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Project leader:	Gordon Hanks
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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GROWER SUMMARY

Headline

- A survey of commercial daffodil bunches showed that low levels of daffodil rust are widespread in the rust-prone ‘Golden Ducat’ but are also surprisingly common in other cultivars. In this survey and in the field trial, while rust was only very rarely found at a level sufficient to cause rejection of the product, these findings will emphasise to growers the importance of thorough pre-picking field inspections.

Background

The physiological disorder known as ‘daffodil rust’ (or ‘stem rust’, or simply ‘rust’) degrades daffodil stem quality and can make affected cut-flowers unmarketable. In mild cases rust may result in a few small, inconspicuous, rusty lesions on the flower stem, but in more severe cases the lesions are more obvious, with groups of larger lesions along the stem which can lead to the product being down-graded or rendered unsuitable for sale. In the most extreme instances rust leads to brittleness, cracking or bending of the stem. Commercial daffodil production in the UK is largely dependent on the sales of cut-flowers, so it is important to avoid anything that might harm the customer’s perception of product quality.

To gauge the extent and economic cost of rust, the HDC (now AHDB Horticulture) organised surveys of daffodil growers in 2002, 2003 and 2011–2013. The findings confirmed that rust was causing ongoing, commercially significant losses, justifying an investigation into its cause and potential management. At those times no pathological or nutritional cause for rust was found, though neither should be ruled out entirely because more structured sampling might have yielded a more robust conclusion. Some physiological disorders of other crops are characterised by the appearance of brown or black spotting and have been linked with some adverse environmental conditions, also suggested as a possible cause of daffodil rust. Project BOF 076 was set up in 2012 to test the hypothesis that the soil-water environment may be involved in the development of rust. In 2012, plots of the rust-susceptible cultivar ‘Golden Ducat’ were planted in ten commercial daffodil fields at varied locations through west Cornwall, where daffodils appeared to be prone to the disorder. It was hoped that this would maximise the likelihood that rust, despite its unpredictability, would occur naturally in at least some of the test locations, potentially enabling predisposing factors to be identified. Project BOF 76 was also used to supply a structured set of plant samples, with and without rust symptoms, that was used to examine possible pathological or nutritional causes of rust. The project extended from 2012 to 2014.

The main findings from BOF 076 were:

In spring 2013 very few rust lesions appeared in the period before picking/flowering, and at only one of the ten sites. The incidence of rust lesions then increased slightly at most sites to give between 0 and 144 affected stems per plot of about 1,000 stems in the post-picking stage. In spring 2014 rust lesions appeared at a higher incidence but still a low severity. By the post-picking stage all sites had mild rust symptoms, most or all stems being affected at seven of the ten sites, one of these exhibiting occasionally more severe symptoms of stem-cracking. None of the assessments found commercially-significant levels of rust (with the possible exception of the stem-cracking mentioned), and it was evident that trace levels of rust, were common, particularly after flowering. Hence, in a susceptible cultivar, rust may commonly persist at a low and insidious level.

Rust incidence varied substantially between sites and between years, and weather patterns (particularly for winter rain) were also markedly different between years. Data on soil water content (SWC) and other meteorological factors, logged at the ten sites, were examined for any associations with rust incidence and severity. The most striking result was an apparent relationship between rust incidence and SWC in the months before flowering. For the first crop-year, three of the four sites with the highest incidence of rust were associated with the highest SWC (the exception was a steeply sloping site). Further analysis showed that high SWC in November and December was closely related to the high levels of rust. For the second crop-year also there was a strong tendency for higher rust incidence where SWC was high, and lower incidence where SWC was low. Again, the apparent relationship was stronger for SWC over the preceding months than for SWC around flowering, suggesting the incidence of rust was related to conditions over a longer period.

Rust incidence did not appear to be associated with:

- Soil and air temperature and relative humidity, which were relatively uniform across all sites in both years;
- Some geographical factors – longitude, latitude, altitude and distance from the sea in the prevailing wind direction (which would affect the amount of salt-laden air);
- Soil structural factors – ‘Visual Soil Structure Quality Assessment’, ADAS soil texture assessment, soil depth and the proportions of clay, silt, sand and stone particles;
- The type of fertiliser applied before planting, or previous cropping;
- The date the bulbs were planted (which had varied substantially between sites);
- The concentrations of N, P, K or Mg or soil pH or (probably) trace elements;
- The current concentrations of major nutrients or trace elements in the leaves.

Laboratory examination showed that a *Stemphylium* species was the fungus most

consistently isolated from rust-affected leaves. *Ramularia* and *Botrytis* spp. were also isolated from some samples, though these are common daffodil pathogens and were probably coincidentally present. There was no evidence of bacterial infection in rust-affected leaves.

Summary

The present work comprised principally a one-year extension to project BOF 076 to cover 2014–2015, allowing a further year of observations. At the time of this report (June 2015) much of the data collection has been completed – crop and rust assessments, logging of SWC and weather data, collection of soil and leaf samples for mineral analysis, and sampling of leaves for diagnostic examination – and analytical, biometrical and diagnostic work is now under way and will be reported in the final project report (January 2016).

In the third crop-year, the overall incidence and severity of rust on the ten plots were broadly similar to those in 2014. At the pre-picking stage (14–16 February) a low level of rust was found, though the sites where rust first appeared were not the same as the previous years, and rust incidence was notably higher at Penventon than at the other sites.

At the picking stage (9–12 March) rust levels had again increased markedly since the pre-picking assessment, and were greater than in 2014. Rust levels varied between sites more than before, with incidence scores (on a 0–5 scale) between 1 and 4, between 3 and 200 affected stems per plot. The severity score, however, was 1 at all ten sites (on a 0–5 scale). Plants at St Buryan and Goonhavern had the lowest incidence of rust, and levels were highest at Roseworthy and Penventon. These low- and high-rust sites did not correspond to the low and high-rust sites of the previous years.

At the post-picking stage (10–12 April), and despite a confounding effect of white mould infestations at some sites, it was clear that the incidence of rust had increased substantially at all sites, with overall levels similar to those of 2014. Rust incidence score varied from 2 (Tregiffian) to 5 (Kelynack, St Buryan, Roseworthy, Bodilly, Mawla, Penventon and Fourburrow), with corresponding extremes of stems with rust per plot of <100 (Tregiffian and Goonhavern) and all (Roseworthy and Bodilly) or most (>900) stems affected (St Buryan and Fourburrow). The severity score remained at 1, except at Roseworthy and Bodilly where it had risen to 2.

The lack of consistency in rust levels over the three years and ten sites, and the substantial differences in weather patterns over the three years, notably a much drier winter in 2014–2015, will be of benefit in seeking associations between rust, SWC and other factors, as the data-set will cover a wide range of conditions – a challenging task but one that should provide a resilient model if indeed the link

between SWC and rust is confirmed in this third year.

Other work carried out in 2014–2015 is summarised below:

The diagnostic work on fungal pathogens at PHS is concentrating on the role of *Stemphylium*, which has again been isolated from stems with rust.

At WCC, work to extract and sequence viral RNA from 'rusted' and clean leaves is underway. The presence of some virus families, such as potyviruses, has been confirmed, while others have not been found.

Little is known about the course of development of rust lesions. Putative 'early-stage' lesions - small patches or larger tracts of 'pitting' and depressed, paler areas on the stems as well as 'blistering' - were often seen during regular assessments. The lesions can be seen soon after shoot emergence, and it might help our understanding of rust if the time of first appearance of (putative) rust lesions were known. Bulbs of 'Golden Ducat' were recovered from one of the fields at intervals from 12 November through 10 March and entire stems dissected out and examined for lesions. Few 'early-stage lesions' were found, and only on the samples taken 26 January (on 2 out of 10 plants, on the yellowish part of the stem passing through the soil) and 10 March (on 1/10 stems, on the white part of the stem within the bulb). More extensive observations would be needed to study fully the significance of these putative lesions.

The results of the project suggest that rust-prone cultivars like 'Golden Ducat' may always carry a low level of rust but the situation is less clear for 'non-rust-prone' cultivars. To assess the incidence and severity of rust on daffodil bunches being traded, random five-bunch samples of 'Golden Ducat', and of 'non-rust-prone' cultivars flowering at about the same time, were obtained from growers/traders and the stems assessed. The survey yielded 103 samples, comprising 42 'Golden Ducat' and 61 other cultivars, 31 being from Cornwall, 47 from Lincolnshire and Norfolk and 25 from Scotland. The 'Golden Ducat' samples gave an average rust severity score of 1.2 (on a scale of 0–6, where 1 is 'almost unnoticeable' and 3 and 4 represent the borderline between acceptability 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 level) and the other cultivars a much lower 21%. However, despite these generally mild rust symptoms, both groups included some bunches with all stems affected by rust at some level, and some with none. This confirmed the susceptibility of 'Golden Ducat' to rust, but the other cultivars displayed more rust, perhaps much more, than expected. Most 'Golden Ducat' samples had values in the highest incidence class (more than 40%). Continued vigilance is needed in carrying out pre-picking crop inspections.

Financial Benefits

On the basis of information provided by growers, rust can result in a 3% average annual loss of revenue from cut-flowers (spread across all years), or losses of 10% in one year in three (with negligible losses in the intervening years). A 3% annual loss is estimated to amount to about £0.7m annually to UK growers, or just over £2m every third year. These are direct monetary losses resulting from reduced flower yields and downgraded or unmarketable product, and there would probably be additional costs associated with finding alternative customers and safeguarding against future unpredictable yields and poor quality. Such losses might be largely eliminated if this project and its extension lead to the provision of solutions for rust and the development of strategies for rust avoidance or risk management. These financial and other benefits should be set against the total project cost of £118k over 3½ years.

Much more importantly, solving the rust problem would remove the likelihood of a gross loss of markets through lowered customer perception of the product – especially important at a time when many other issues are impinging on the profitability of daffodil growing.

Action Point

The project needs to be completed before recommendations are made. However, the high rate of rust incidence (and sometimes of rust severity) in ‘Golden Ducat’, coupled with the finding that other cultivars can also display not inconsiderable rust symptoms, suggest that continued vigilance is needed by growers in carrying out pre-picking crop inspections.

SCIENCE SECTION

Introduction

Since the early-1990s daffodil growers in the UK have been concerned over rust-like lesions sometimes found on the flower stems. The symptoms may be insignificant, through in severe cases there are prominent rusty lesions along a large part of an increasingly brittle stem, making the product unmarketable. The symptoms have continued to be found sporadically to the present time – perhaps their most predictable feature is its unpredictability. As the symptoms did not appear to be caused by a pathogen, the condition became known as 'physiological rust' and later 'stem rust' or simply 'daffodil rust'.

The cause of daffodil rust is unknown. *Ad hoc* examinations of affected tissues failed to find a pathogen associated with the rusty lesions, while further *ad hoc* analyses appeared to show no linkage between soil nutritional levels and the occurrence of daffodil rust. A pathogen- or nutrition-based explanation of rust was therefore largely ruled out, though it can be argued that, since the diagnostic and analytical tests may have been carried out in a less than systematic way, further testing was required. Anecdotal information circulating in the industry seemed to suggest that daffodil rust developed following specific weather or ground conditions, such as rapid crop growth following a frosty period, or mainly in crops growing in waterlogged areas.

In 2012 a project (BOF 076) was set up to examine the possible effects of weather and ground conditions on rust outbreaks, and to investigate systematically any relationships between rust lesions, the occurrence of pathogens and soil nutrient concentrations. As the triggers to rust development were not known, it was not practical to simulate rust-inducing conditions using an experimental approach. In project BOF 076 the approach used was therefore to plant plots of a rust-prone cultivar, 'Golden Ducat', on ten varied sites across west Cornwall, the bulb-growing area where rust appeared most prone to develop. At each site loggers were set up to collect meteorological data and SWC. Crop growth and rust incidence and severity were recorded, with a view to look for relationships between them. Soil and plant nutrient concentrations were also determined to investigate any relationship with rust levels, while plant samples were examined for any lesion-associated pathogens at an independent laboratory.

This project ran over the first and second years of the crops (2012–2014), and the methodology and results have been reported in detail in the final report of the project. In essence the tentative findings were (a) that higher rates of rust were associated with higher SWC in the previous winter months, (b) that there were no associations between rust levels and soil or plant nutrient concentrations, nor soil type, topography, etc., and (c) that a fungal pathogen not previously reported on daffodil crops was detected in rust lesions.

In Cornwall, commercial daffodils are usually planted and left ‘down’ for at least a three-year crop cycle. Winter and spring weather patterns over the two growing seasons of the crops were very different for the two crop-years, differences that can be exploited in studies of this type if robust conclusions are to be drawn. Although rust occurred at all sites in both growing-years, and at levels varying between the sites, it rarely approached the severity level that would be of concern in commercial cut-flower production. For all three reasons, a project extension was proposed, and approved as project BOF 076a, to extend the study to the third year of the crop, 2014–2015.

At the outset of BOF 076 it was reported that daffodil rust does not appear to have been described in key advisory literature or research reviews in the UK, the Netherlands or the USA, the three countries producing most daffodil bulbs and flowers. Further literature searches failed to reveal references to daffodil rust.

The core of the project extension, BOF 076a, is to carry out a third year of field-work on the ten plots of ‘Golden Ducat’ in Cornwall, involving continued collection of weather and soil water data, assessment of rust incidence and severity, further analysis of nutrients in soil and plants, continued pathogen testing, and the study of associations between these factor and rust levels in an attempt to understand the cause of rust. In this interim report (June 2015) crop growth and rust occurrence up to the end of growing season 2015 is described, while further nutritional, pathological and biometrical work is on-going and will be reported in the final report (January 2016). The project extension added three additional elements: (1) RNA extraction and sequencing from stems to determine any link between viruses and rust, which is on-going, (2) the examination of stems before shoot emergence and in the early stages of shoot extension, to determine the earliest time of appearance of rust lesions, which is reported here, and (3) a survey of commercial cut-flowers to assess the overall frequency of rust lesions in ‘Golden Ducat’ and ‘non-rust-prone’ cultivars, which is also reported here.

Materials and methods

Objective

The aim of the field-work was to test the proposition that the soil-water environment (soil structure, water availability, temperature, nutritional status, etc.) affects the occurrence, incidence or severity of daffodil rust. Since the occurrence of rust is unpredictable, the work was structured to increase the likelihood that the disorder would occur in at least some of the experimental plots: the cultivar used, ‘Golden Ducat’, is very susceptible to rust, ten sites with a variety of soil types and topography were used, and the work was located in west Cornwall, the region of the UK where it was considered (at the time) that daffodil crops seem most prone to rust. The ten plots were also used as sources of rusty and healthy plant material for disease diagnostics, and of soil and plant samples for measuring the concentrations of nutrients.

Bulb material

A suitable stock of narcissus 'Golden Ducat' was sourced and 250kg of bulbs of each of two grades, 10–12 and 12–14cm circumference obtained. The bulbs had received standard hot-water treatment at the growers. Twenty-five kg of each grade were allocated for planting at each of the ten sites. Since 25kg of bulbs is equivalent to *ca* 610 and 425 bulbs of the smaller and larger grades, respectively, each plot consists of *ca* 1,000 bulbs.

Sites

Following discussion with bulb growers, ten commercial daffodil fields were selected, taking into account the requirement to locate them throughout west Cornwall and to include varied soil types, topography and husbandry. Sites are listed in west-to-east order throughout the reports. Site locations, topography, pre-planting soil analysis, previous cropping, fertiliser and lime applications, dates of bulb planting, soil texture, soil type and other soil attributes were given in the final report for project 76.

Husbandry

At each site the two grades of bulbs were planted in two adjacent lengths of ridge each *ca* 20m long (except at Rosevidney and Roseworthy where they were *ca* 30m long). The inter-ridge distance varied according to the growers' usual practices, but typically gave planting densities of *ca* 14t/ha (20m-long plots) or *ca* 9t/ha (30m-long plots). Due to the prolonged and exceptionally wet autumn in 2012, some bulb planting had to be delayed until the advent of better conditions, so planting dates ranged from 12 September to 5 November 2012. Bulb planting and subsequent husbandry at the sites followed each grower's usual practice, though it was requested that flowers were not picked but left *in situ* to allow the full development of any daffodil rust symptoms for assessment. Each grower was asked to provide details of fertilisers and sprays applied and of other field operations for reference purposes.

Environmental monitoring

After planting the bulbs an 'Active Irrigation Scheduling' monitoring station, with added air temperature and humidity sensors, was set up in the plot at each site by Plant systems who continued to monitor and maintain them. Each station included a sensor to measure each of the following: percentage SWC at 0–10, 10–20 and 20–30cm depth, soil temperature at 15cm depth, rainfall, air temperature and RH. The soil sensors were inserted in the ridge centre, while air temperature and humidity sensors were positioned 20 to 30cm above the ridge tops, roughly corresponding to mid-canopy height for the fully-grown crops. The measure 'percentage SWC' is equivalent to the amount of water in mm per 100mm depth (1% = 1mm of water in 100mm of soil), and could also be expressed as the average across the three depths (i.e. mm of water in 100mm of soil) or the total of the three

depths (i.e. mm of water in 300mm of soil). All measurements were logged at 15-minute intervals, accumulated on Plantsystems' data-base, and checked and downloaded at appropriate intervals for analysis. As of the time of writing this report (June 2015) crop monitoring has recently been completed.

Soil and plant sampling and analysis

Soil sampling and examination techniques were guided by 'RB209', the standard text and standard analytical methods were employed. For consistency, all regular soil samples were taken from half-way up the ridges.

In spring 2015 (17–20 April), by which date reasonable levels of rust were apparent in many plots), further samples were collected from each site.

Soil samples were taken 0 to 20cm-deep for analysis by Natural Resource Management (NRM) for pH, top-soil organic matter and the available concentrations of major nutrients (P, K and Mg) and trace elements (SO4, Ca, Na, Fe, Mn, Cu, Zn, B, Mo and Al).

Soil samples were taken from 0 to 45–50cm-deep for analysis by WCC of total mineral N (NO3-N and NH4-N).

Leaf samples, comprising ten groups of leaves per plot, were excised at soil level and wiped free of soil, for analysis by NRM for total concentrations of N, P, K, Mg, S, Ca, Na, Fe, Mn, Cu, Zn, B, Mo and Al.

At the time of writing soil and plant analyses are in progress and the results will be presented in the final report of the project in January 2016.

Fungal and bacterial disease diagnostics

Using procedures similar to those of spring 2014, stem samples were collected from each site on 10–12 April 2015 for disease diagnostics. At each site six stems with typical rust lesions, and (where available) three stems with no rust lesions were selected; where white mould was a problem stems with white mould symptoms were avoided as far as practical. At St Buryan, Roseworthy, Bodilly and Fourburrow most or all plants were affected by rust, so no rust-free stems could be obtained (at Fourburrow rust-free stems of the same cultivar were taken from an adjacent stock). Hands and implements were wiped with disinfectant wipes between handling successive samples. Stems were cut off at ground level and the remains of the flower removed before placing each sample into a sealable polythene bag to isolate it for transport to Plant Health Solutions (PHS) for examination along the lines of the previous year. Examination is on-going and the findings will be presented in the final report. In 2015 the emphasis is on examining further the significance of *Stemphylium* in relation to rust, including using re-inoculation tests.

Additionally, (1) commercial samples of cut-flowers with rust were despatched from various sources to PHS for examination, including a sample of Cornish-grown daffodil 'Watford' with rust lesions at a borderline level for rejection, and samples of Cornish-grown 'Irish Luck' and 'Counsellor', cultivars reported by a grower to be rust-prone, and (2) also on 10–12 April ten to fifteen plants were dug-up at random from each trial plot and surface-dried under fans at ambient conditions for *ca* 2 weeks, after which the bulbs were cleaned by hand and delivered to PHS for follow-up studies (on-going).

Viral RNA analysis

In April 2014 three stems with typical rust lesions and (where available, see previous section) three stems with no rust lesions were also collected, avoiding stems with white mould symptoms. The handling and storage of these samples – which was all that had been agreed for project BOF 076 - was described in the final report of that project. The extraction and sequencing of RNA from the stored samples was left as an option for any follow-up work, depending on what other results were found. Extraction and sequencing was agreed as part of project BOF 076a and is now well under way at WCC.

On 10–12 April 2015 further stem samples were collected from each site and transported to WCC for sub-sampling, freezing in liquid nitrogen and storage at -70°C as previously described. These samples will be held at WCC as a back-up and for possible further work.

Crop and rust assessments

Each year plots were routinely assessed at three growth stages (GS, see Appendix, Table 3) based around flower-picking, when the appearance of rust is most commercially relevant, i.e. pre-picking, at picking and post-picking. The assessment made in 2013 and 2014 were detailed in the final report for project BOF 076, and in 2015 the assessment dates were 14–16 February, 9–12 March and 10–12 April.

To assess crop growth the minimum, most usual and maximum GS were recorded, including stem and foliage heights where applicable. This information served to check the rate of crop development over each year of the project.

To assess rust levels, all emerged stems in a plot were checked individually for the incidence and severity of lesions. The total number of stems per plot showing any rust was recorded. In assessing the stems the nature of the lesions present was noted, such as "one or two small spots per stem", "several groups of larger lesions per stem", etc. From these observations, rust incidence and severity scores, each of 0 to 5, were recorded (Table 1). In isolated instances it was not appropriate to assess the whole plot, e.g. when the end of the plot had been damaged by tractor movements, in which case the length of damaged plot was noted, the remainder of

the plot assessed, and rust incidence scaled-up to the equivalent of a full plot. Similarly, at some sites in some years some stems had been lost before recording, e.g. by wind damage or unauthorised picking: when this was the case, the total number of 'missing' stems was recorded by counting cut or broken stem-ends, and of the stems remaining the number with rust was scaled-up to a full-plot equivalent. In 2015 some plots were seriously affected by white mould by the end of the growing season, and the effect of this is considered under 'Results'.

Table 1. Rust (stem rust) severity and incidence scores used in plot assessment.

Severity	**Score**	**Incidence**	**Score**
None seen	0	None seen	0
Slight markings or blistering that may not be rust-coloured, and/or one or two inconspicuous, small but typical rust spots	1	Up to 1% of stems affected	1
Sparse but typical rust spots or rust-like streaks/blotches, no commercial significance but worth watching	2	Up to 5% of stems affected	2
Moderate lesions that are becoming dis-figuring; commercially might lead to down-grading	3	Up to 10% of stems affected	3
Severe rust with many lesions, some cracks across stems or across the keel of the stem, very disfiguring; flowers un-marketable	4	Up to 50% of stems affected	4
Very severe rust with very obvious cracking and stem bending; flowers un-marketable	5	Up to 100% of stems affected	5

In 2015 the incidence and severity of rust lesions was also assessed on leaves (14–16 May). From each plot ten clumps of ten healthy, full-sized leaves were removed by cutting at ground level at intervals along each plot (at Fourburrow it was difficult to exclude all leaves affected by white mould because of the extent of the disease at that site). The samples were loosely enclosed in polythene bags, kept out of the sun, and examined within a few hours. The incidence of leaf rust lesions was assessed as for stem rust (see Table 1) and severity was assessed using an adaptation of the stem rust severity score that took account of the wide variation of symptoms on leaves and the absence of a criterion of unacceptable appearance such as exists for cut-flowers for market (Table 2). At the same time root growth was checked by digging out the soil one spit deep at one end of each plot.

Table 2. Rust severity scores as adapted for recording (a) leaf lesions in the field crop assessment and (b) stem lesions in the survey of commercial bunches.

Severity	Scores	
	(a)	(b)
None seen	0	0
Slight markings or blistering that may not be rust-coloured, and/or one to three inconspicuous, small but typical rust spots	1	1
Small groups of typical rust spots (say, three to ten), one or two rust-like streaks/blotches, and/or larger groups of inconspicuous spots	2	2
Larger groups of conspicuous rust spots (>10) and/or rust-like streaks/blotches	3	3[1]
		4[2]
As above, but spreading along a significant proportion of the stem or leaf and becoming disfiguring	4	5
Many conspicuous rust spots and/or rust-like streaks/blotches along a significant proportion of the stem or leaf, clearly disfiguring	5	6

[1] Stems would probably be commercially acceptable

[2] Stems would probably not be commercially acceptable

Relationships between levels of rust, SWC and other factors

Findings for the first two years of the project were reported in the final report for project BOF 076. They suggested an association between high SWC in the preceding winter months and high levels of rust. Once data from the third-year of the crop has been analysed, multiple regression analysis will be used to explore the relationships of the whole data-set.

Examination of stems for early-stage lesions

Preliminary observations indicated that putative rust lesions may begin to develop on the stem before it is exposed above ground, which may have implications for the understanding of the onset of the disorder. By arrangement with one of the participating growers and using a commercial stock of daffodil ‘Golden Ducat’ growing adjacent to one of the trial plots, random ten- to fifteen-plant samples were dug-up in mid-November 2014 and then at 2- to 4-week intervals and sent to Spalding for examination. Excluding any bulbs damaged by bulb rots or large narcissus fly, ten intact stems were dissected out from each sample. They were examined under a hand-lens for the presence of putative early-stage rust lesions in the three regions of the stem – the white part within in the bulb, the yellowish part growing through the soil, and the green above-ground part.

Survey of commercial cut-flowers

Although some information was gathered in 2002, 2003 and 2011–2013 on the extent of rust occurrence in commercial crops in England and Wales (see annual or final report on project BOF 076), this was based on sending survey forms to growers and the response rates elicited were variable. In order to update this information, samples of commercial bunches were obtained from England and Scotland through the help of four grower-packers in the 2015 flower season. An important goal was to determine how widespread rust is in 'Golden Ducat' but also in other, 'non-rust-prone' cultivars.

For 'Golden Ducat', the grower-packers were each asked to provide, as far as practical, samples of three sendings (usually three picking dates from one crop) from each of five sources (usually five separate growers), making 15 samples for each grower-packer and 60 samples in all. Not all growers were able to provide three sendings from each crop, and sample numbers were made up by crops from distinct fields or from other growers. Each sample consisted of five bunches of at least ten stems, and all samples were to be taken in a random fashion without regard to their rust status or the rust status of the bulb stocks from which they came. The grower-packers were asked to provide picking date and county of origin for each sample. After transport the samples were collected locally after interim cold storage.

For 'non-rust-prone' cultivars, an equivalent set of random samples was to be taken as described above for 'Golden Ducat', using cultivars that could be picked around the same dates as the 'Golden Ducat' and which they did not consider to be rust-prone. Because of differences in growing practices and the cultivars grown in the regions it was not practical to select a singe cultivar to provide all these samples (they were made up of 18 different cultivars in all and included 18 samples of 'Standard Value', ten of 'Lowan' and seven of 'Carlton').

Each bunch was examined individually, recording its GS (usually 3.2 to 3.3) and its freedom from rust lesions or extent of rust lesions where present. The severity of rust lesions was expressed as a score based on the categories shown at (b) in Table 2, with category 3 split into 3- and 3+ to denote a boundary between damage that was serious but "acceptable" or "not acceptable". In a few cases the bunches received contained 15 or 18 stems, in which case all were examined and the counts scaled to the equivalent of a ten-stem bunch. Data were averaged across the five bunches to give sample means.

Results

Crop and rust assessments

The results from 2013 and 2014 were given in the final report on project BOF 076. The results for 2015 and a comparison with the previous years' data are presented here.

2015: Pre-picking stage

The first crop and rust assessment of 2015 was carried out on 14–16 February (Figure 1). As in previous years the plants at most sites were at an early stem extension stage with many buds visible; however, at the two most westerly and three most easterly sites the most advanced plants had reached GS 3.1 (straight pencils becoming clear of the foliage) so the crop was already close to an early picking stage. The results appear to confirm the previous year's finding that the effect of planting date had lessened over time.

Overall, a low level of rust was found, similar to that of the previous year at the pre-picking stage (Figure 2). However, the sites where rust first appeared were different in the three years of the study; for example, rust incidence was higher at this date at Penventon (>40 stems/plot with rust) than at the other sites this year or at the pre-picking stage in previous years.

White mould lesions, often sporulating, were seen on the plots at Rosevidney, Bodilly and Fourburrow, but they were not extensive at this stage and may have been under control through the fungicide spray programme.

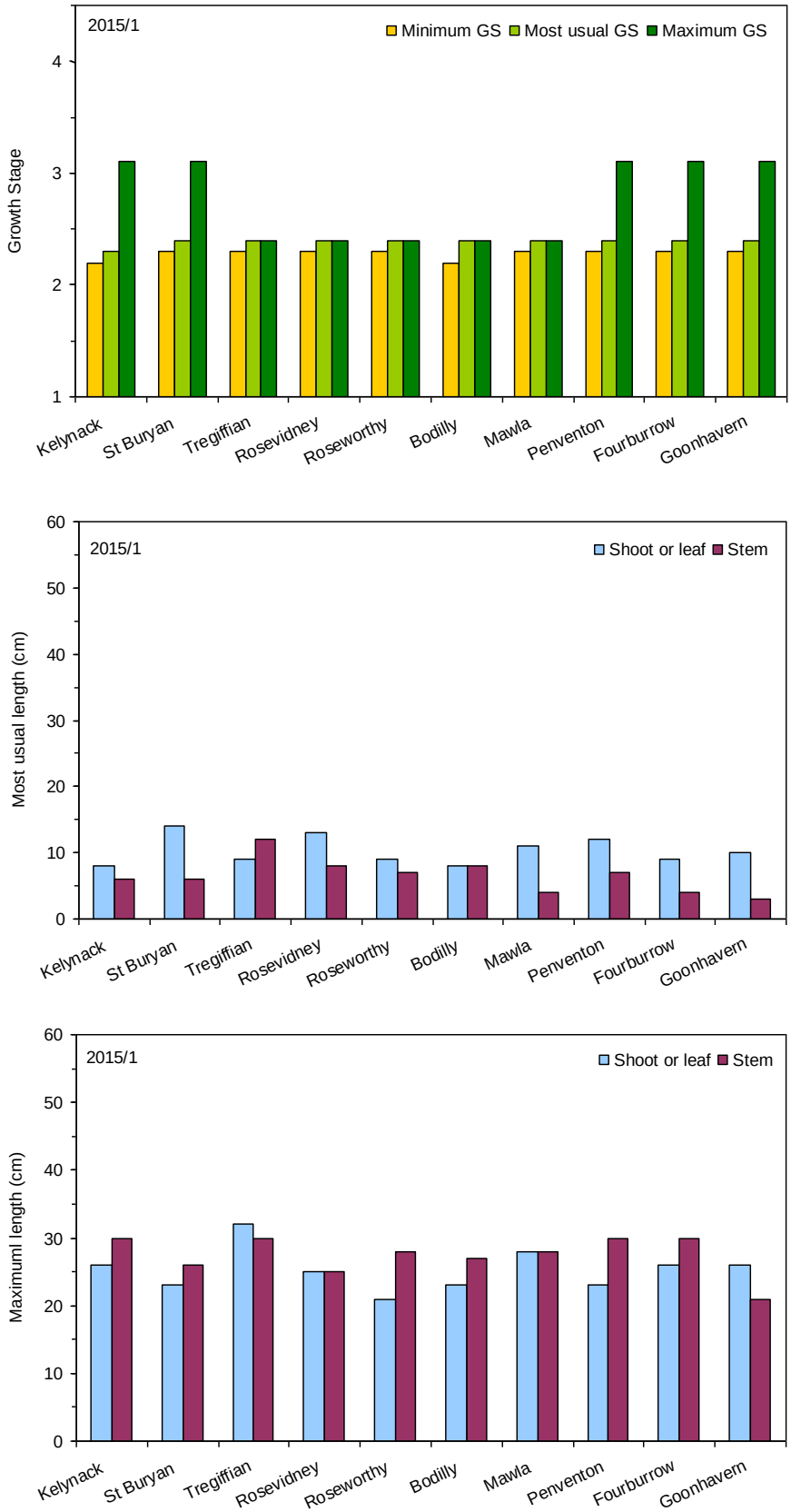

Figure 1. Crop development at ten sites assessed at pre-picking stage in 2015: (top) minimum, most usual and maximum GS, (middle) most usual shoot/leaf and stem lengths and (bottom) maximum shoot/leaf and stem lengths.

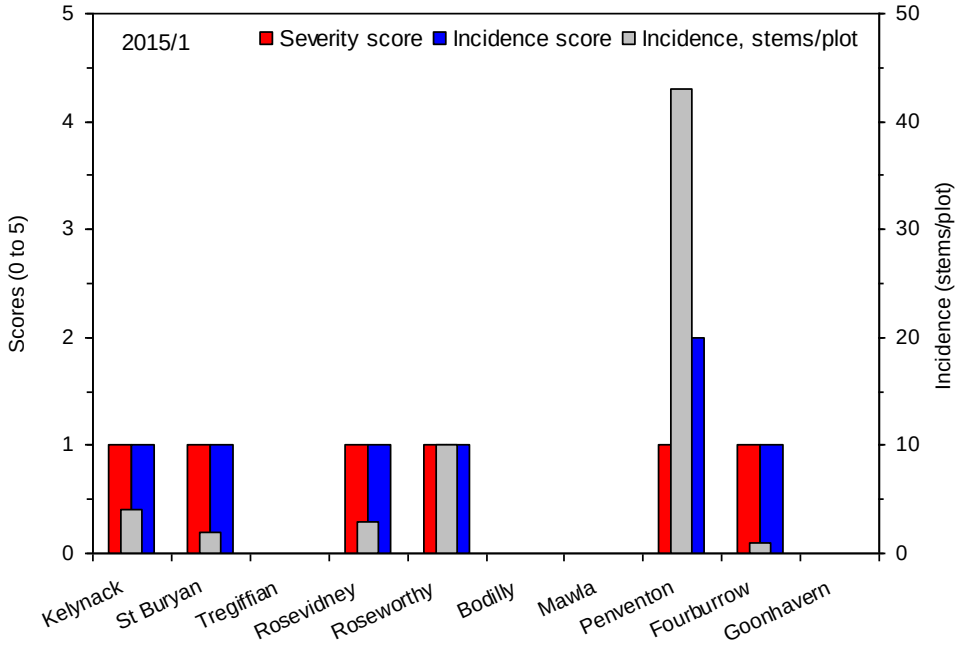

Figure 2. Severity and incidence scores for rust at ten sites assessed in 2015 at the pre-picking stage. Incidence is shown as both a 0–5 score and as the number of stems per plot with rust.

2015: Picking stage

The second assessment of 2015 was carried out over 9–12 March, when all crops were generally ready for picking although a few plants at some sites now had fully open flowers (Figure 3). Shoot/leaf and stem lengths varied across the sites – for example, plants were taller at Tregiffian and Penventon – though these two sites did not appear to have any particular characteristics in common.

Rust levels had again increased markedly since the pre-picking assessment (Figure 4). Levels were substantially greater than in 2014, and also varied between sites more than seen previously: the incidence score varied between 1 and 4, and the numbers of stems with rust between 3 and 200 per plot. In contrast, the severity score was 1 at all ten sites. Plants at St Buryan and Goonhavern had the lowest incidence of rust and levels were highest at Roseworthy and Penventon, providing potentially useful data for examining the associations between rust, weather and SWC. These low- and high-rust sites did not appear to relate to the low and high-rust sites in previous years.

White mould lesions were noted at St Buryan, Roseworthy, Goonhavern and, particularly, at Fourburrow (which had also been seriously affected by white mould in 2014), where leaves and the upper stems were becoming more seriously affected, though not so seriously as to affect the assessment of rust lesions.

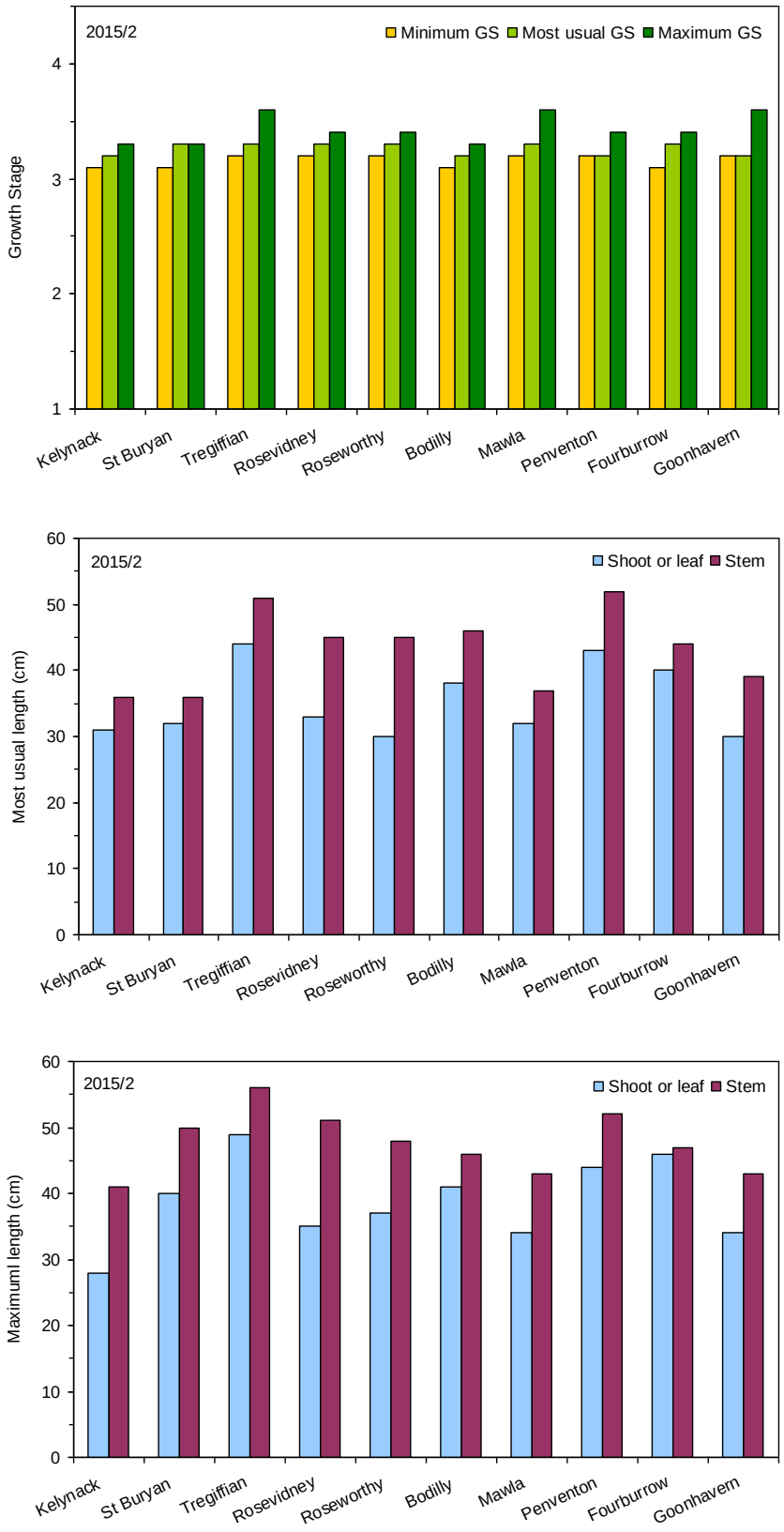

Figure 3. Crop development at ten sites assessed at picking stage in 2015: (top) minimum, most usual and maximum GS, (middle) most usual shoot/leaf and stem lengths and (bottom) maximum shoot/leaf and stem lengths.

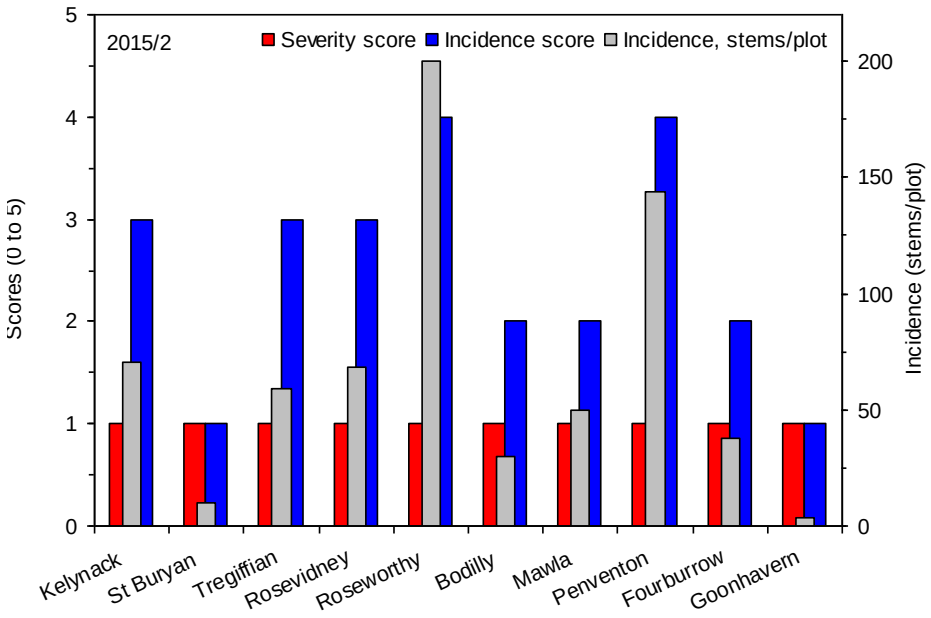

Figure 4. Severity and incidence scores for rust at ten sites assessed in 2015 at the picking stage. Incidence is shown as both a 0–5 score and as the number of stems per plot with rust.

2015: Post-picking stage

The third assessment of 2015 was carried out on 10–12 April. The developmental stage of crops was the same at all sites, GS 3.7 (flowers beginning to senesce) (Figure 5). At this time root growth was also checked. The usual rather meagre daffodil root system was evident at each site, with no site offering obviously better or poorer root systems, and the upper 10 to 30cm of soil appeared to have remained friable in all cases, with no obvious compaction (other than the capping noted at some sites).

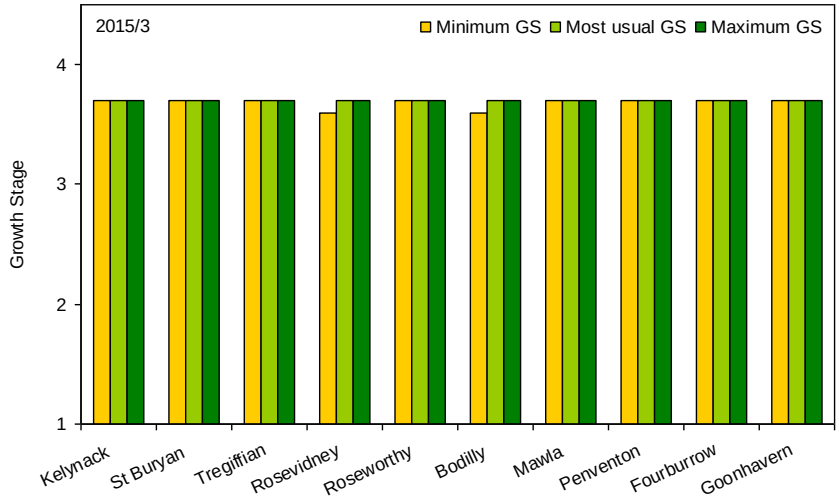

Figure 5. Crop development at ten sites assessed at post-picking stage in 2015, showing minimum, most usual and maximum GS.

Five of the sites – Kelynack, Tregiffian, Rosevidney, Penventon and Goonhavern – appeared to be free of white mould or had only small amounts. Plots at the remaining sites were seriously affected by white mould, with significant amounts of green-leaf area (GLA) and upper parts of the stems becoming affected. At Mawla the areas affected by white mould were patchy and could be avoided in assessing rust. At Fourburrow the main effect of the serious white mould attacks of 2014 and 2015 was that few flowers were produced, but these could be assessed for rust, with the figures scaled-up to represent the equivalent of a full plot. At St Buryan, Roseworthy and Bodilly much of the GLA had been affected by white mould, though rust lesions could still be assessed against this background, and it was clear that most or all of the stems had rust lesions.

Despite the confounding effect of white mould, it was clear that the incidence of rust had again increased substantially at all sites, with overall levels being similar to those of the previous year (Figure 6). The rust incidence score varied from 2 (Tregiffian) to 5 (Kelynack, St Buryan, Roseworthy, Bodilly, Mawla, Penventon and Fourburrow), with corresponding extremes of stems per plot with rust of <100 (Tregiffian and Goonhavern) and all (Roseworthy and Bodilly) or most (>900) stems affected (St Buryan and Fourburrow). The large increase of rust at St Buryan since the previous assessment confirmed that rust symptoms can develop very quickly at times. The severity score remained at 1, except at Roseworthy and Bodilly where it had risen to 2.

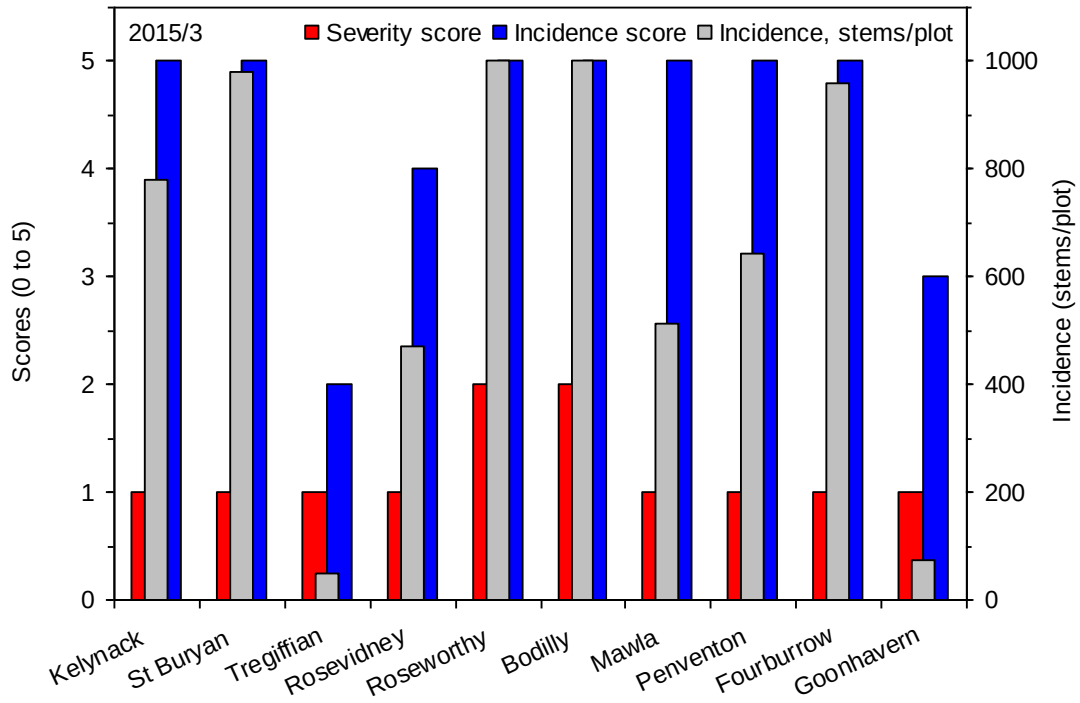

Figure 6. Severity and incidence scores for rust at ten sites assessed in 2015 at the post-picking stage. Incidence is shown as both a 0–5 score and as the number of stems per plot with rust.

Rust levels 2013 to 2015

The severity and incidence of rust are shown for all three years in Figure 7, **Figure 8** and Figure 9. To summarise generally, rust appeared in most plots, its level increased steadily through the flowering period each year, as well as between years 1 and 2. The severity of the disorder was generally low, with a score of 1 – a mild, almost unnoticeable disorder, and only in two cases did the severity score reach 3, the borderline level between a commercially insignificant effect and a crop that might give concern about its stem quality. Rust incidence, however, varied widely, from one or two affected stems per plot, up to all stems affected.

Rust severity and incidence scores are obviously linked since severity cannot be scored without incidence. Before picking in the first year, 2013, rust lesions were found only at Tregiffian. At the picking stage three weeks later rust had appeared at six sites, and subsequently at the post-picking stage only one site, Fourburrow, remained unaffected by rust. In 2014 and 2015 four and six sites, respectively, were affected by rust at the pre-picking stage, and all were affected at the subsequent assessments. The middle year saw the highest rust severity scores, with most sites having scores of 2 or even 3, the borderline of acceptability.

In 2013 rust incidence scores did not exceed 1 (up to 1% of stems affected) at the first two assessments, but by the post-picking stage scores had increased to between 2 and 4 at eight of the sites, meaning that they had up to 50% of stems affected. In 2014 more sites were affected early, and incidence scores increased faster and to a greater extent – by the post-picking stage all sites scored 4 or 5, with seven sites having up to 100% of stems affected (the exceptions being Rosevidney, Penventon and Fourburrow). However, as the incidence scores were not accompanied by high severity scores, commercially speaking there would be no concerns about loss of product quality; in any case, rust symptoms after flower picking would be of little concern to the industry, though it could indicate a problem in waiting. In 2015 the final incidence of rust was lower overall than in 2014, with Tregiffian and Goonhavern having a low incidence of rust. Figure 7 shows rust incidence as the number of stems affected with rust in each plot, and (though time-consuming) it is probably less crude than expressing incidence as a five-point scale that has to cover the full range from 0 to 100% of stems affected.

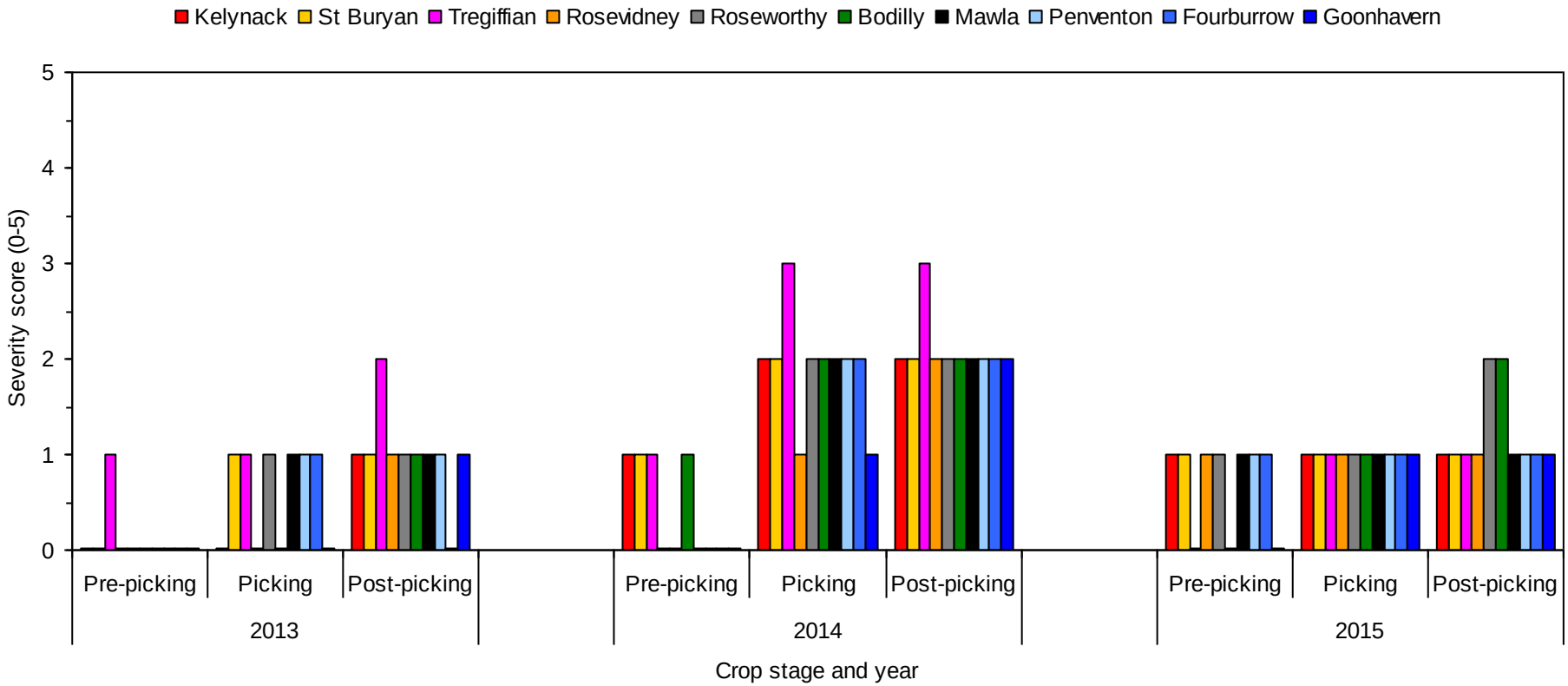

Figure 7. Severity scores for rust at the ten sites assessed in 2013, 2014 and 2015 at pre-picking, picking and post-picking stages.

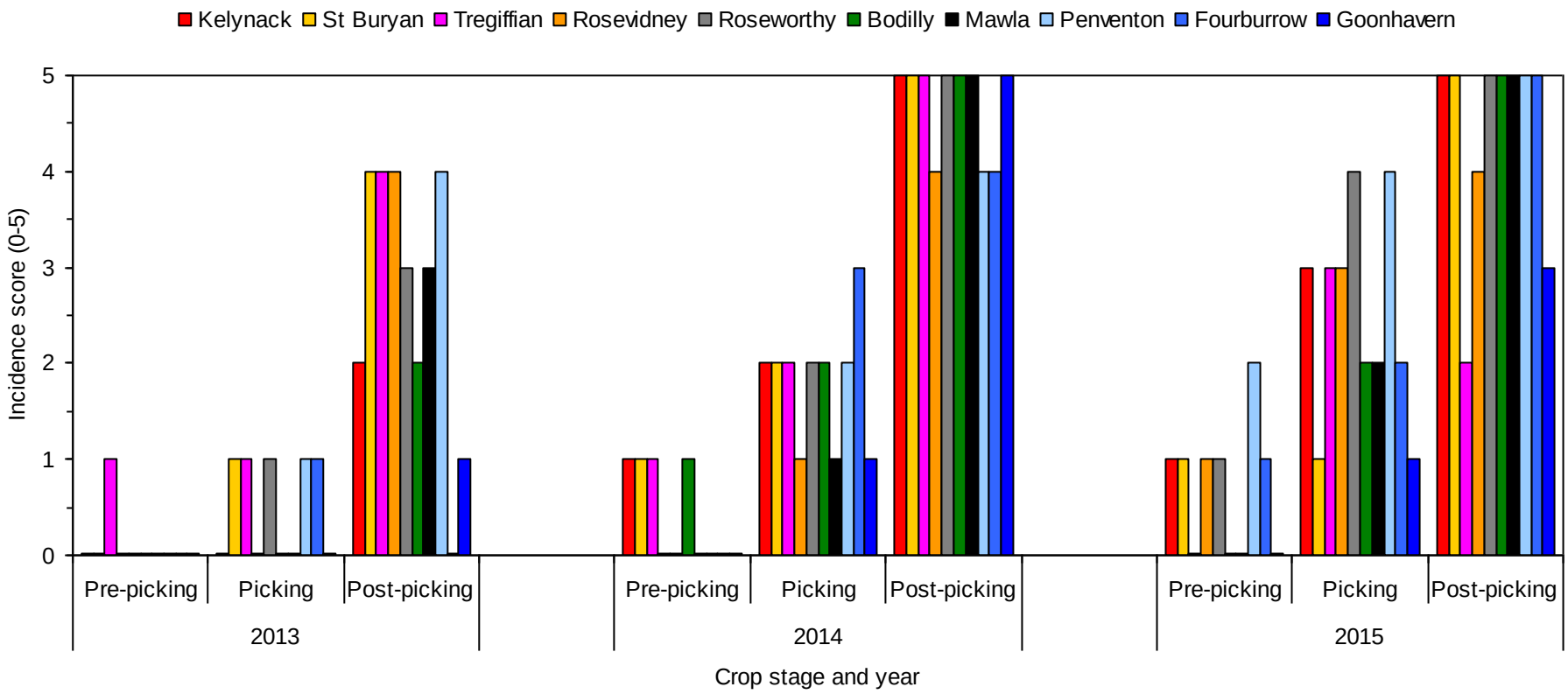

Figure 8. **Incidence scores for rust at the ten sites assessed in 2013, 2014 and 2015 at pre-picking, picking and post-picking stages.**

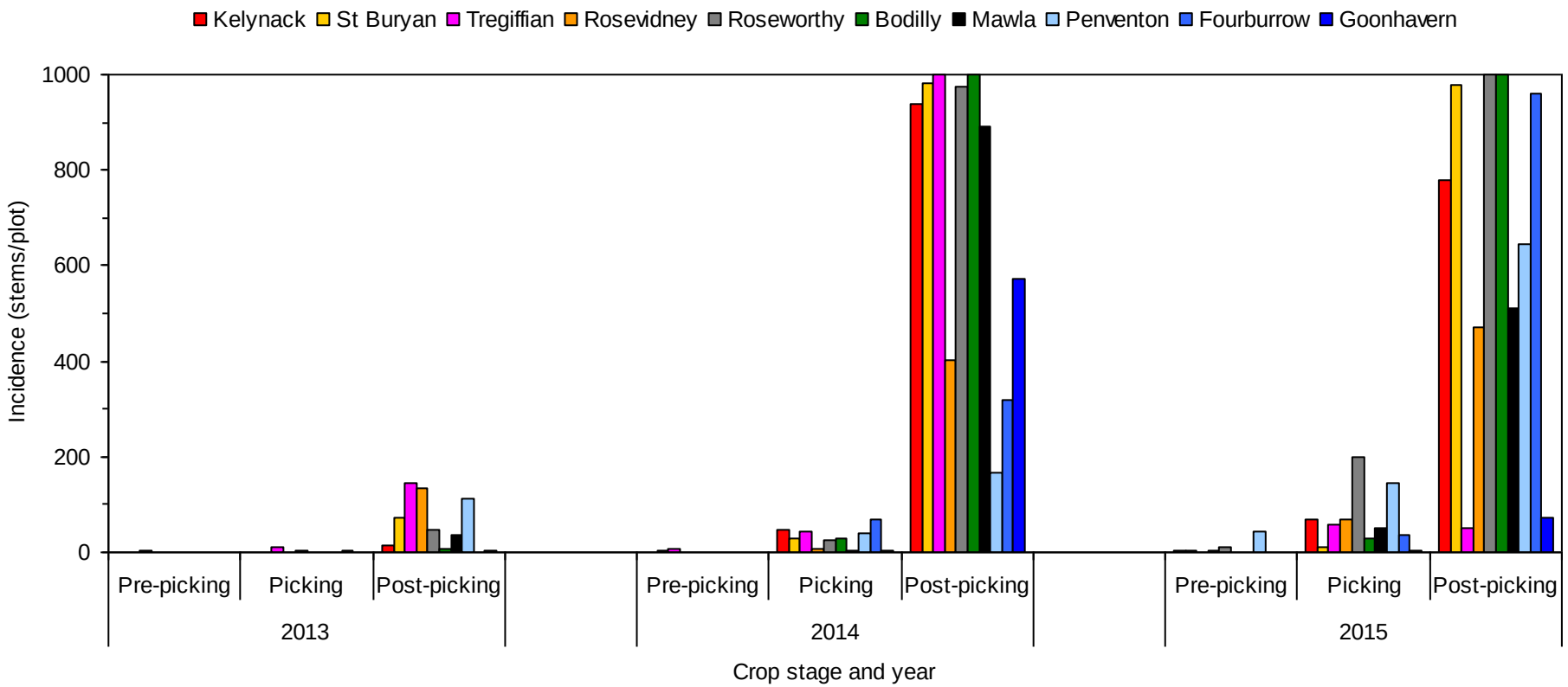

Figure 9. **Incidence (stems per plot) of rust at the ten sites assessed in 2013, 2014 and 2015 at pre-picking, picking and post-picking stages.**

Rust lesions on leaves

Rust lesions also occur on leaves, though their significance is less clear since there is no obvious effect on flower quality. In 2013 greater levels of leaf rust were seen at Kelynack, St Buryan and Tregiffian. In both 2014 and 2015 the assessment of leaf rust was made more difficult by the occurrence of white mould at many of the sites.

Over 14–16 May 2015 samples of 100 leaves per plot were scored for rust lesions. Overall, 81% of the samples were free of rust lesions, while 6, 10 and 4% of leaves received severity scores of 1, 2 or 3, respectively (no leaves merited the use of scores 4 to 6). The extremes were 61% rust-free lesions for Mawla and 94% for Goonhavern, with the sites appearing to fall within two groups: with 60–70% rust-free leaves at Mawla, St Buryan and Rosevidney, and >80% at the remaining seven sites (Figure 10). The percentage of leaves affected by rust was much lower than found in the previous year (though the methodology was different).

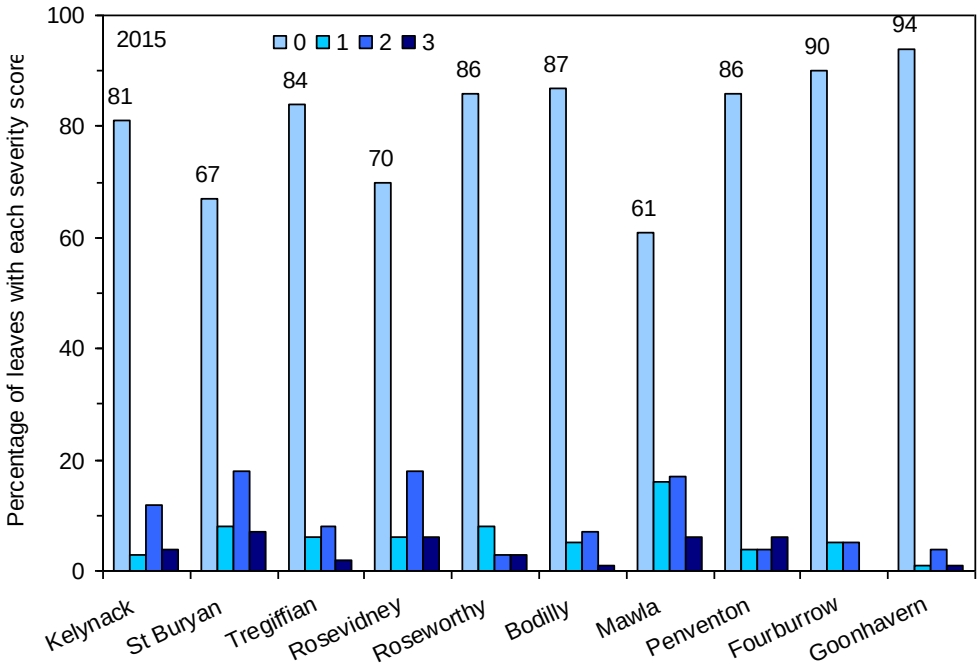

Figure 10. The incidence and severity of rust lesions on 100-leaf samples taken from the ten sites in May 2015.

Examination of stems for early-stage lesions

As well typical rust lesions, putative ‘early-stage’ lesions were often noted during regular stem assessments. They consisted of small patches or larger tracts of ‘pitting’ and depressed, paler areas on the stems as well as the ‘blistering’ previously described by Andrew Tompsett. Casual observations of these small lesions suggested that they do not develop further following picking. It might help understanding of daffodil rust if the time of first appearance of rust lesions were

known, and in February 2014 samples of bulbs were recovered from each site and the entire stem dissected out and examined (see final report on project BOF 076). Of 89 stems examined, just five showed a single, typical, small rust lesion, of which only one occurred below the green stem, i.e. in the yellowish part of the stem in the ground.

To investigate the first appearance of rust lesions further, bulbs from a stock of 'Golden Ducat' were dug from the field in 2014–2015, on 12 November (GS 1.3), 15 December (GS 2.1), 13 January (GS 2.2), 26 January (GS 2.2–2.3), 15 February (GS 2.4) and 10 March (GS 3.3). Few lesions were found when the entire stems (10 stems per sample date) were dissected out and carefully examined under a hand lens. In the 26 January sample two of the ten stems had two or three small groups of a few more or less rounded, up to 1mm diameter, dull flat lesions mid-way along the stem. In the 10 March sample typical rust lesions were found on the green (above-ground) part of the stems, and just one of the ten stems examined showed a zone of blistery irregularities about 2 x 1cm in extent part-way along the white zone of the stem (usually within the bulb). No lesions were seen on stems of the four remaining samples. More detailed observations would be needed to shed more light on these putative lesions.

Survey of commercial cut-flowers

The survey yielded 103 five-bunch samples, made up of 42 'Golden Ducat' and 61 other ('non-rust-prone') cultivars; there were 31 samples from Cornwall, 47 from Lincolnshire and Norfolk and 25 from Scotland.

The 'Golden Ducat' samples gave an average rust severity score of 1.2 from a typically wide range of values (0–3.2) and the other cultivars a notably lower average (0.3) from a similar range (0–2.7). As described under 'Materials and methods', a severity score of 0 represents an absence of rust, 1 represents a very slight (hardly noticeable) level of rust, 2 a low level of rust, 3 an increasing level of rust that is still not commercially significant, 4 a greater level of rust that may cause commercial concerns, and 5 and 6 increasingly serious levels of rust that render the product unmarketable (in this survey no stems reached a score of 5 or 6).

For rust incidence, 'Golden Ducat' averaged just over 50% of stems with rust (27.4 stems/50 stems) and the other cultivars a much lower 21% (10.7 stems/50 stems). However, these averages, both for 'Golden Ducat' and for the other cultivars, were drawn from almost the total range of possible individual values (0–50 stems/50 stems). Its higher rust severity score and incidence confirmed the rust-susceptibility of 'Golden Ducat', though the other cultivars displayed perhaps much more rust that was expected and in some cases incidences as high as with 'Golden Ducat'. The frequency plots (Figure 11) show that, while the severity scores were concentrated in

the lowest class (scores up to 0.5), for rust incidence most ‘Golden Ducat’ samples had values in the highest class (incidence of more than 40%). Most ‘Golden Ducat’ stems had at least a low level of rust, whereas this was not the case in the other cultivars.

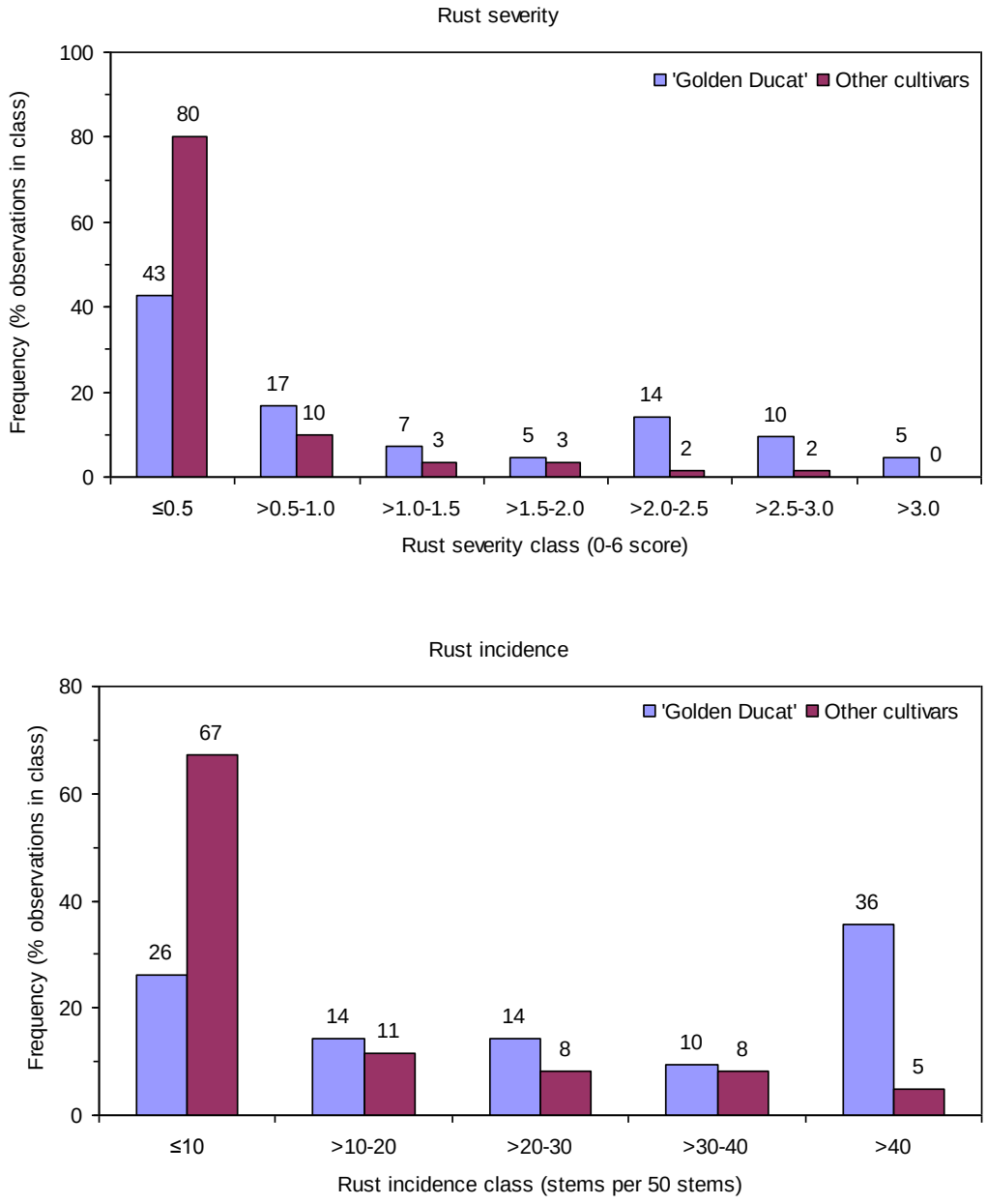

Figure 11. The frequency of rust severity scores (from 0 to 6, top) and of rust incidence (stems with rust per 50 stems, bottom) for samples of ‘Golden Ducat’ and other cultivars. The numbers above the bars are the percentage of observations in each of the classes along the horizontal axis; for example, in the top figure, reading from the left, 43% of ‘Golden Ducat’ samples and 80% of samples of other cultivars fell into severity scores up to 0.5, 17% and 10% fell into severity scores >0.5 and up to 1.0, and so on.

While the survey data were considered ample for making comparisons between ‘Golden Ducat’ and ‘non-rust-prone’ cultivars, the numbers were insufficient for making comparison either between the three regions growing daffodils or between the various cultivars in the ‘non-rust-prone’ group. However, with a good number of ‘Golden Ducat’ samples, it seemed worthwhile to analyse the severity and incidence of rust in relation to sampling (picking) dates, which ran from 9 March to 26 April. Scatter plots of severity scores and rust incidence (stems/50 stems) are shown in Figure 11. There was no clear change in rust levels as the flowering season progressed, and further analysis confirmed this, with regression coefficients (r^2) of 0.1819 (severity) and 0.2018 (incidence). The take-home lesson from the survey is the frequency – albeit at generally low severity – with which rust appears on cut-flowers in the trade.

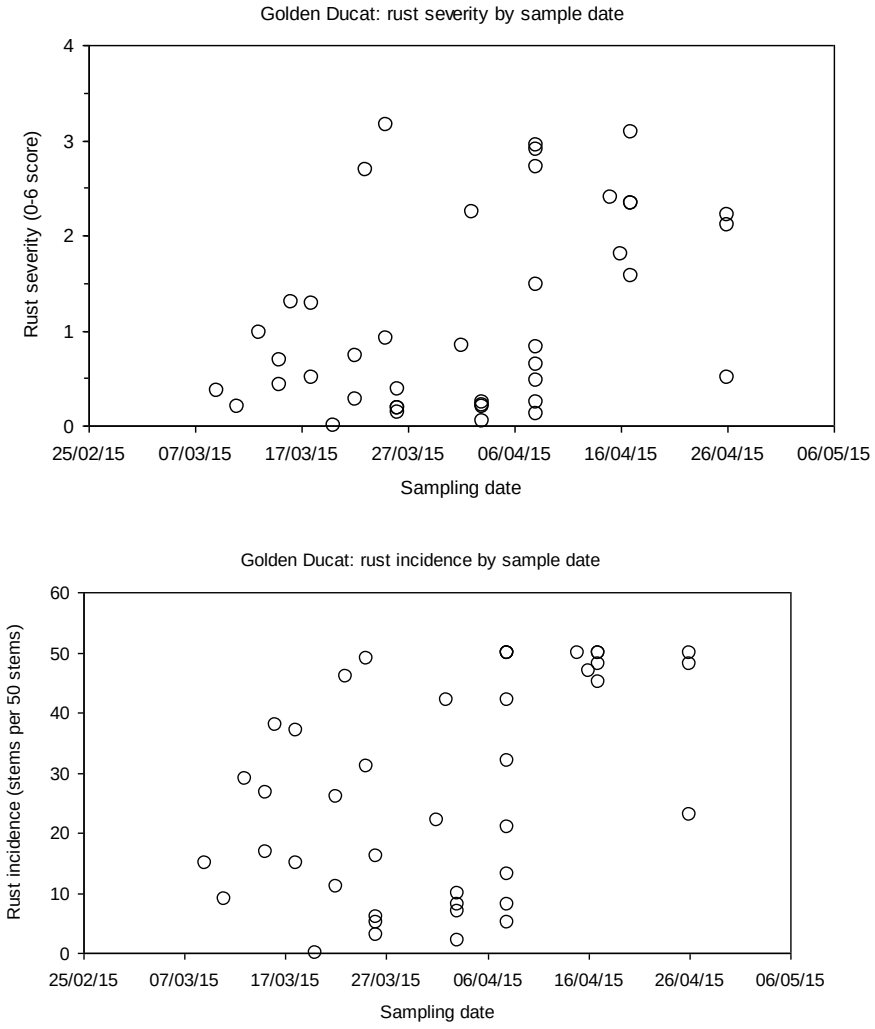

Figure 11. Scatter plots of rust severity scores (top) and rust incidence (stems with rust per 50 stems, bottom) for 42 samples of commercial ‘Golden Ducat’ stems picked between 9 March and 26 April 2015. The lack of any pattern in the data-points indicates a random distribution of rust levels across all picking dates.

Discussion

Significance of rust to the UK industry

The discussion in the final report of project BOF 076 considered the question of whether daffodil rust poses a threat to UK horticulture. It was concluded that, while the sporadic loss of quality and profit due to rust were currently relatively modest – perhaps a loss of 3% in turnover amounting to about £0.7m annually, or just over £2m in every third year – there is a danger that any increase in rust incidence or severity on bunches of UK-grown daffodils, particularly when seen by supermarket or overseas customers, could damage the esteem in which the UK product is held. Despite the commercial success of UK daffodil flowers since the 1970s, growers and traders already face a number of issues, including low returns, uncertainties in continuity of supply, structural changes in the industry and the loss of pesticides (particularly to control basal rot). Adverse publicity from daffodil rust might cause further difficulties, and from the outset it has been the intention to maintain a 'low profile' around the 'physiological disorders' projects, though it has also been necessary to accept that rust does constitute a threat and does need to be remedied.

At the start of project BOF 076 it was surprising to find that a low incidence of rust was common on most 'Golden Ducat' trial plots, and indeed some level of rust has been seen on all ten trial plots at flower picking time over the course of field-work, albeit at a low severity and, usually, a low incidence – generally far below levels that would cause a grower to downgrade a product or withdraw it from sale. From this developed the notion that perhaps rust was universally present at a low level on susceptible cultivars such as 'Golden Ducat'. And, if it were looked for, would low levels of rust be found on other cultivars not considered prone to rust? To begin looking at these questions, a survey of daffodils collected from the commercial chain was carried out in spring 2015. While it would probably be prohibitively expensive to carry out a survey on a completely statistically satisfying scale, it was considered that the survey of just over 100 five-bunch samples from Cornwall, the east of England and Scotland would, at least, be a reasonable representation.

Although the survey found few stems in need of rejection as unsuitable for sale, it was concerning that many bunches showed some rust symptoms. As expected, the severity and incidence of rust were greater in 'Golden Ducat' than in 'non-rust-prone' cultivars, but the latter nevertheless carried a surprising level of symptoms. Any stocks obviously affected by severe rust and unsuitable for the market would usually be identified by the grower in the field during regular inspections, and would be excluded from the picking programme. Once a stock is being picked, however, it is generally accepted that it would be unreasonable to expect pickers to check every stem for the more serious rust symptoms. It is evident from the survey, however, that even in stocks generally unaffected by rust, some unsuitable stems do get through to packing and sale, and this will emphasise to growers the importance of thorough pre-

picking field inspections, and of assuring that supervisors know at what level affected stems are unsuitable for market.

Towards finding the cause of rust

With one exception rust symptoms were found at all ten sites and in all three years of the project, and in almost all cases the symptoms were mild and well below a level that would have resulted in the rejection of stems on the commercial grounds of poor quality. In general terms rust severity was always low, while the incidence of rust was low at flowering in 2013 and notably higher in 2014 and 2015; there was, however, no consistency between years in the sites that had low or high rust levels. Following the first year, examination of SWC, weather and other variables for any associations with rust levels revealed that sites with high SWC in the months preceding flowering usually had high levels of rust. Rainfall in winter 2012–2013 had been high, but the following winter was wetter, with widespread flooding. Despite this different weather pattern, rust levels around flowering in 2014 confirmed the possible link between high winter SWC and high rust levels. Winter 2014–2015 was more 'normal', and data analysis over the next months will show whether the relationship between high SWC and high rust incidence has held for a third year. The lack of consistency over the three years as to which sites are rust-prone and which are resilient is challenging, giving no clue on the topography, soil type, etc., that might favour rust development.

None of the other meteorological, soil, geographical or husbandry factors recorded showed any association with rust levels. Of particular interest was the lack of clear effects on rust due to the concentrations of major soil nutrients or trace elements, since a nutritional factor had been previously suggested as a possible cause of rust. A pathological cause had also been suggested, so of equal interest were the preliminary findings regarding bacterial and fungal pathogens and their association with rust lesions. A bacterial cause has been ruled out, but a fungal pathogen, a *Stemphylium* species, not before reported from daffodils, was recovered from rust lesions.

Rust symptomatology

Despite the information gained on the incidence and severity of rust on daffodil stems as a result of this project, little is known of the overall development of the disorder. On the assumption that they are not already present on the stem when it emerges from the ground and from its enveloping leaves, the typical lesions become apparent in the period between stem emergence and the post-flowering stage – a brief window of a few weeks in which rust lesions develop, in some cases sufficiently and rapidly enough to degrade the appearance of the stem. Putative early-stage rust lesions – often appearing as inconspicuous 'blistering' but perhaps more variable in form - are easily seen on daffodil stems during the same period, and also, infrequently (perhaps because they have not been looked for), on the part of the

stem that is still enclosed underground at the same time as the upper portion is extending towards anthesis. In this project a number of early-stage lesions on stems in one of the experimental plots were marked for further observations, but all were lost due to unauthorised flower picking. Limited post-picking observations of typical lesions have shown that little further development appears to take place in storage or in the vase. What is the trigger that can lead to rapid lesion development? This period of rapid development needs further research.

At this point any relationship between typical rust lesions and the larger, irregular brown blotches and streaks that appear on stems and leaves, to the physiological disorder known as chocolate spot, or to rust lesions on leaves - none of which apparently cause any concerns to growers - are unknown. Microscopy of chocolate spot lesions has reportedly shown small patches of dead surface cells, but this has not been carried out to any extent on rust lesions.

Rust management

At this stage little guidance can be given on the management or control of rust. If the effect of SWC is confirmed, risk-avoidance (such as avoiding unsuitable wet sites and replacing rust-prone cultivars) may be the chief answer. Knowing the environmental cause(s) of rust would at least lead to better risk-assessment regarding suitable sites for rust-prone cultivars. On the other hand, some practical steps might be worthwhile, such as attention to improving drainage in potentially waterlogged fields or low-lying parts of fields by digging temporary ditches or running tines along the furrows before the winter rains.

The bunch survey demonstrated the widespread, if mild, incidence of typical rust lesions on daffodils in the supply chain – and not only in ‘Golden Ducat’. More vigilance may be needed on the part of growers to ensure that a watch is kept for stems with disfiguring rust lesions.

Technology Transfer

Hanks, G, Collier, R & Jones, J (2013). *Update on project BOF 76: daffodil rust.* Presentation to the HDC/BDGA Narcissus Growers Meeting, Redruth, UK, 4 December 2013.

Hanks, G, Collier, R & Jones, J (2013). *Update on project BOF 76: daffodil rust.* Presentation to the HDC/BDGA Narcissus Growers Meeting, Spalding, UK, 11 December 2013.

Hanks, GR (2014). The enigma of daffodil rust. *HDC News*, no.199, 20-21.

Hanks, GR (2014). *Review of HDC-funded daffodil projects.* Presentation to the South Holland Growers Club, Holbeach, UK, 17 February 2014.

Hanks, G.R. (2014). Soil water may have link to daffodil rust. *Ornamentals Review 2014* (HDC News supplement), 19.

Acknowledgements

The project leader thanks the growers and traders who hosted trial plots, provided samples, facilities and ideas, allowed access to their analytical and diagnostic data, and provided samples for use in the survey of commercial bunches.

APPENDIX: daffodil growth stages

Table 3. A scale of growth stages (GS) for daffodils

Period	GS	Description	Notes
Unplanted bulb (GS 0)	**0.1**	**'Dormant' bulb in storage**	**Bulbs would normally be planted at GS 0.1 or 0.2**
	0.2	**Root initial development evident close to the surface of the bulb**	
	0.3	**Shoot and/or roots emerging from stored bulb**	**Apply only to stored bulbs**
	0.4	**Bulb becoming desiccated with loss of skin, emerging roots or shoots becoming moribund**	
	0.5	**Bulb shrivelled, light in weight, or rotted**	
Planted bulb (GS 1)	**1.1**	**No clear emergence of shoot and/or roots**	
	1.2	**Roots and/or shoot emerging, <1cm in length**	
	1.3	**Roots and shoot elongating**	
	1.4	**Shoot tip close to soil surface**	
Emergence (GS 2)	**2.1**	**First shoots starting to emerge**	**Foliage height nominally 0**
	2.2	**Shoots elongating, but no buds obviously visible**	**Record foliage height (and stem height for 2.3 and 2.4) 2**
	2.3	**Shoots elongating, tips of flower buds visible without pulling shoots apart**	

	2.4	**Full length of buds visible ('upright pencils')**	
Anthesis (GS 3) 3	**3.1**	**Flower buds still 'upright pencils' with no colour showing, but becoming clear of the foliage; flower cropping could have begun if a very tight stage is required and stem length is adequate**	**Record foliage and stem heights**
	3.2	**Flower buds are 'fat pencils' with no colour showing, flower cropping should have begun**	**Record stem height**
	3.3	**Pedicels bending and/or spathes splitting, colour may be showing; a very late picking stage**	
	3.4	**Pedicels fully 'goose-necked' but flowers not open**	**This stage may pass quickly and variably**
	3.5	**Flowers (or florets) starting to open**	
	3.6	**Flowers fully open**	**For multi-headed types, 50% of florets open, senescing or senescent**
	3.7	**Flowers at least starting to senesce (petal tips dying) but not fully senescent**	
	3.8	**Flowers (or florets) fully senescent, leaves still fully green and upright**	
Post-flowering (GS 4)	**4.1**	**Leaves still fully green, but at least some leaves starting to bend to ground**	
	4.2	**As 4.1, but some leaves bending conspicuously and at least some leaves with senescent (yellowing and dying) tips**	

	4.3	**Most leaves almost flat, with general incidence of senescence at the leaf ends**
	4.4	**Some 50% of leaf area senescent**
	4.5	**Less than 10% leaf area remaining green**
	4.6	**None (or a trace) of leaf area remaining green**
'Summer dormancy' **(GS 5)**	**5.1**	**Small amounts of green foliage remaining attached to bulbs**
	5.2	**Any foliage attached to the bulbs now dead**
	5.3	**Dead foliage lost or removed**
Lifted bulb **(GS 6)**	**6.1**	**Bulb surface damp and/or not cleaned**
	6.2	**'First stage' drying (surface drying) complete**
	6.3	**'Second stage' drying complete**
	6.4	**Bulbs cleaned (and graded if appropriate)**

[1] Avoid the following when recording: plot or row ends; obvious rogues, off-types and atypically damaged/diseased plants; late flowers from lateral bulbs; and the most advanced plants if these are about 1% or less of the total.

[2] Record shoot height from the point of emergence from the soil to the uppermost tip of foliage, and stem height from the point of emergence from the soil to the topmost tip if the bud, spathe or flower.

[3] If flowers cropped and no remnants left to estimate exact GS, record as '3.C' (cropped).

