pathogen was found. In 2014 fungal cultures from typical rust lesions from eight of the ten sites consistently yielded an as-yet unidentified species of *Stemphylium*, a pathogen not know to have been previously reported on daffodils. On stems from the other two sites, which had rust-coloured blotches or streaks rather than typical rust lesions, *Stemphylium* was not found. In a similar exercise in 2015, fungal cultures from typical rust lesions from eight of the ten sites again consistently yielded a *Stemphylium* species. On stems from the other two sites, one bearing typical rust lesions and one which had rust-coloured blotches or streaks rather than typical rust lesions, no fungi were isolated. The *Stemphylium* species was also isolated from typical rust lesions on samples of two cultivars supplied by a Cornish commercial grower. Independently, cultures from typical rust lesions on further stems from the experimental plots were found to yield the same *Stemphylium* species as before, as well as *Alternaria infectoria*. The five *Stemphylium* isolates obtained were identified as such by sequencing. At the time of writing there is no proof that the *Stemphylium* species is pathogenic on daffodils, and nothing to contradict the physiological nature of rust. However, tests are under way to determine whether the *Stemphylium* isolates can cause typical rust symptoms when re-inoculated to fresh daffodil leaves. *Stemphylium* species occur on many crops, causing a variety of diseases.

To investigate whether viruses are associated with rust lesions, RNA was extracted from stem samples from the ten sites, either with typical rust lesions or free of lesions. The resultant sequences were tested against four important virus genera that together include most of the significant viruses attacking daffodils. Products from all samples, with or without rust lesions,

were positive for Potyviruses (the group including the important aphid-borne viruses of daffodils). The dominant sequence was a 90% match to Narcissus Late Seasons Yellows Virus. In addition another sequence was a 76% match to Artichoke Latent Virus (not previously reported from daffodils). Testing for Nepoviruses (the group including the important nematode-borne viruses of daffodils) all samples tested negative for Nepovirus sub-groups B and C, but all were positive for sub-group A; the dominant sequence corresponded with Arabis Mosaic Virus, which is known to infect daffodils. All samples tested negative against viruses of the Carlavirus group (which includes the aphid-transmitted daffodil virus Carnation Latent Virus) and the Tospovirus group (which includes the thrips-borne Tomato Spotted Wilt Virus that can also attack daffodils). These tests confirmed the widespread infection of daffodil stocks by a number of viruses, but there was no evidence that viruses were specifically associated with the rust lesions.

The findings suggested that a rust-prone cultivar like ‘Golden Ducat’ may always carry a low level of rust – what about other cultivars? In spring 2015 a survey was carried out of five-bunch daffodil bunches sampled from the trade. The survey totalled 42 ‘Golden Ducat’ samples and 61 samples of other cultivars not considered prone to rust by the growers. The ‘Golden Ducat’ samples gave an average rust severity score of 1.2 (on a scale of 0–6, where 1 was ‘almost unnoticeable’ and 3 and 4 represented the borderline between marketability and rejection). The other cultivars had a notably lower average, 0.3. For rust incidence, ‘Golden Ducat’ averaged just over 50% of stems with rust (at any severity level), and for the other cultivars a much lower 21%. Both groups included some bunches with all stems affected by rust at some level, and some with none. The survey confirmed the susceptibility of ‘Golden Ducat’ to rust, but warned that other cultivars displayed more rust than expected.

Financial Benefits

At the start of the project and on the basis of information provided by growers, rust could result in an average 3% annual loss of revenue from cut-flowers (spread across all years), or losses of 10% one year in three (with negligible losses in the intervening years). A 3% annual loss was estimated to amount to about £0.7m annually to UK growers, or just over £2m every third year. In the past three years these values are not thought to have changed substantially. These are direct monetary losses resulting from reduced flower yields and downgraded or unmarketable product: there would probably be additional costs associated with finding alternative customers and safeguarding against unpredictable yields and poor quality in the future. These losses might be largely eliminated if the industry were able to implement a low-cost solution for rust and/or strategies for rust avoidance or risk management. In that case the financial benefits quoted, around £700k annually, should be set against the total project cost

of £118k over 3½ years. The findings advocate avoiding potentially water-logged sites and taking steps towards improving soil management – admittedly not procedures that could be implemented immediately or easily – and suggest that some three-quarters of rust outbreaks might be preventable by these means. As pathogens are probably *not* involved in rust there is no reason to apply pesticides in a 'just-in-case' attempt to control the problem, which could result in savings. The option of avoiding growing the rust-prone 'Golden Ducat' is probably impractical because of the continuing demand for this variety; in any case the project has shown that many other daffodil cultivars can have rust symptoms and that it is probably present in many stocks at a low level.

Much more importantly, eliminating the rust problem would remove the possibility of rust resulting in a loss of markets through lowered customer perception of our product. This seems especially important at a time when many other issues are impinging on the profitability of daffodil growing.

Action Points

- In trials in west Cornwall, a high soil water content in the few months before flower-picking was a 'predictor' of higher-than-usual levels of rust next spring. In no way does this mean the flower crop will be spoilt to the extent of non-marketability after a wet winter, because it seems the damage from rust will, in most cases, remain at a relatively low or inconspicuous level. It does serve as a warning of a potential issue, however, and when planting cultivars known to be rust-prone, poorly drained fields, low areas, and sites known to be compacted should be avoided. It is appreciated that finding suitable land for bulb-growing in the South-West is not easy.
- The level of rust is not affected by the concentrations of N, P, K, Mg and trace elements in the soil, so fertiliser practice should continue to follow standard advice for the crop.
- A fungal pathogen, *Stemphylium* species, not previously reported on daffodils, was cultured from Cornish daffodil leaves with typical rust lesions, though at this point it has not been proved that this species is pathogenic to daffodils. Further information on this point will be available later in 2016, but there is no need for a change in fungicide programmes at this time. No other pathogen (fungal, bacterial or viral) was isolated specifically from rust lesions in this project.
  Random samples of commercial cut-flower samples from across the UK, including apparently non-rust-prone cultivars, were often found with rust symptoms. In a few cases they were affected seriously enough to suggest they should have been eliminated from the supply chain.