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Project leader:	Gordon Hanks
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The results and conclusions in this report are based on investigations conducted over a three-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

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.

SCIENCE SECTION

Introduction

Since the early-1990s daffodil growers in the UK have been concerned over rust-like lesions that were sometimes found on the flower stems of some cultivars. Since the condition did not appear to be caused by an obvious pathogen, it became known as ‘physiological rust’, and later ‘stem rust’ or simply ‘daffodil rust’. The symptoms were very often insignificant, but in severe cases took the form of prominent rust-coloured lesions along the stem, sometimes accompanied by transverse cracks across the keel, the stem becoming increasingly unsightly and brittle and rendering the product unmarketable. In 2002–2003 the erstwhile HDC Bulbs and Outdoor Flowers (BOF) Panel instigated surveys of growers to establish the economic importance of daffodil rust, seek possible relationships between cultural practices and the onset of the condition and collect the industry’s ideas about the factors that predispose crops to rust. The survey was repeated over 2011–2013 and confirmed that rust was continuing to cause occasional but substantial losses in product quality and output. The data from all five years of the survey were summarised as Appendix 2 of the final report on project BOF 076 in 2015. Since these surveys were completed growers have continued to report that rust outbreaks have continued to occur sporadically. Perhaps the only predictable feature of daffodil rust is its unpredictability.

As suggested above, the cause of daffodil rust is unknown. Examinations of affected stems by growers and advisors failed to find either a pathogen associated with the lesions or any obvious association between soil nutrient levels and the occurrence of rust. A pathogen- or nutrition-based explanation of rust was therefore largely ruled out, though it could be argued that the diagnostic and analytical testing done had been carried out in an opportunistic, *ad hoc* way, and that more systematic testing was needed. Anecdotal information circulating in the industry suggested that rust developed following specific weather or ground conditions, such as during rapid crop growth following a cold, frosty period, or mainly in crops growing in waterlogged areas. At the outset of the project 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 have also failed to reveal references to daffodil rust.

Since the triggers for rust development were not known, it was not practical to simulate rust-inducing conditions and take an experimental approach. Therefore 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 the development of rust lesions, the occurrence of pathogens and soil and plant nutrient concentrations. Plots of a rust-prone daffodil cultivar, ‘Golden Ducat’, were planted on ten sites representing a range of topographies and soil conditions across west Cornwall, the bulb-growing area where rust appeared most likely to develop. At each site loggers were set up to record meteorological and soil water content (SWC) data. Crop growth and the incidence and severity of rust were recorded with a view to seeking any associations with SWC and meteorological data. Soil and plant nutrient concentrations were measured and plant samples examined for lesion-associated pathogens.

Standard plots of bulbs were planted in autumn 2012 and the project covered the first and second years of the crops (2012–2014). Rust occurred at all sites in both years and its incidence and severity varied between the sites. Rust levels were low in the first flowering season and considerably higher in the second, but even in the second year rarely approached the incidence or severity needed to cause concern in commercial cut-flower production. These simple findings changed the thinking on rust, from being a disorder that appeared only from time to time, to one that was routinely present in daffodil crops at a low level but which occasionally developed into a significant outbreak. At that point it was tentatively concluded that (a) higher rates of rust were associated with higher SWC in the previous winter months, (b) there were no associations between rust levels and soil or plant nutrient concentrations, nor soil type, topography, etc., and (c) a fungal pathogen not previously reported on daffodils was associated with typical rust lesions. In 2014 a year’s project extension (BOF 076a) was funded so that the experimental plots could be studied over the typical three-year crop cycle used in commerce. This provided an additional data-set, an important factor when considering an unpredictable problem, and especially so at a time of atypical weather patterns.

In addition to extending the main object of the investigation, the project extension added three elements designed to investigate the involvement of viruses in rust, extend knowledge of rust symptomatology and gauge the prevalence of rust in commercial cut-flowers:

- RNA extraction and sequencing from stems and lesions to investigate whether there is any association between virus infection and the development of rust;
- Examination of stems from before shoot emergence to determine the earliest time that rust lesions appear;

- Surveying commercial cut-flowers to assess the frequency of rust in ‘Golden Ducat’ and other cultivars in the wider industry.

In order to provide a complete account of the daffodil rust project, the present document summarises all of the work carried out under projects BOF 076 and 076a, though the earlier reports may need to be consulted to get complete sets of results.

Materials and methods

Objective and strategy

The occurrence of daffodil rust is unpredictable. The aim of the project 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 rust.

Daffodils were planted at a number of sites and the levels of rust and aspects of the weather and soil-water environment were recorded, seeking any associations between rust levels and environmental factors. The field-work was structured so as 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, the plants were grown for three years to ‘capture’ a range of weather patterns, 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) daffodil crops seem most prone to rust. The ten plots were also used as sources of ‘rusty’ and ‘healthy’ plant material for disease diagnostics (fungal, bacterial and viral) and of soil and plant samples for measuring the concentrations of nutrients.

Bulb material

Following consultation with the HDC Industry Representative and others, a suitable Cornish 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 consisted of *ca* 1,000 bulbs. For convenience the results are usually quoted on a ‘per plot’ basis, effectively meaning ‘per 1,000 bulbs planted’.

Trial sites

Ten commercial daffodil fields were selected as test sites following discussion with bulb growers, taking into account the requirements to locate them throughout west Cornwall and include varied soil types, topography, husbandry, etc. Throughout this report the sites are listed in a standard, west-to-east order.

Figure **1** shows the location of the sites. The sites are illustrated in Figure 2 and details of their location, topography and aspect are provided in Table 1. Information on pre-planting soil analyses, supplied by the growers, is given in Table 2, and previous cropping, fertiliser and lime applications and dates of bulb planting in Table 3. Grower assessments of soil texture, soil information from the Soil Map of England and Wales (Soil Survey of England and Wales, 1983) and other soil quality assessments are provided in Table 4. No subsequent soil analyses or fertiliser applications were carried out by the growers at the sites during the course of the project.

Crop 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 each *ca* 30m long). The inter-ridge distance (centre-to-centre) varied according to the growers' usual practices, between 0.76 and 1.06m. A typical arrangement would be to plant ridges in pairs at 0.76m centres between tractor wheelings (centre-to-centre) of 1.82m, giving an inter-ridge distance of either 0.76m between the ridge-pairs or 1.06m between adjacent ridges separated by a wheeling. This gave a planting density of *ca* 14t/ha with 20m-long plots and *ca* 9t/ha with 30m-long plots. Due to the prolonged and exceptionally wet autumn of 2012, bulb planting at some sites was delayed until the advent of better conditions, so planting dates ranged from 12 September to 5 November 2012 (Table 3).

Bulb planting and subsequent husbandry at the sites followed each grower's usual practice, although it was asked that flowers were not picked but left *in situ* since without this the full development of any rust symptoms could not be assessed. The trial plots were marked (with posts and high-visibility tape) to emphasise this requirement.[1] These markers also served to draw tractor drivers' attention to the location of the plots and monitoring stations.[2] Each grower was asked to provide details of sprays applied and other relevant field operations carried out.

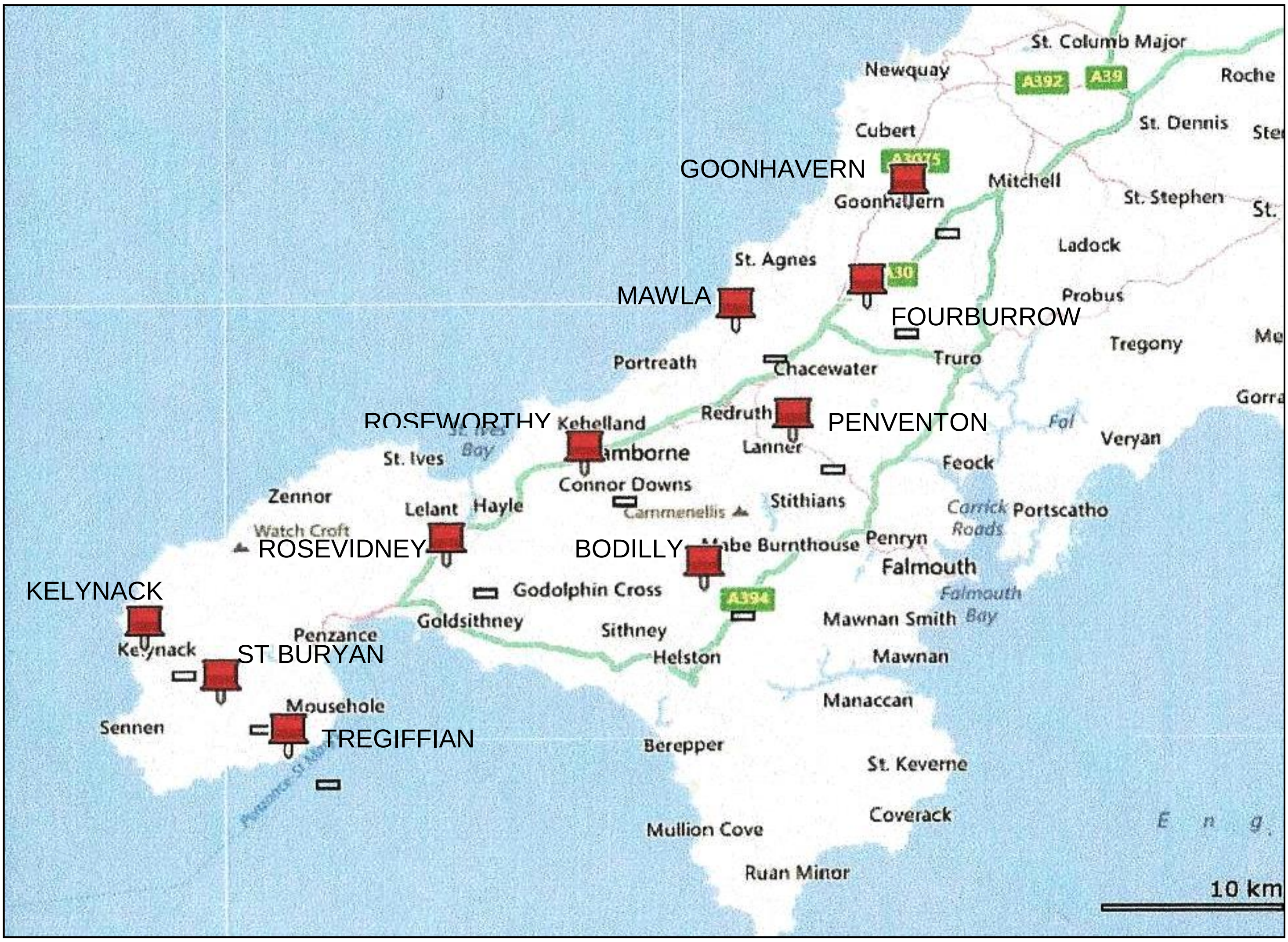

Figure 1. Locations of the ten experimental plots of 'Golden Ducat', indicated by red pins and place names in block capitals (the site names used reflected current usage locally and were not necessarily definitive or unique)

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[1] This was broadly successful, though a better protocol for future work might be to have the plots surrounded by varieties that flower substantially earlier or later than 'Golden Ducat', so that pickers are unlikely to be on-site while the 'Golden Ducat' plots are flowering. In the few instances where some flowers were taken the number of cut-stems was recorded and used to scale-up the results to take account of it.

[2] Despite this, at one site one-half of one ridge, and at a second site 1m at one end of each ridge, were damaged by vehicles 'cutting corners'; these damaged portions of plots were not assessed but counts were simply scaled-up to take account of it.

Kelynack

St Buryan

Tregiffian

Rosevidney

Roseworthy

Bodilly

Figure 2. The ten sites of ‘Golden Ducat’ plots (continued on next page)

Mawla Penventon

Fourburrow Goonhavern

Figure 2. (continued)

Table 1. Location, elevation and aspect of the sites (listed in standard west to east order)

Site reference and name	O.S. grid reference	Latitude (°)	Longitude (°)	Elevation (m)	Aspect, shelter, plot position and drainage
A Kelynack	SW 36413 29971	50.111180	-5.688128	107	In higher but ±level part of SW-facing field. Close to sea (0.7km). Plot situated at end of outside rows oriented E-W. Water easily pools in furrows here, exacerbated by adjacent heavy compaction from vehicle movements. No shelter except nearby hedgerows to north and west.
B St Buryan	SW 40491 26646	50.083135	-5.628959	121	Slightly undulating site, plot at lower end of field sloping gently to E. Plot situated at end of middle row oriented E-W. Furrows very liable to water-logging. No shelter.
C Tregiffian	SW 44071 23250	50.054204	-5.576742	73	In almost lowest part of S-facing field, near cliff edge (0.2km from sea). Plot situated at middle of outside headland rows oriented E-W. Drainage across ridges but satisfactory. Large, very exposed field: no shelter.
D Rosevidney	SW 53346 33870	50.153462	-5.454283	77	In lower corner of gently S-sloping site. Plot situated at end of outside headland rows oriented SW-NE. Water can pool in furrows here. Sheltered on SW and SE by hedgerows.
E Roseworthy	SW 61155 38796	50.200885	-5.348260	77	At higher end of site sloping gently N. Water can sometimes pool in furrows here. Open, exposed site

						away from hedgerows. Plot situated at end of middle rows oriented N-S.
F	Bodilly	SW 67519 31844	50.140991	-5.254984	138	In lower part of gently SW-sloping field. Water can sometimes pool in furrows here. Some broken shelter (trees and buildings) to W. Plot situated at end of outside rows oriented SW-NE.
G	Mawla	SW 69899 46588	50.274311	-5.230672	102	At higher end of field on NW-SE sloping ridge. Water can easily pool in furrows here, exacerbated by adjacent heavy compaction from vehicle movements. Some shelter from S and W (hedgerow). Old mining area. Plot situated at end of outside rows oriented SW-NE.
H	Penventon	SW 72769 40130	50.217433	-5.186588	91	Plot at top of steep SE slope, draining down well though sometimes pooling seen in furrows of plot. Broken shelter (hedgerow) to NW. Plot situated at end of middle rows oriented SE-NW.
I	Fourburrow	SW 77193 47636	50.286518	-5.129086	96	SW-facing steep slope, large, very exposed field. Plot situated at middle of outside rows oriented NE-SW. Drainage satisfactory.
J	Goonhavern	SW 79638 53300	50.338305	-5.098118	83	In low corner of field on W-facing slope. Some shelter (trees) except on S. Plot situated at end of outside rows oriented E-W. Adjacent compaction and a

tendency for water to pool in furrows.

Table 2. Soil chemical analysis for August 2012 (provided by growers)

Site reference and name		pH	Nutrient index		
			P	K	Mg
A	Kelynack	6.3	4	2+	2
B	St Buryan	5.5	3	2+	3
C	Tregiffian	6.2	3	1	3
D	Rosevidney	6.6	3	2-	2
E	Roseworthy	n/a	n/a	n/a	n/a
F	Bodilly	5.9	3	1	2
G	Mawla	7.3	4	3	2
H	Penventon[1]	-	-	-	-
I	Fourburrow	n/a	n/a	n/a	n/a
J	Goonhavern	5.4	0	1	2

[1] Soil analysis not done at this site
n/a = information not available

Table 3. Previous cropping and fertilizer and lime application for the sites (provided by growers)

Site reference and name		Last crop and previous brassicas or grass (if any)	History of organic fertiliser	Fertiliser applied in 2012	Lime applied in 2012	Planting date (2012)
A	Kelynack	1-year ley, silage taken Previously brassicas	No	300kg/ha sulphate of potash	None	25 Sep
B	St Buryan	Potatoes Previously brassica	No	450kg/ha 0:11:34	7.2t/ha	5 Oct
C	Tregiffian	Long-term pasture	No	450kg/ha 0:11:34	3.5t/ha	25 Sep
D	Rosevidney	Spring barley Previously brassicas	No	450kg/ha 0:11:34	None	20 Oct
E	Roseworthy	Brassicas for last 3 years	No	500kg/ha 5:10:30[1]	na	12 Sep
F	Bodilly	Barley Previously brassicas	No	500kg/ha 0:11:34	4.8t/ha	5 Nov
G	Mawla	Barley Previously brassicas	No	450kg/ha 0:11:34	None	5 Nov
H	Penventon	3 to 4-year ley, mainly grazed, some silage taken	FYM, cattle	None	None	28 Sep
I	Fourburrow	Winter wheat	No	600kg/ha 0:18:36	n/a	17 Sep
J	Goonhavern	Brassicas	No	400kg/ha 0:11:34	9.1t/ha	1 Nov

[1] The N applied at this site was unlikely to have been necessary due to the presence of brassica residues
n/a = information not available

Table 4. Soil texture assessments and soil descriptions (assessed October 2012), drainage (assessed February 2014) and soil associations for the sites

Site reference and name		Soil texture[1]	Soil texture[2]	VSS QA[3]	Observations on soil, surface state, horizons and drainage	Soil association[4]
A	Kelynack	Sandy silt loam	Loamy fine sand	1.00	Surface: many stones and rock chips up to 15cm across, occasionally more 0-25cm: uniform, friable dark brown soil with small stones 25-45cm: becoming increasingly compacted, lighter in colour, clayey >45cm: stony layer not easily penetrable Draining well except in wheelings where compacted, area of plots liable to pool in furrows, adjacent area heavily compacted	Moor Gate (612b)
B	St Buryan	Sandy silt loam	Medium sandy loam	1.25	Surface: many stones and rock chips up to 15cm across, occasionally more; extensive effect of precipitation with fines washed into furrows, becoming capped From surface to between 30 to 50cm deep (variable across plot): uniform, friable dark brown soil with small stones, becoming increasingly compacted Below 30 to 50cm deep (variable across plot): yellow, sandy and progressively stonier, generally not easily penetrable below 40cm Water pools in some furrows including those of the plot	Morton-hampstead (611b)
C	Tregiffian	Sandy silt loam	Medium sandy loam	1.00	Surface: liberally strewn with quartz or blue-grey angular stones up to 20cm across; windward sire of ridges dried out 0-25 to 0-40cm: uniform, friable dark brown soil with small stones >25 to >40cm: clayey, stonier but still more or less friable >45cm: stony, not easily penetrable Furrows can flood with heavy rain but drains well down steep slope	Morton-hampstead (611b)
D	Rosevidney	Sandy clay loam	Silty clay loam	2.50	Surface: liberally strewn with cobbles up to 2.5cm across, occasionally to 15cm. 0-15cm: uniformly dark brown with small stones, friable 15-40cm: clayey with small stones 40-45cm: hard, sandy layer	Denbigh 2 (541k)

					>45cm: not easily penetrable Furrows can flood with heavy rain but drains down, only furrows at the lower end of field (including plot) with standing water	
E	Roseworthy	Silt loam	Medium sandy loam	1.00	Surface: liberally strewn with quartz or blue-grey angular stones up to 20cm across 0-58cm: soil uniform, brown 58-60cm: soil lighter in colour, gritty >60cm: stony layer not easily penetrable Drains reasonably well but some standing water in furrows	Trusham (541n)
F	Bodilly	Sandy silt loam	Medium sandy loam	1.00	Surface: much gravel evident on surface, and strewn with quartz or brown/pink stones up to 10cm across 0-12cm: uniform, brown, friable soil 12-25cm: sticky clay 25-50cm: clay with sand and grit >50cm: sandy or gritty layer not easily penetrated Patches of standing water in furrows	Morton-hampstead (611b)
G	Mawla	Silt loam	Silty clay loam	1.50	Surface: liberally strewn with quartz or blue-grey stones or chips mostly up to 15cm across 0-20cm: soil uniform, dark brown and friable 20-40cm: soil becoming increasingly clayey and compacted 40-45cm: brick-red compacted layer >45cm: not easily penetrable Water appears to drain down slope well, but still some standing water in furrows exacerbated by adjacent compacted area	Denbigh 2 (541k)
H	Penventon	Sandy silt loam	Silt loam	1.00	Surface: some small stones to 3cm (rarely 10cm) across. Ridge tops gravely and draining 0-30cm: uniform dark-brown soil 30-45cm: increasingly stony and clayey >45cm: stony or sandy layer not easily penetrable Water drains down slope well, but still some standing water in furrows at times	Denbigh 2 (541k)
I	Fourburrow	Silt	Silty	1.00	Surface: very liberally strewn with quartz or blue-grey angular stones up	Denbigh 2

		loam	clay loam		to 15cm across 0-30cm: soil uniform, light brown 30-40cm: soil becoming increasingly clayey and pink-coloured >40cm: stony layer not easily penetrable Well drained down steep slope	(541k)
J	Goonhavern	Sandy silt loam	Silty clay loam	2.00	Surface: strewn with quartz or blue-grey stones or chips up to 15cm across; ridge top with many cobbles up to 1cm across and clods up to 10cm across 0-20cm: loose brown soil with cobbles and small stones 20-40cm: soil becoming increasingly clayey and compacted 40-45cm: sandy compacted layer >45cm: not easily penetrable Water drains down well but some water standing at lower end of field	Denbigh 2 (541k)

[1] ADAS soil texture (0-30cm) as determined by particle size analysis (see Figure 7)

[2] Soil texture as provided by growers

[3] 'Visual Soil Structure Quality Assessment' (Sq1 friable, Sq2 intact, Sq3 firm, Sq4 compact and Sq5 very compact) (Ball, Batey & Munkholm, undated)

[4] Soil association and map symbols (Soil Survey of England and Wales, 1983, 1:250,000 series) as follows:

541: typical brown earths – non-alluvial loamy soils with a non-calcareous subsoil without significant clay enrichment. The 541k subgroup (the Denbigh 2 soil association) represents well drained, fine loamy soils over slate or slate rubble, typical of early potato and broccoli growing in west Cornwall, while 541n (Trusham) are also well drained, fine loamy soils, but over deeply weathered rock, some shallow and some deeply sloping and with bare rock locally and typically used for "horticultural crops in drier districts."

611: typical dark brown podzolic soils resulting from pedogenic accumulation of iron and aluminium or organic matter or some combination of these, normally formed as a result of acidic weathering conditions and, under natural or semi-natural vegetation, having an unincorporated acidic layer at the surface. The 611b subgroup (Moretonhampstead) represents well drained, gritty loamy soils with a humose surface horizon in places, some with steep slopes and with boulders and rocks locally, some used for early potato and broccoli growing in west Cornwall.

612: humic podzolic soils, as 611 but with a humose or peaty topsoil. The 612b (Moor Gate) subgroup represents well drained, humose gritty loamy soils, occasionally with a thin iron-pan, many with steep slopes, often with boulders or rocky, and not traditionally used for horticultural crops.

Environmental monitoring

After planting the bulb plots an 'Active Irrigation Scheduling' monitoring station, with added air temperature and humidity sensors, was set up in the plot at each site by Plantsystems,[3] who continued to monitor and maintain them through the project. Each station

Figure **3**) included sensors to measure % soil water content (SWC) at 0–10, 10–20 and 20–30cm depth, soil temperature at 15cm depth, precipitation, air temperature and relative humidity (RH). The soil sensors were inserted in the ridge centre, while the air temperature and RH sensors were positioned 20–30cm above the ridge tops (roughly corresponding to mid-canopy height for the fully-grown crops) and the rain-gauge was clear of the crop. The %SWC is equivalent to the amount of water in mm per 100mm depth (1% = 1mm of water in 100mm of soil); in 'Results' %SWC is often expressed as the average across the three depths (i.e. mm of water in 100mm of soil). Monitoring was started on the completion of bulb planting and continued until after the final crop assessment had been made in June 2015. All measurements were logged at 15-min intervals, accumulated on Plantsystems' data-base, and checked and downloaded at appropriate intervals for analysis; this produced a large volume of data but allowed more accurate calculations to be made.

Figure 3. One of Plantsystems 'Active Irrigation Scheduling' monitoring station on site

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[3] Plantsystems Ltd (now Agrovista UK Ltd, trading as Plantsystems); http://www.plantsystems.co.uk/

Soil and plant sampling and analysis

Soil sampling, examination and analysis techniques were guided by the standard text '*RB 209*' (Defra and Welsh Assembly Government, 2010-2011). The concentrations of soil nutrients quoted refer to available or extractable (not total) nutrients. Soil samples were taken from each of the ten plots at the start of the trial in October 2012 (well after the pre-planting fertiliser had been applied) and in spring 2013, 2014 and 2015 (no further fertiliser having been applied). However, analysis of a full range of trace elements was not begun until 2014, once it was clear that worthwhile and varying levels of rust were present in the plots. For consistency, all soil samples were taken from half-way up the bulb ridge.

Leaf samples were taken from each of the ten plots in spring 2014 and 2015. Leaves were chosen for sampling since they were considered better to represent the current nutrient status of the plant than flower stems (already becoming senescent) or bulbs (with their large nutrient reserves). The concentrations of leaf nutrients quoted refer to total nutrients.

Because of equipment failure there was an unavoidable change of analytical laboratories in 2014, which in the case of soil analysis for Al, Fe and Na incurred changes in the analytical techniques used. In 2013 Warwick Crop Centre, University of Warwick (WCC) used cation exchange with barium chloride for Al, Fe and Na determination, while in 2014 and 2015 NRM Laboratories (NRM) used DTPA-extraction (chelation) for Al and Fe and ammonium nitrate-extraction for Na. Since the analysis of a full range of trace elements in soil had not commenced until 2014, this change had relatively little impact on results, but it means that Al, Fe and Na levels can only be compared between sites with a year, not between years.

Autumn 2012

On 25–27 October 2012 the soil in each of the ten plots was examined and samples taken for analysis.

- Using a 'cheese corer' (2.5cm diameter) a soil sample 0–20cm deep was taken by bulking at least ten cores from across a whole plot. Standard agricultural soil analysis on air-dried samples was carried out at WCC to determine the concentration of major nutrients (nitrate-N, P, K and Mg).
- Using a 90cm soil auger (2.5cm diameter) soil samples were taken from 0–30 and (as far as practical) 30–60cm deep, the maximum practical auger depth being 40cm at St Buryan and Fourburrow, 50cm at Bodilly, 60cm at Roseworthy and 45cm at the six other sites. Soil from both layers was physically examined and a bulked sample of three to five

0–30cm cores from each site was subjected to mechanical (particle size) analysis by Anglian Soil Analysis Ltd. The results were used to define the soil texture at each site.

- In addition a 15cm-thick soil block was removed to full spade-depth, placed on a tray and each layer was examined and described (a) by assessment of soil texture by hand (following '*RB 209*') and (b) using VSSQA which defines soil structure as an easily quantifiable score from 1 (friable) to 5 (very compact) (Ball, Batey & Munkholm, undated).

Spring 2013

On 26–28 March 2013, after the bulk of the winter rain, soil samples were taken at each site as listed below, otherwise using the methods previously described.

- Samples 0–20cm deep were analysed for pH and the concentrations of major nutrients and some trace elements (P, K, Mg, Ca, Fe, Mn and Na) on air-dried samples by WCC.
- Samples were taken as deep as practical, from 0 to between 40 and 60cm at the different sites (see above), and bulked samples of three to five cores were analysed as fresh samples for total mineral N (NO_3-N and NH_4-N) by WCC. The resultant total mineral N concentrations given under 'Results' are therefore equivalent to the concentrations across the rooting zone or available soil zone.

Spring 2014 and 2015

On 11–15 April 2014 and 17–20 April 2015 soil and leaf samples were collected from each site. Soil samples were taken as described for 2013, but see above for changes in the analytical methods used for Al, Fe and Na. Leaf samples were as described below.

- Soil samples 0–20cm deep were analysed for pH, topsoil organic matter (OM) and the concentrations of nutrients (P, K, Mg, SO_4, Ca, Na, Fe, Mn, Cu, Zn, B, Mo and, in 2015 only, Al) by NRM.
- Soil samples were taken from as deep as practical and samples analysed for total mineral N (NO_3-N and NH_4-N) by WCC.
- Leaf samples consisted of ten to 15 leaves taken from across each plot, cutting at soil level and wiping them free of soil. Obviously diseased leaves and damaged 'outside' leaves were avoided. They were analysed for total concentrations of N, P, K, Mg, S, Ca, Na, Fe, Mn, Cu, Zn, B, Mo and Al by NRM.

Fungal and bacterial diagnostics

Sampling

Stem and whole plant samples were collected from each site for laboratory examination on 5–7 April 2014 and 10–12 April 2015. Stems or plants with white mould or other symptoms were avoided as far as possible when sampling. Stems were cut-off at ground level, the remains of the flower removed and discarded, and each stem placed into a sealable polythene bag for transport to the laboratory. Hands and implements were cleaned with disinfectant wipes between handling successive samples. All samples were delivered to Plant Health Solutions (PHS), Warwick, for examination. In 2015 a Cornish grower provided for testing some additional samples of commercial daffodils with rust lesions.

Microscopy

Sections of symptomatic tissue were mounted in a drop of sterile nutrient broth (SNB) on a microscope slide and observed under bright-field, dark-field and phase contrast illumination.

Surface slivers of symptomatic tissue were placed on three layers of filter paper in a Petri dish. The paper was soaked with 3ml peroxyacetic acid (as ‘Jet 5’) and ethanol 1:1 v/v. Plates were sealed with ‘Parafilm’ and incubated at room temperature (RT) until the tissues had cleared. Tissues were then stained with lactophenol cotton blue and observed microscopically.

In 2014, to encourage further development of symptoms and (or) sporulation of any fungi present, sections of symptomatic tissue, both surface-sterilised (see below) and non-surface-sterilised, were placed in humid boxes and incubated at RT for up to four weeks.

Surface sterilisation

Tissues pieces (~2cm^2) containing lesions were placed in a solution of 0.3% chlorine (prepared using ‘Presept’ tablets) for 30–60sec, blotted on paper towel, rinsed in sterile de-ionised water and blotted dry.

Isolation of fungi

In 2014 and 2015 sections of surface-sterilised tissue containing lesions were excised with a sterile scalpel and placed on the surface of two agar media, Cornmeal Agar (CMA; relatively nutrient-poor) and Potato Dextrose Agar (PDA; nutrient-rich) in 9cm-diameter Petri dishes. The media were selected on the basis that they may select for and encourage, or limit, sporulation of different fungi. Up to four pieces per sample were placed in each Petri dish.

The plates were incubated for 7–14d at 20°C in the dark. Representatives of the different fungal ‘types’ growing on the plates were sub-cultured to fresh plates of PDA and CMA and examined microscopically.

Isolation of bacteria

For the isolation of bacteria, in 2014 only sections of tissue (2–4mm^2) containing rust lesions were excised with a sterile scalpel and crushed in a drop of SNB on a sterile microscope slide. The resulting suspensions were streaked onto plates of two bacterial culture media, *Pseudomonas* Agar F (PAF) and 5% Sucrose Nutrient Agar (SNA). Plates were incubated at 25°C for 48h before examination.

Re-inoculation of fungal isolates to daffodils

Following the unexpected but consistent isolation of *Stemphylium* from lesions in 2014 and 2015, cultures were maintained and used to attempt inoculation to stems of a fresh stock of Cornish-grown ‘Golden Ducat’ bulbs in spring 2016. The bulbs, 12-14cm grade, were obtained in autumn 2015 and planted in a coir and vermiculite growing medium (‘Fertile Fibre Seed Compost’, Fertile Fibre, Hereford, UK) in 3L planter bags, five bulbs per pot. They were maintained outdoors until the advent of cooler conditions in November 2015 when they were moved to an un-heated, well ventilated polythene tunnel. This work is ongoing and will be reported later in 2016.

Identification of fungal isolates by sequencing

The daffodil stem samples collected in 2014 for the analysis of viral RNA (see next section, ‘Analysis of viral RNA…’) were also used to isolate fungi from rust lesions for identification purposes. Lesions from a number of sites were excised and placed on PDA containing chlorotetracycline at 20°C for 6d. Any fungal growth was sub-cultured onto fresh PDA and incubated at 20°C for a further 17d. Three agar plugs (5mm-diameter) were added to 25ml of potato dextrose broth (PDB) which was incubated at 20°C for 7d. The resultant mycelium was freeze-dried prior to DNA extraction. DNA was extracted using a method based on the ABI Ultra Sample Preparation Reagent (personal communication, S Rehner, USDA). PCR was carried out using 1µl of diluted extract (diluted 1 in 10 using Tris-EDTA buffer) and the fungal bar-coding primers ITS5 and ITS4 (White et al. 1990). Cycling conditions were as follows: 1 cycle of 94°C for 2min; 35 cycles of 94°C for 2min, 55°C for 30sec and 72°C for 1min; and 1 cycle of 72°C for 4min. Products were run on a 1.5% agarose gel to confirm successful amplification before sequencing using the ITS4 primer. Sequences were identified using the National Center for Biotechnology Information Basic Local Alignment Tool (NCBI BLAST)[4] and aligned as described in the next section. A maximum-likelihood tree was constructed using the calculated best model, Kimura-2-parameter (Kimura, 1980) and sequences from NCBI were included for comparison.

Analysis of viral RNA in stems

Sample preparation

Representative daffodil stems were sampled on 5–7 April 2014 from each of the ten sites and delivered to WCC. On examination three stems with typical rust lesions, and three symptomless stems, were selected from each of the Kelynack, Mawla and Penventon samples. An appropriate, *ca* 2cm-long, piece of each stem (either free of lesions or with typical rust lesions) was excised, cut into five or six smaller sections, flash-frozen in liquid N and stored at -70°C until analysis. Among samples from the other seven sites, no stem was considered totally free of rust or rust-like lesions, and in these cases three stems that included both typical rust lesions and clear symptomless areas were selected from each site. Two appropriate, *ca* 2cm-long, pieces of each stem (either from a section free of lesions or from a section with typical rust lesions) were excised, and treated and stored as described above.

Stem samples with and without rust lesions from Kelynack, Mawla and Penventon, and stem samples with rust lesions from the other seven sites, were sub-sampled for RNA extraction. The symptomless samples from the seven sites were retained in storage with the intention of RNA extraction should an association be found between the presence of virus and rust symptoms, in which case whether the virus particles were systematic, or localised to lesions, would be investigated. RNA was extracted using Trizol® reagent (Life Technologies, UK), any DNA removed using DNase I (Sigma, UK) and RNA further purified by precipitation with lithium chloride (Ambion®, Life Technologies, UK), following the manufacturers' guidelines. RNA was also extracted, as already described, from material used as positive control samples. As a control for the Potyvirus group, a leaf infected with Turnip Mosaic Virus (TuMV; Max Newbert, WCC) was used. Freeze-dried samples of leaves infected with Potato Virus M (PVM) and Tomato Spotted Wilt Virus (TSWV) (Bioreba, Switzerland) were obtained as controls for the Carlavirus and Tospovirus groups. As an additional control stems were sampled from a daffodils (cultivar unknown) growing alongside the main Wellesbourne

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[4] http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome

Campus entrance and showing no symptoms of rust. RNA integrity was examined on a 1.5% agarose gel, and re-extraction carried out for any degraded samples.

On 10–12 April 2015 stem samples were again collected from each site and sub-sampled, frozen and stored at WCC as previously described to provide material for further work if required.

Synthesis of cDNA and detection of Potyviruses

For the Potyvirus group, first-strand cDNA was synthesised from 16 selected samples, including a positive control, using Superscript II reverse transcriptase (Life Technologies, UK) and a modified protocol (Max Newbert, WCC) (samples 1-16, Table 5). RNA (1µg) was added to a final concentration of 0.5µM of Potyvirus-specific NIB3R primer (Zheng *et al.*, 2010) in a 20µl reaction volume which was incubated at 70°C for 10min and 25°C for 10min before placing on ice. The following components were then added: 8µl 5x buffer, 4µl DTT, 2µl dNTPs (10mM), 1µl RNaseOUT, 1µl Superscript II reverse transcriptase and 4µl DEPC-treated water. The mixture was incubated at 25°C for 10min, 37°C for 45min, 42°C for 45min, 70°C for 15min and 4°C for 5min before storing at -20°C. PCR of the cDNA was then carried out using published primers and conditions (Zheng *et al*., 2010;

Table 6) in order to assess the presence of Potyviruses. Each 20µl reaction contained 1µl cDNA, 10µl REDTaq® ReadyMix® (Sigma-Aldrich, UK) and 0.5µM of each primer. Products were visualised on a 1.2% agarose gel and the remainder purified using a PCR purification kit (Qiagen, UK) and sequenced using the forward primer. Sequences were identified using the NCBI BLAST resource. Further cDNA was synthesised for 14 selected samples using Superscript II reverse transcriptase with Oligo dT21V as the primer (Pappu *et al*., 1993) and following the manufacturers' guidelines (samples 17-30, Table 5).

A selection of the resulting PCR products was cloned, due to mixed sequences, using a CloneJET PCR Cloning kit (ThermoFisher Scientific, UK) following the sticky end protocol. The ligation (2µl) was transformed into 20µl of *Escherichia coli* DH5α chemically competent cells by placing on ice for 30min, shocking at 42°C for 30s, and then adding 250µl SOC and shaking for 1h at 37°C. A range of volumes was plated on LB agar containing 50mg/ml ampicillin and plates incubated at 37°C overnight. Colony PCR was carried out as described by the manufacturer and 63 positive products of the correct size purified and sequenced as previously described, using the pJET 2.1 forward primer. Sequences were identified using NCBI BLAST and aligned sequences were aligned (CLUSTALW method) using MEGA version 6.06 (Tamura *et al*., 2011) and a maximum-likelihood tree constructed using the calculated best model, Kimura-2-parameter (Kimura, 1980).

Primers were designed for a putative new virus using Primer3Plus (Untergasser *et al*., 2012). PCR was set up as previously described using ALVL FOR (5'TCGCGCATAAAATGGCACCGT-3') and ALVL REV (5'CCTGGTTGGTTGCACGGGAG-3') primers and the following cycling conditions: 1 cycle of 94°C for 2min, followed by 30 cycles of 94°C for 45s, 61°C for 30s and 72°C for 1min, then 1 cycle of 72°C for 7min. Products were visualised on a 1.5% agarose gel. Selected products were sequenced to confirm their identity.

Synthesis of cDNA and detection of Tospoviruses, Carlaviruses and Nepoviruses

Further cDNA was synthesised for 14 selected samples (samples 36-49, Table 5) using the method described for Potyviruses and the primer gL3920c (Chen *et al*., 2012) to determine the presence of Tospoviruses. PCR was carried out as described using the published primers and conditions (Chen *et al*., 2012;

Table 6). Products were visualised on a 1.5% agarose gel.

Using the set of cDNA synthesised using OligodTV (samples 17-30), PCR was carried out as described above using published primers and conditions (Gaspar *et a*l., 2008;

Table 6) in order to determine the presence of Carlaviruses. Products were visualised on a 1.2% agarose gel. The same set of cDNA was also used to test for the presence of Nepoviruses using primers (

Table 6) and conditions described for subgroup A and B (Wei and Clover, 2008) and C (Digiaro *et al*., 2007).

Further synthesis of cDNA and detection of all four virus groups

Stems of daffodil 'Scarlet Royal' derived from a Scottish 'virus-free' stock were obtained from a commercial bulb grower on 28 April 2015. RNA was extracted and cDNA synthesised as described previously, using OligodT21V. These samples were tested for the presence of all four virus groups as previously described.

Table 5. Details of cDNA samples synthesised and control samples (green, lesion-free; black, with lesions; red, positive controls and blue, 'virus-free' samples)

Sample	Site	Rust lesions	cDNA primer
1	Kelynack	No	Nib3R
2	Mawla	No	Nib3R
3	Penventon	No	Nib3R
4	Tregiffian	Yes	Nib3R
5	Mawla	Yes	Nib3R
6	Kelynack	Yes	Nib3R
7	Penventon	Yes	Nib3R
8	St Buryan	Yes	Nib3R
9	Roseworthy	Yes	Nib3R
10	Fourburrow	Yes	Nib3R
11	Goonhavern	Yes	Nib3R
12	Bodilly	Yes	Nib3R
13	Rosevidney	Yes	Nib3R
14	Wellesbourne	No	Nib3R
15	Wellesbourne	No	Nib3R
16	TuMV	Yes	Nib3R
17	Kelynack	No	OligodT21V
18	Mawla	No	OligodT21V
19	Penventon	No	OligodT21V
20	Tregiffian	Yes	OligodT21V
21	Mawla	Yes	OligodT21V
22	Kelynack	Yes	OligodT21V
23	Penventon	Yes	OligodT21V
24	St Buryan	Yes	OligodT21V
25	Roseworthy	Yes	OligodT21V
26	Fourburrow	Yes	OligodT21V
27	Goonhavern	Yes	OligodT21V
28	Bodilly	Yes	OligodT21V
29	Rosevidney	Yes	OligodT21V
30	Wellesbourne	No	OligodT21V
31	TuMV	Yes	OligodT21V
32	PVM	Yes	OligodT21V
33	TSWV	Yes	OligodT21V
34	'Virus-free' 1	No	OligodT21V
35	'Virus-free' 2	No	OligodT21V
36	Kelynack	No	gL3920c
37	Mawla	No	gL3920c
38	Penventon	No	gL3920c
39	Tregiffian	Yes	gL3920c
40	Mawla	Yes	gL3920c
41	Kelynack	Yes	gL3920c
42	Penventon	Yes	gL3920c
43	St Buryan	Yes	gL3920c
44	Roseworthy	Yes	gL3920c
45	Fourburrow	Yes	gL3920c
46	Goonhavern	Yes	gL3920c
47	Bodilly	Yes	gL3920c
48	Rosevidney	Yes	gL3920c

49	Wellesbourne	No	gL3920c

Table 6. Primers used in this study and their associated publications

Target	Forward primer (5’-3’)	Reverse primer (5’-3’)	Publication
Potyviruses	GTITGYGTIGAYGAYTTYAAYAA	TCIACIACIGTIGAIGGYTGNCC	Zheng *et al*., 2010
Unknown Potyvirus	TCGCGCATAAAATGGCACCGT	CCTGGTTGGTTGCACGGGAG	This study
Tospoviruses	ATGGGDATNTTTGATTTCATGRTATGC	TCATGCTCATSAGRTAAATYTCTCT	Chen *et al.,* 2012
Carlaviruses	GGBYTNGGBGTNCCNACNGA	TTTTTTTTTTTTTTTTTTTTTV	Gaspar *et al.,* 2008
Nepoviruses subgroup A	ACDTCWGARGGITAYCC	RATDCCYACYTGRCWIGGCA	Wei and Clover, 2008
Nepoviruses subgroup B	TCTGGITTTGCYTTRACRGT	CTTRTCACTVCCATCRGTAA	Wei and Clover, 2008
Nepoviruses subgroup C	TTRKDYTGGYKAAMYYCCA	TMATCSWASCRHGTGSKKGCCA	Digiaro *et al*., 2007

Crop and rust assessments

Each year plots were routinely assessed at three stages around flower-picking (referred to as pre-picking, picking and post-picking) when the appearance of rust is most commercially relevant. The assessment dates were 7–8 February, 27 February–1 March and 26–28 March 2013, 12–14 February, 4–6 March and 5–7 April 2014, and 14–16 February, 9–12 March and 10–12 April 2015. Rust lesions on leaves were assessed on 26–28 March 2013, 1–2 June 2014 and 14–16 May 2015. Over winter 2014 to spring 2015 and using a commercial stock of ‘Golden Ducat’ at the Roseworthy site, rust lesions were assessed on stems at intervals before, during and after emergence. Root growth was checked on 14–16 May 2015.

Crop assessments

Different rates or timing of crop growth in different years or at different sites might affect the levels of rust, so crop growth stage (GS; see Appendix) was recorded at each assessment. In each case the minimum, most usual and maximum GS were recorded, including (at the earlier stages) stem and foliage heights. Overall crop vigour and the general occurrence of pest, disease, disorder and other problems was noted. Towards the end of the project root growth was checked after digging out the soil one spit deep at one end of each plot.

Rust assessments

To assess rust levels it had been intended to use a scoring system to record incidence and severity at the flower picking stage. On-site it was immediately evident that the initially low incidence of rust would require a more quantitative method and that rust incidence could increase rapidly around this time. Therefore all emerged stems were checked individually for the incidence and severity of lesions at three stages, about 2 weeks before, close to, and about 2 weeks after picking stage. The number of stems per plot (of 1,000 bulbs) showing any rust symptoms was recorded, the number, size and nature of the lesions being noted to enable rust incidence and severity scores to be given (see Table 7 and Figure 4). A range of typical lesions is illustrated in Figure 5; very occasionally rust-coloured lesions appeared as streaks or blotches rather than typical lesions, and provided these had the characteristic rust colour they were included. Rust lesions were quite distinct from the larger, dark-chocolate-coloured symptoms of daffodil ‘chocolate spot’ disorder.

Putative ‘early-stage’ rust lesions were often seen during regular stem assessments, consisting 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 (Figure 5). Casual observations of these small lesions showed they do not develop further following picking, but it might help the understanding of rust if the time of first appearance of rust lesions were known. In February 2014 samples of typical plants were recovered from each site and entire stems dissected out. The three regions of the stem – within in the bulb (white), growing through the soil (yellowish) and above-ground (green) - were examined under a hand-lens and scanned under incident light at x40 magnification for the presence of putative early-stage lesions. Preliminary observations indicated that a few such lesions may begin to develop on the stem before it is exposed above ground. Further examples were examined from mid-November 2014 until flower picking in 2015. Using a commercial stock of daffodil ‘Golden Ducat’ (growing adjacent to the trial plot at Roseworthy), random ten- to fifteen-plant samples were dug-up on 12 November 2014 (GS 1.3) and then at 2- to 4-week intervals (15 December, GS 2.1; 13 January 2015, GS 2.2; 26 January, GS 2.2–2.3; 15 February, GS 2.4 and 10 March, GS 3.3) and couriered to Spalding where ten intact stems were dissected out from each sample and examined as described above.

Rust lesions also occur on leaves, though this seems to have no commercial significance as far as stem quality is concerned. Foliage was checked for the presence and nature of any rust lesions once each year. How this was done varied from year to year in an attempt to find a cost-effective method. In 2013 leaf rust was recorded in the field at the post-picking stage (GS 3.4–3.8) by scoring rust incidence (Table 7) and severity (Table 8, score a) clump-by-clump (rather than leaf-by-leaf) along the plots. In 2014 leaf rust was recorded in the field at an early senescence stage (GS 4.1 or 4.2) by assessing ten clumps of leaves at random along the plots. In 2015 leaf rust was recorded at the post-flowering, pre-senescence stage (GS 3.8–4.2) by cutting-off ten clumps of ten healthy, large leaves at random from each plot, loosely enclosing them in polythene bags and keeping them out of the sun for assessment a few hours later.

Table 7. Rust severity and rust incidence scores used in assessing stem symptoms.

Types of rust lesions	Severity score
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
Sparse but typical rust spots or rust-like streaks/blotches, no commercial significance but worth watching	2
Moderate lesions that are becoming disfiguring; commercially might lead to down-grading	3
Severe rust with many lesions, some cracks across stems or across the keel of the stem, very disfiguring; flowers un-marketable	4
Very severe rust with very obvious cracking and stem bending; flowers un-marketable	5

Numbers of stems affected	Incidence score
None seen	0
Up to 1% of stems affected	1
Up to 5% of stems affected	2
Up to 10% of stems affected	3
Up to 50% of stems affected	4
Up to 100% of stems affected	5

Table 8. Rust severity scores as adapted for recording (a) leaf lesions in the field crop and (b) stem lesions in the commercial bunch survey

Severity	Score a	Score 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
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] Many stems fell into this middle group, which is marginal for marketability, and the scores were split to those that would probably be commercially acceptable (score 3) and those that would probably be unmarketable (score 4)

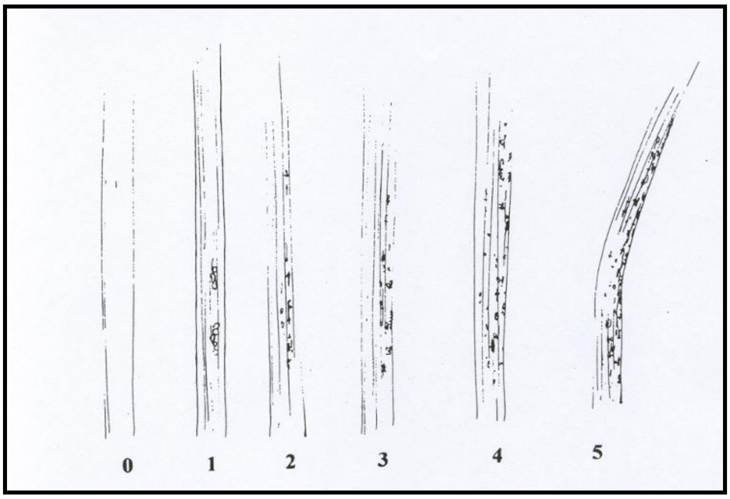


Figure 4. Daffodil rust lesions showing severity scores of 0 to 5 (by courtesy of Andrew Tompsett)

Figure 5. Typical rust symptoms: top, increasing rust severity with blistering (left), a few rust lesions (middle) and larger, coalescing lesions (right); bottom, close-up of blistering (left) and rust lesions with cracking (right)

Relationships between levels of rust, SWC and other factors

In preliminary data assessments potential relationships between the severity and incidence of rust with SWC and weather data were examined using graphical summaries in the form of box-and-whisker plots. This format provided convenient visualisation of the means and ranges of the multitude of values collected at 15-min intervals (a box-and-whisker plot covering a one-month period is derived from almost 3,000 data points at each site for each factor). The layout of these plots is described in the legend to Figure **41**. Suggested associations, for example between SWC or soil nutrient concentrations and rust incidence, were examined using linear regression analysis. The regression coefficient (R^2) is usually quoted under ‘Results’, along with the statistical significance of the regression from analysis of variance. Following comparisons of the different measures of rust ‘level’ - the rust incidence score, severity score and the number of stems with rust per plot - the number/plot was invariably used in these analyses. Since not all factors of interest were easily quantifiable, for example previous fertiliser practice or soil texture, and in these cases simple histograms are presented as an aid to visualising the results.

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 growers in England and Scotland through the help of four 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 packers were each asked to provide, as far as practical, sample bunches of three sendings (usually three picking dates from one crop) from each of five sources (usually five separate growers), making 15 samples for each packer and 60 samples in all. Not all growers were able to provide three sendings from every 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 was to be taken in a random fashion and without regard to the amount of rust seen or the rust status of the bulb stocks from which they came. The packers were asked to provide picking date and county of origin for each sample. After transport the samples were collected locally following a short period of 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 ‘Golden Ducat’ and which the packers did not consider to be rust-prone. Because of differences in growing practices and in the cultivars grown in the regions it was not practical to select a single cultivar to provide all these samples: in practice the samples comprised 18 different cultivars, and included 18 samples of ‘Standard Value’, ten of ‘Lowan’ and seven of ‘Carlton’.

Each bunch was examined and its GS recorded (usually 3.2 to 3.3 at receipt). Stems were assessed individually and (where lesions were present) scored for rust severity according to the 0–6 score shown as score b in Table 8. In this system stems with serious damage but probably marketable were scored as ‘3’, or ‘4’ if probably unmarketable. Where the bunches received contained >10 stems 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

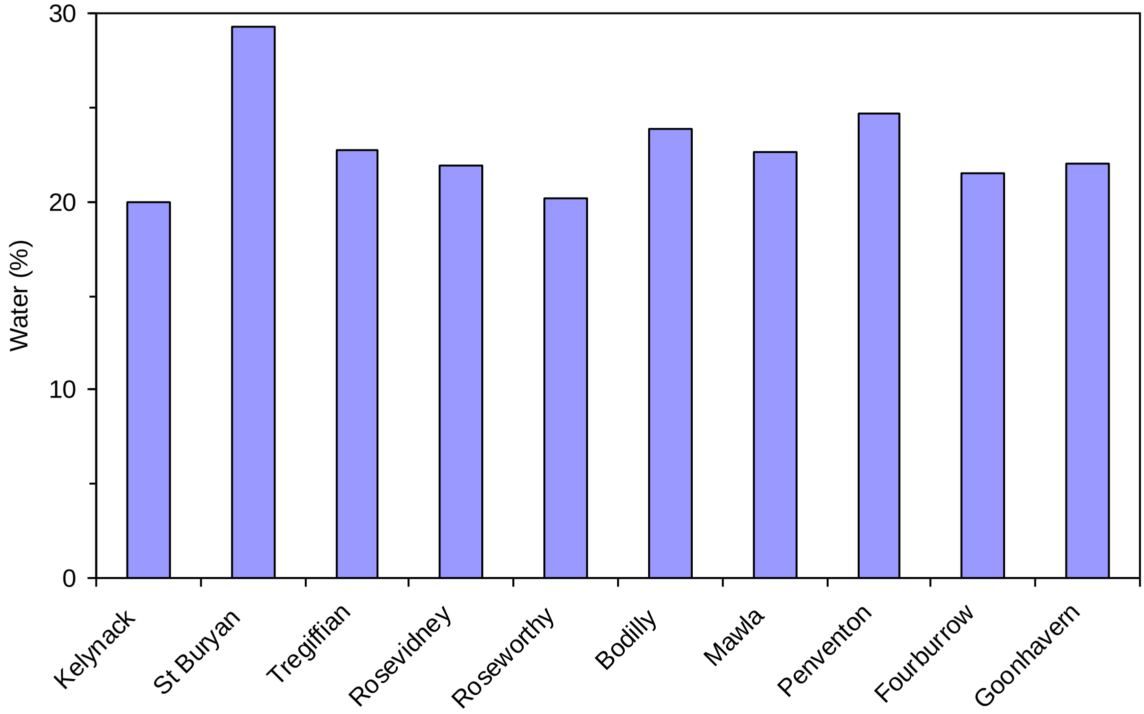
Soil analysis

Soil texture and particle size analysis

Particle size analysis was carried out at the start of the project in autumn 2012 (Figure 6 and Figure 7). The soil water content at or just above 20% was adequate for growing daffodils, though it reached nearly 30% at St Buryan, later noted as a site with a tendency to water-logging. At around 20 to 30%, the content of particles >2mm (stones, gravel, etc.) was rather high at most sites for bulb production, likely resulting in damage to bulbs and difficulties at lifting. At St Buryan the content of particles >2mm was lower, about 13%. De-stoning machines are not regularly used before planting daffodil crops and were not used in the fields studied. High stone contents would also damage potato crops, and it is interesting to note that the relatively stone-free St Buryan site was the only one at which potatoes were recorded as having been grown recently.

The soil at all ten sites contained <20% v/v clay and variable amounts of sand, from <10% sand at Rosevidney (defining the soil texture as a sandy clay loam), 10 to 30% sand at Fourburrow, Mawla and Roseworthy (silt loam) and 30 to 50% sand at the remaining six sites (sandy silt loam) (Figure 7) Soils with a low clay content are preferred for bulb production as they allow relatively soil-free bulbs to be harvested. The soil textures quoted by the growers were also recorded (Table 4) but differed somewhat from those defined here and may not have been derived using standard ADAS methods.

Soil structure was also assessed using ‘Visual Soil Structure Quality Assessment’ (VSSQA), which produces an easily quantifiable score from 1 (‘friable’) through ’intact’, ‘firm’ and ‘compact’ to 5 (‘very compact’) (Table 4). Most sites scored 1.0 to 1.5 (friable or friable/intact), with Goonhavern scoring 2.0 (intact) and Rosevidney 2.5 (intact/firm); this indicated a degree of compaction at Rosevidney that could begin to affect bulb growth.



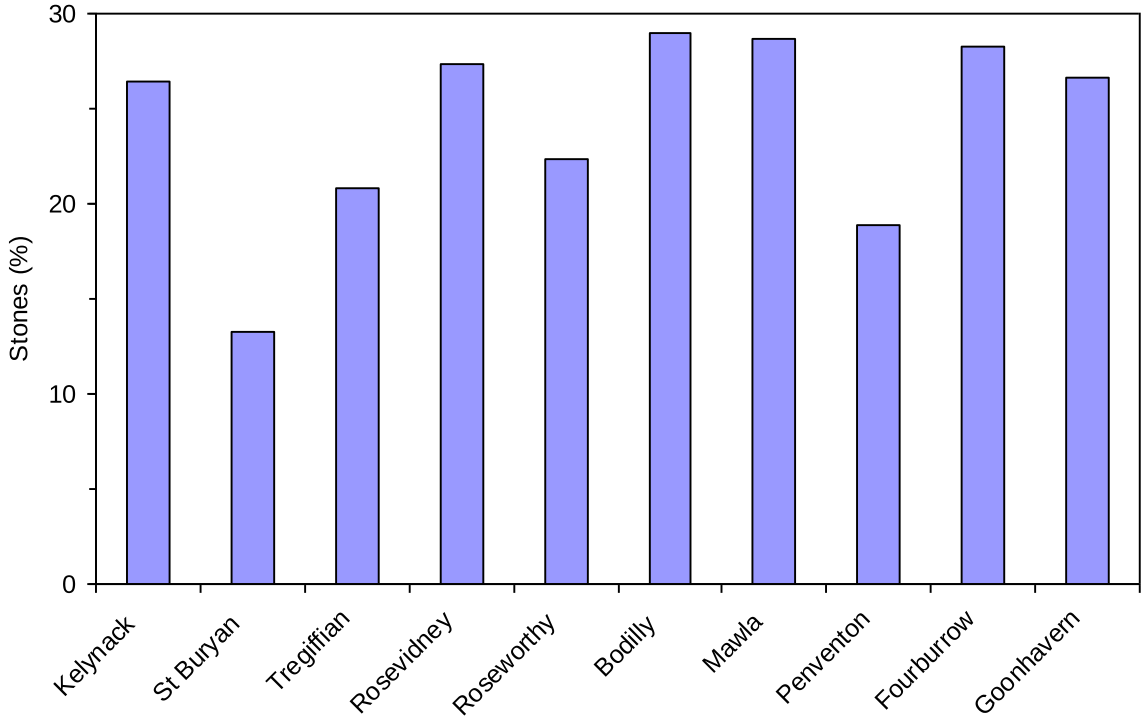
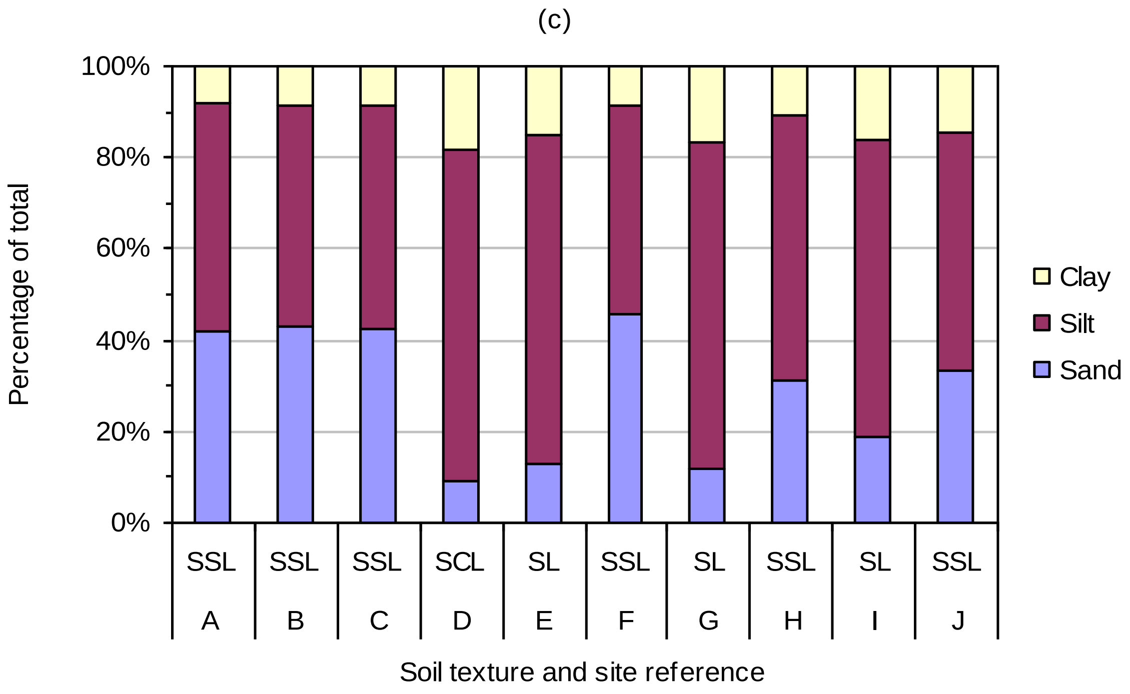


Figure 6. Soil particle size analysis at 0–30cm for the ten sites: top, percentage water content (w/w) prior to drying; bottom, all particles >2mm diameter as percentage of dry soil; data continued in Figure 7



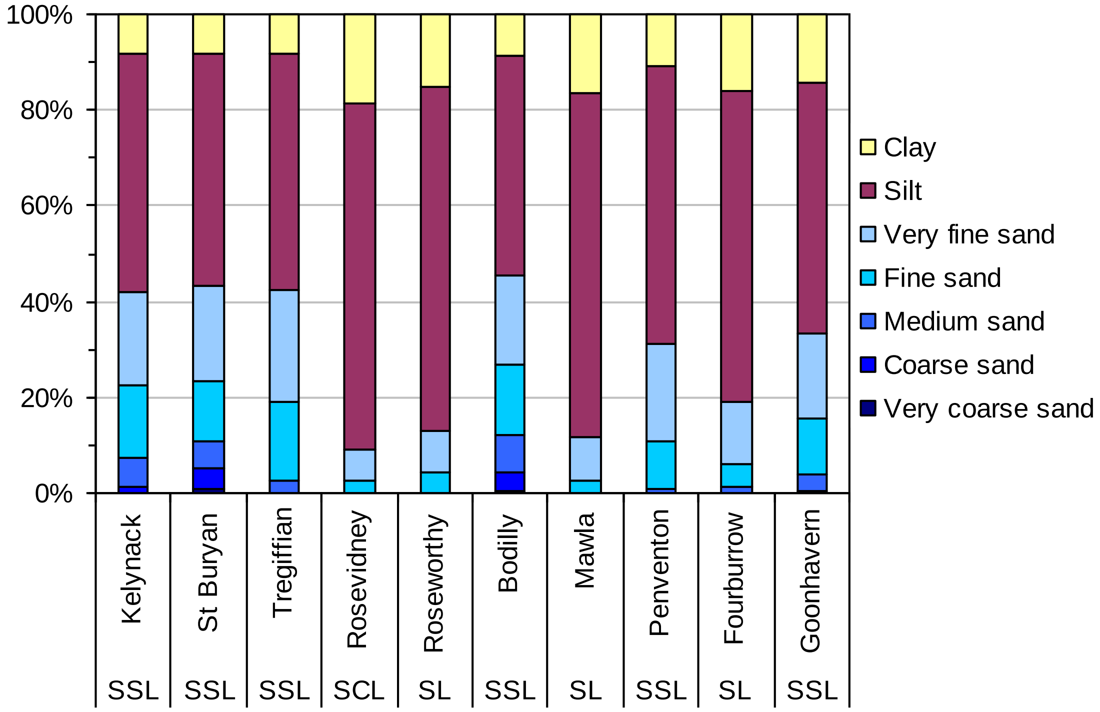


Figure 7. Soil particle size analysis to 30cm (data continued from Figure 6): top, clay, silt and sand as percentage of dry soil after gravel and stones (>2mm) removed, with standard ADAS soil textures determined by particle size analysis shown as ‘SCL’ (sandy clay loam), ‘SL’ (silt loam) or ‘SSL’ (sandy silt loam); bottom, as above but with sand broken down to grades

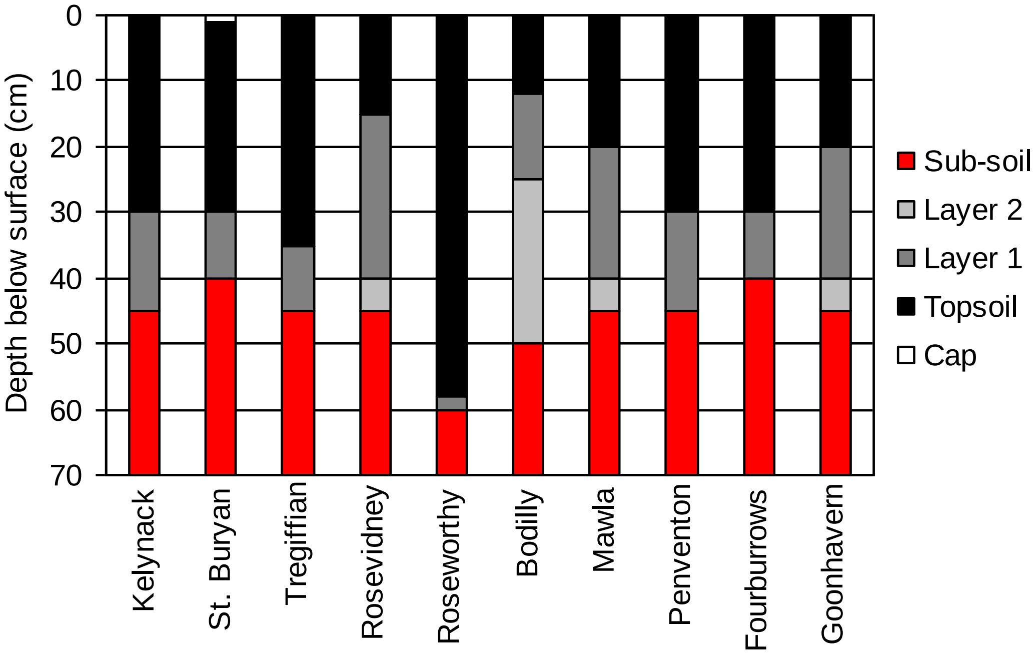


Figure 8. Soil layers at the test sites

Soil descriptions and associations

Physical examination of the soils in autumn 2012 showed that most sites had a relatively shallow, well worked upper layer 20 to 30cm-deep (Figure 8; see also Table 4). However, Bodilly and Rosevidney had a shallower upper layer (12 to 15cm-deep) while Roseworthy had a uniform upper layer almost 60cm-deep. Other than at Roseworthy, the soil then became progressively more compact and either clayey, sandy, gritty or stony with depth. Except at Roseworthy the soil below 40 to 50cm was difficult to penetrate. The surface contained gravel, stones or rock chips at all sites, liberally so at Tregiffian, Rosevidney, Roseworthy, Mawla and Fourburrow. The soil at Mawla, on or near an old mining site, had had much old building material incorporated. The soil appeared particularly prone to capping at St Buryan. In general all sites appeared to drain well down slopes, though at St Buryan and Rosevidney water stood in the furrows where the plots had been located in lower parts of the fields (areas of standing water are not unusual in daffodil fields). Ideally bulbs require deep soils with good structure, a criterion met at Roseworthy but probably not at the other sites and certainly not at Bodilly.

The soil associations, as defined by the Soil Survey of England and Wales, were at most sites described as typical brown earths commonly used for horticultural crops in west Cornwall (Table 4). At St Buryan, Tregiffian and Bodilly they were typically dark brown podzolic soils resulting from acidic weathering, also commonly used for horticultural crops in west Cornwall, and at Kelynack the soil was similar but with a humose or peaty topsoil, a soil type not traditionally associated with horticultural crops in the area. In the soil analyses reported below, however, the Kelynack sample did not have a high OM content, likely to have been a result of local variation in the indicative soil type.

Soil analysis

Table 9 shows the results of standard agricultural soil analysis at the start of the project in October 2012, which followed the growers' own sampling, analysis and fertiliser applications *ca* August 2012. A pH of 6.0–7.5 is considered suitable for daffodil production, so all sites were acceptable in this respect, Roseworthy being a little higher at pH 7.7. Nutrient concentrations indicated that all sites were low in N (indices 0 to 2), well supplied with P and Mg, and (except at St Buryan and Rosevidney) low in K. N fertiliser had been applied only at Roseworthy, possibly unnecessarily since the bulb crop was following brassicas (Table 3), but this was not evident from the October 2012 analysis. Avoiding excessive levels of N is advised in growing daffodil crops, as it may lead to an increase in basal rot, but there was no evidence for this. Equally, pale foliage was not seen in the trial plots over 2013–2015, so there was no indication of N-deficiency either. The generally low K levels suggest some sites would subsequently require fresh K fertiliser, once K indices reached 0 or 1.

Table 9. Agricultural soil analysis October 2012

Site reference and name		pH	NO_3-N ppm [index]	P ppm [index]	K ppm [index]	Mg ppm [index]
A	Kelynack	7.4	43 [1]	165 [7]	213 [2-]	133 [4]
B	St Buryan	6.7	42 [1]	164 [7]	461 [4]	233 [5]
C	Tregiffian	7.0	51 [2]	128 [6]	171 [2-]	144 [4]
D	Rosevidney	6.8	15 [0]	102 [6]	443 [4]	99 [3]
E	Roseworthy	7.7	18 [0]	115 [6]	318 [3]	102 [4]
F	Bodilly	7.4	56 [2]	149 [7]	171 [2-]	156 [4]
G	Mawla	7.3	24 [0]	82 [5]	315 [3]	101 [4]
H	Penventon	6.3	20 [0]	167 [7]	118 [1]	113 [4]
I	Fourburrow	6.3	14 [0]	57 [4]	274 [2+]	58 [3]
J	Goonhavern	6.8	6 [0]	57 [4]	164 [2-]	118 [4]

More detailed soil analysis was carried out in spring 2013, 2014 and 2015 and the results for total mineral nitrogen are shown in Table 10 with the other analyses in Table 11 to Table 13. The results are described and shown graphically after the tables. Starting in 2014 there were unavoidable changes in the analytical methods used for soil Al, Fe and Na (see 'Materials and methods') - where this applies it is noted in the results that comparisons should be made only across sites and within one year.

Total mineral nitrogen

In spring 2013–2015 total mineral N was measured at all sites (Table 10). In spring 2013, following a very wet winter, all sites showed similarly low concentrations, with 1 the highest index for nitrate-N (). Both the only site to which N had been applied in 2012 (Roseworthy), and the only site having a history of organic fertiliser (Penventon), shared these low levels. Seven of the sites had previously been cropped with brassicas (Roseworthy for 3 years), but lower or higher concentrations of nitrate-N occurred irrespective of whether there had been previous brassicas. By 2014 more N was available, with nitrate-N indices of 1 to 3, ammonium-N levels having fallen to ca 3ppm. By 2015 the available nitrate-N was variable, with indices between 0 and 3, the Kelynack site having high levels of nitrate-N but also of increased ammonium-N.

Table 10. Total mineral N in the root zone (ppm) with (in brackets) ADAS indices, measured in spring 2013–2015

Site reference and name	2013		2014		2015	
	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N
A Kelynack	10.6 [0]	6.0 [0]	23.7 [1]	3.1 [0]	73.6 [3]	34.4 [1]
B St Buryan	11.0 [0]	5.5 [0]	34.6 [2]	3.2 [0]	43.5 [2]	5.3 [0]
C Tregiffian	16.7 [1]	6.0 [0]	52.3 [3]	3.2 [0]	21.8 [1]	13.4 [0]
D Rosevidney	12.5 [0]	4.6 [0]	21.5 [1]	2.9 [0]	1.2 [0]	3.8 [0]
E Roseworthy	10.8 [0]	6.4 [0]	22.4 [1]	3.0 [0]	17.1 [1]	4.5 [0]
F Bodilly	17.0 [1]	5.9 [0]	43.1 [2]	3.3 [0]	48.6 [2]	10.3 [0]
G Mawla	11.3 [0]	6.7 [0]	22.5 [1]	3.0 [0]	26.5 [2]	4.9 [0]
H Penventon	9.0 [0]	6.2 [0]	30.4 [2]	3.1 [0]	16.0 [1]	8.5 [0]
I Fourburrow	7.5 [0]	4.7 [0]	38.6 [2]	3.0 [0]	11.2 [0]	9.0 [0]
J Goonhavern	11.7 [0]	6.2 [0]	38.2 [2]	3.1 [0]	28.2 [2]	15.4 [0]

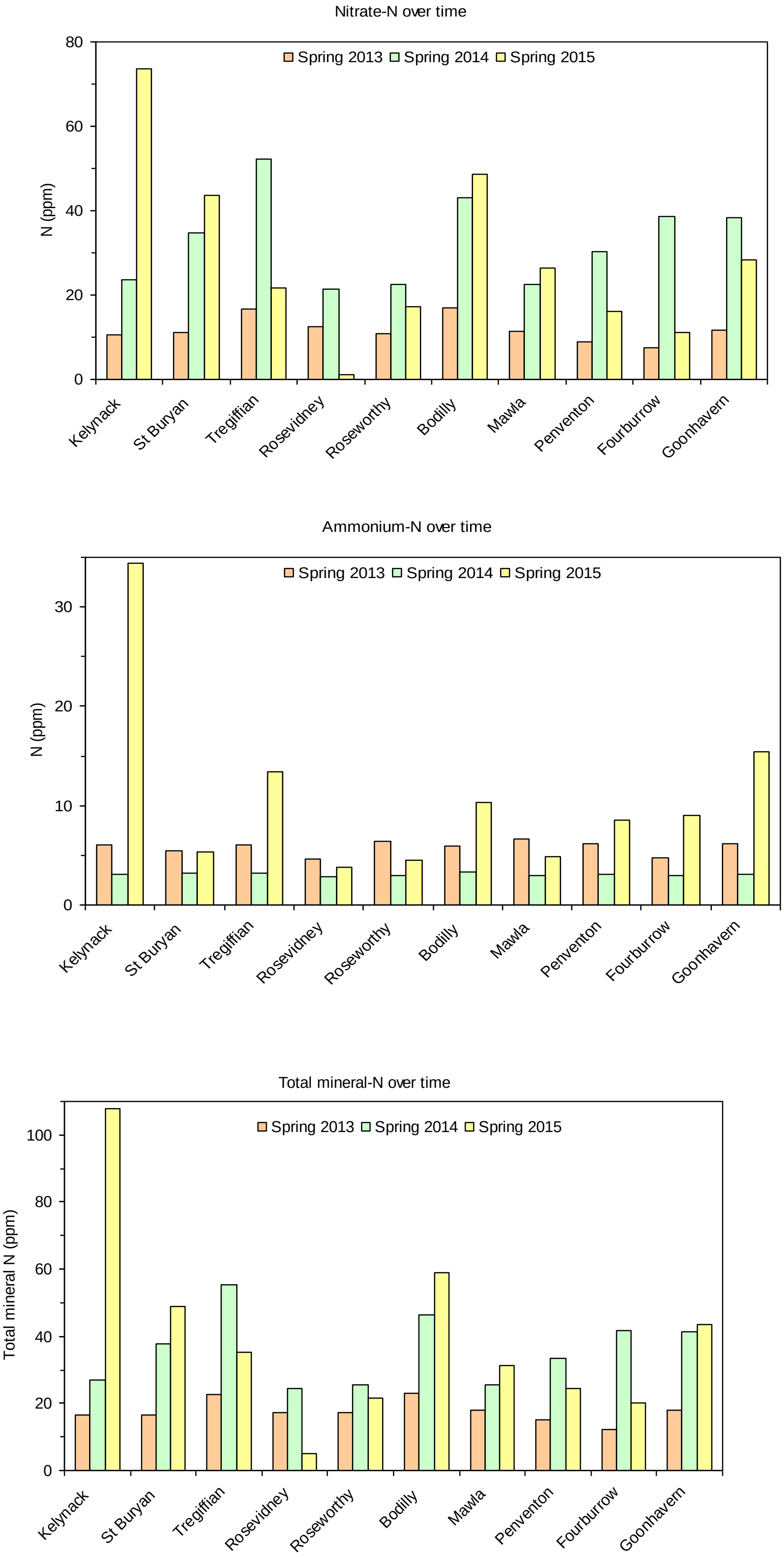


Figure 9. (Top) nitrate-N, (middle) ammonium-N and (bottom) total mineral N in the root zone, 2013 to 2015

Table 11. Soil analysis March 2013 (ppm, ADAS indices for P, K and Mg in brackets)

Site reference and name	pH	Nutrient concentration (ppm)		
		P	K	Mg
A Kelynack	7.0	58 [4]	200 [2-]	151 [4]
B St Buryan	6.6	51 [4]	385 [3]	245 [5]
C Tregiffian	6.8	38 [3]	186 [2-]	149 [4]
D Rosevidney	6.7	49 [4]	401 [4]	104 [4]
E Roseworthy	7.4	53 [4]	303 [3]	107 [4]
F Bodilly	7.0	49 [4]	170 [2-]	145 [4]
G Mawla	7.3	42 [3]	330 [3]	78 [3]
H Penventon	6.1	62 [4]	105 [1]	113 [4]
I Fourburrow	5.9	23 [2]	209 [2-]	58 [3]
J Goonhavern	6.9	22 [2]	176 [2-]	120 [4]

	Al	Ca	Fe	Mn	Na
A Kelynack	21.3	4116	1.4	0.5	920
B St Buryan	16.4	4090	1.9	1.1	866
C Tregiffian	16.7	3101	2.9	1.4	922
D Rosevidney	14.3	3011	1.1	3.5	976
E Roseworthy	15.1	4134	2.9	0.5	985
F Bodilly	35.2	4097	3.8	1.6	1107
G Mawla	17.8	3911	1.4	0.9	772
H Penventon	20.1	2803	1.6	5.1	780
I Fourburrow	24.5	1709	1.0	5.8	887
J Goonhavern	19.6	2898	1.2	2.1	799

Table 12. Soil analysis April 2014 (ppm, ADAS indices for P, K and Mg in brackets)

Site reference and name	pH	Topsoil OM (%w/w)	Nutrient concentration (ppm)		
			P	K	Mg
A Kelynack	7.4	7.0	67 [4]	105 [1]	88 [2]
B St Buryan	6.2	10.3	45 [3]	225 [2]	163 [3]
C Tregiffian	6.4	8.4	30 [3]	94 [1]	114 [3]
D Rosevidney	6.8	5.8	53 [4]	324 [3]	74 [2]
E Roseworthy	7.6	6.2	60 [4]	194 [2]	72 [2]
F Bodilly	7.2	7.8	43 [3]	94 [1]	81 [2]
G Mawla	7.6	7.0	44 [3]	251 [3]	55 [2]
H Penventon	6.0	6.0	61 [4]	59 [0]	87 [2]
I Fourburrow	5.6	5.6	19 [2]	152 [2]	39 [1]
J Goonhavern	7.4	5.7	16 [2]	77 [1]	66 [2]

	Ca	Fe	Mn	Na	Cu	Zn	Mo	SO_4	B
A Kelynack	1849	97	0.7	89	3.0	2.2	0.3	29.3	1.0
B St Buryan	1707	181	0.7	67	1.3	2.2	0.3	37.1	0.9
C Tregiffian	1561	42	2.2	278	4.0	1.1	0.5	59.5	0.9
D Rosevidney	1497	66	4.2	41	23.7	4.9	0.4	18.4	1.3
E Roseworthy	2189	23	4.1	47	18.6	4.2	0.4	20.7	1.3
F Bodilly	2085	86	1.6	48	5.3	2.2	0.4	52.0	0.6
G Mawla	1886	50	4.3	40	39.3	7.0	0.4	23.6	1.3
H Penventon	1576	134	4.0	46	49.3	7.9	0.4	21.9	0.6
I Fourburrow	880	46	5.8	37	1.9	1.1	0.2	22.0	0.9
J Goonhavern	1425	41	4.7	28	7.4	18.1	0.3	30.8	0.7

Table 13. Soil analysis April 2015 (ppm, ADAS indices for P, K and Mg in brackets)

Site reference and name	pH	Topsoil OM (%w/w)	Nutrient concentration (ppm)		
			P	K	Mg
A Kelynack	7.8	7.5	63 [4]	167 [2-]	85 [2]
B St Buryan	6.5	11.4	49 [4]	257 [3]	153 [3]
C Tregiffian	7.0	8.6	30 [3]	162 [2-]	112 [3]
D Rosevidney	7.0	5.9	56 [4]	416 [4]	77 [2]
E Roseworthy	8.0	6.5	68 [4]	242 [3]	70 [2]
F Bodilly	7.5	8.5	45 [3]	142 [2-]	78 [2]
G Mawla	7.5	7.3	47 [4]	294 [3]	65 [2]
H Penventon	6.4	6.6	62 [4]	67 [1]	86 [2]
I Fourburrow	5.9	5.7	28 [3]	227 [2+]	47 [1]
J Goonhavern	7.3	6.1	21 [2]	127 [2-]	81 [2]

	Al	Ca	Fe	Mn	Na	Cu	Zn	Mo	SO_4	B
A Kelynack	275	2265	125	1.2	97.5	4.7	3.0	0.3	23.7	1.2
B St Buryan	477	1970	245	1.2	68.5	3.1	3.8	0.5	35.2	1.1
C Tregiffian	309	1740	58	4.4	238.0	5.0	1.3	0.5	45.4	1.1
D Rosevidney	150	1970	81	8.9	50.5	24.8	5.5	0.4	18.6	1.4
E Roseworthy	85	2615	27	8.1	42.5	20.8	4.5	0.4	16.6	1.5
F Bodilly	252	2165	82	2.5	33.5	7.1	2.7	0.4	30.3	0.7
G Mawla	137	2027	63	9.6	66.3	49.5	8.2	0.4	24.8	1.6
H Penventon	240	1519	160	6.9	40.8	63.1	9.8	0.4	17.4	0.7
I Fourburrow	240	864	69	13.6	48.8	2.8	2.1	0.2	30.6	1.1
J Goonhavern	84	1698	56	11.2	30.8	10.0	20.1	0.2	24.5	0.8

Soil pH

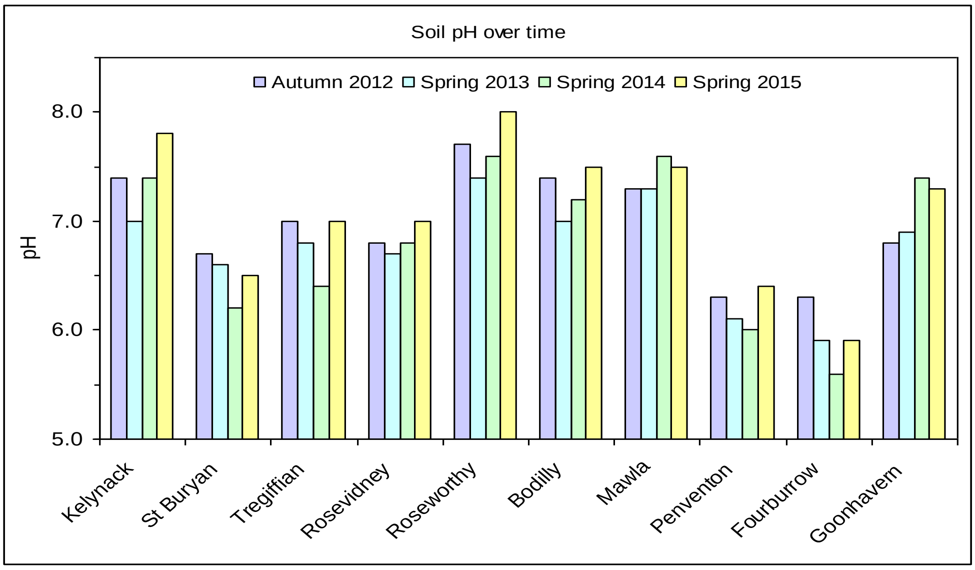


Figure 10. Soil pH in 2012 to 2015

Soil pH initially varied from slightly acidic (pH 6.3) at Penventon and Fourburrow to slightly alkaline at Roseworthy (pH 7.7, indicating a naturally lime-rich soil or a tendency to over-lime), the remaining sites being neutral (pH 7.0±0.4) (Figure 10) Over the course of the project the higher-pH sites remained slightly alkaline and the lower-pH sites remained close to pH 6.0. By the end of the project pH levels remained in a range suitable for daffodil growing (pH 6.0–7.5) at all sites but two, Kelynack and Roseworthy having become slightly more alkaline (pH 7.8–8.0).

Topsoil organic matter

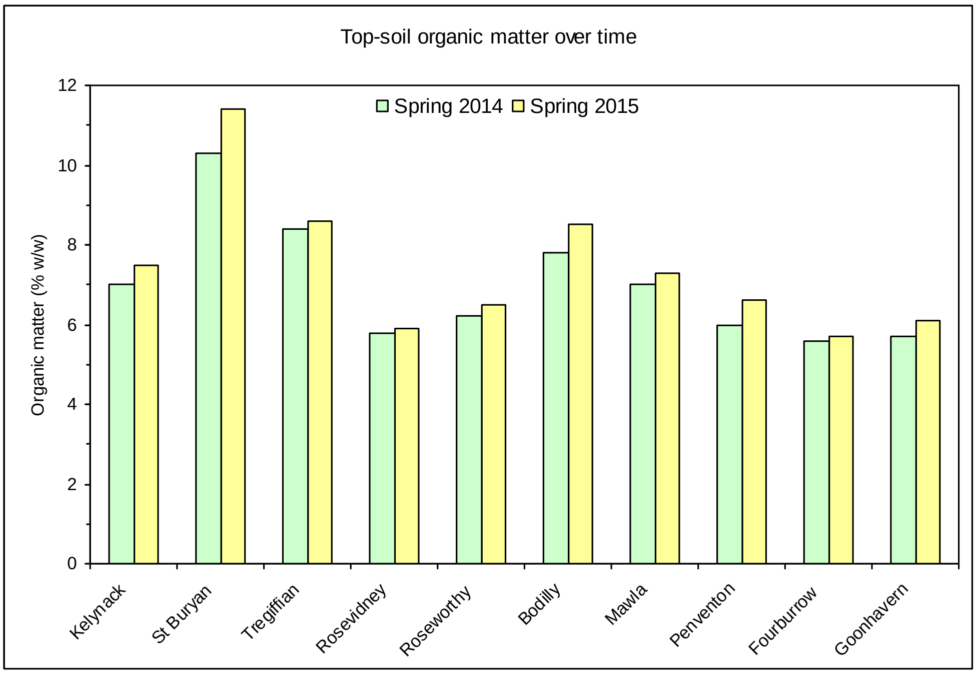


Figure 11. Percentage topsoil OM in 2014 and 2015

Topsoil OM was recorded in 2014 and 2015 (Figure 11). The percentage of OM varied relatively little from site to site, between *ca* 6% at Rosevidney, Roseworthy, Penventon, Fourburrow and Goonhavern and *ca* 11% at St Buryan. Although it is not feasible to suggest an ‘ideal’ percentage of OM, soils with between 10% and 20% OM are regarded as organic and may release more N, P and S by microbial action than non-organic soils. While a higher OM content is generally desirable, it could also be indicative of poor drainage.

Phosphorus

In autumn 2012, after fertiliser had been applied earlier, soil P concentrations would have been adequate at all sites, despite the concentrations found varying between about 60ppm (index 4) at Fourburrow and Goonhavern and *ca* 160ppm (index 7) at Kelynack, St Buryan, Bodilly and Penventon (Figure 12). Goonhavern appears to have been depleted previously, with an index of 0 in the grower's analysis in August of that year. The autumn measurements did not relate obviously to the amount of P fertiliser that had been applied, since Kelynack and Penventon, with high P levels and the only sites not needing it, had *not* previously received a substantial applications of P fertiliser.

By spring 2013 the concentrations of P were between 22 and 62ppm (index 2 to index 4) at the different sites, then changing little over the next two years. It is unlikely that any of the plants would have been deficient in P, even at Fourburrow and Goonhavern, where levels of P were lowest. Before planting bulbs, only small amounts of phosphate fertiliser would be applied to soils that had P indices of 3, in order to maintain an appropriate level in the soil.

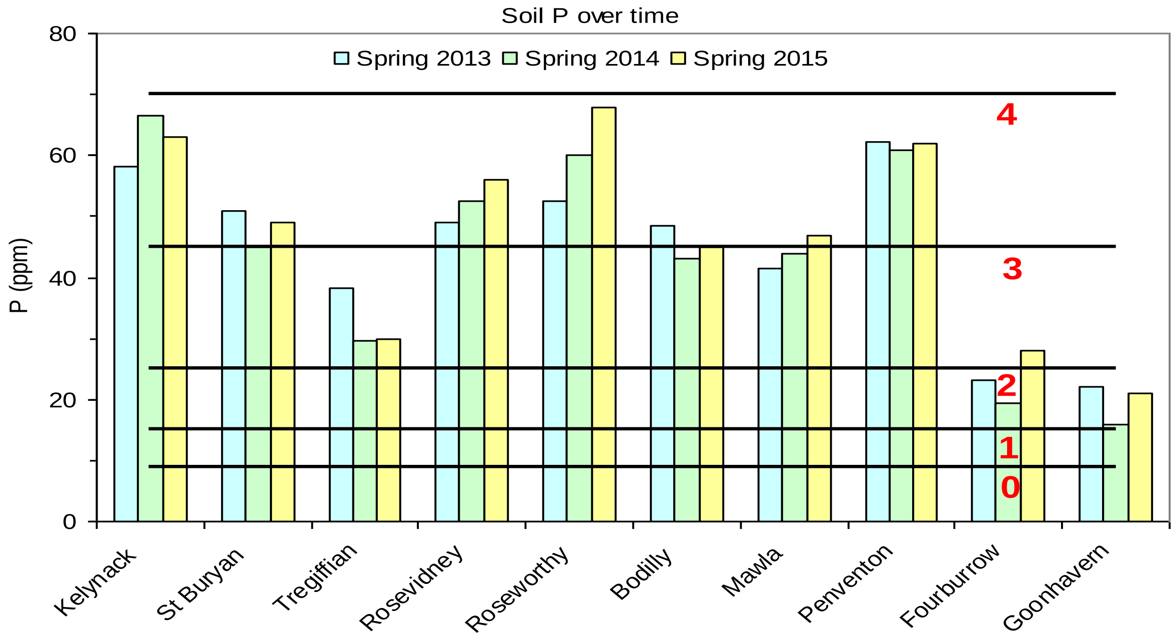


Figure 12. Soil P concentrations in 2013 to 2015; the horizontal lines show the upper limits of ADAS P-indices (in red) from 0 (9ppm) through 4 (70ppm)

Potassium

In autumn 2012, after fertiliser had been applied earlier, soil K levels ranged from 118ppm (index 1) at Penventon to around 450ppm (index 4) at St Buryan and Rosevidney (Figure 13). K concentrations were lower over the next two years, probably enhanced by the wet winters, to between 59ppm (index 0) at Penventon and 324ppm (index 3) at Rosevidney. By spring 2015 K concentrations appear to have increased slightly at all sites, between 67ppm (index 1) at Penventon and 416ppm (index 4) at Rosevidney. It is unlikely that any of the plants would have been deficient in K, though when planting bulbs potash fertiliser would normally be applied to soils with K indices of 3 in order to maintain appropriate levels in the soil.

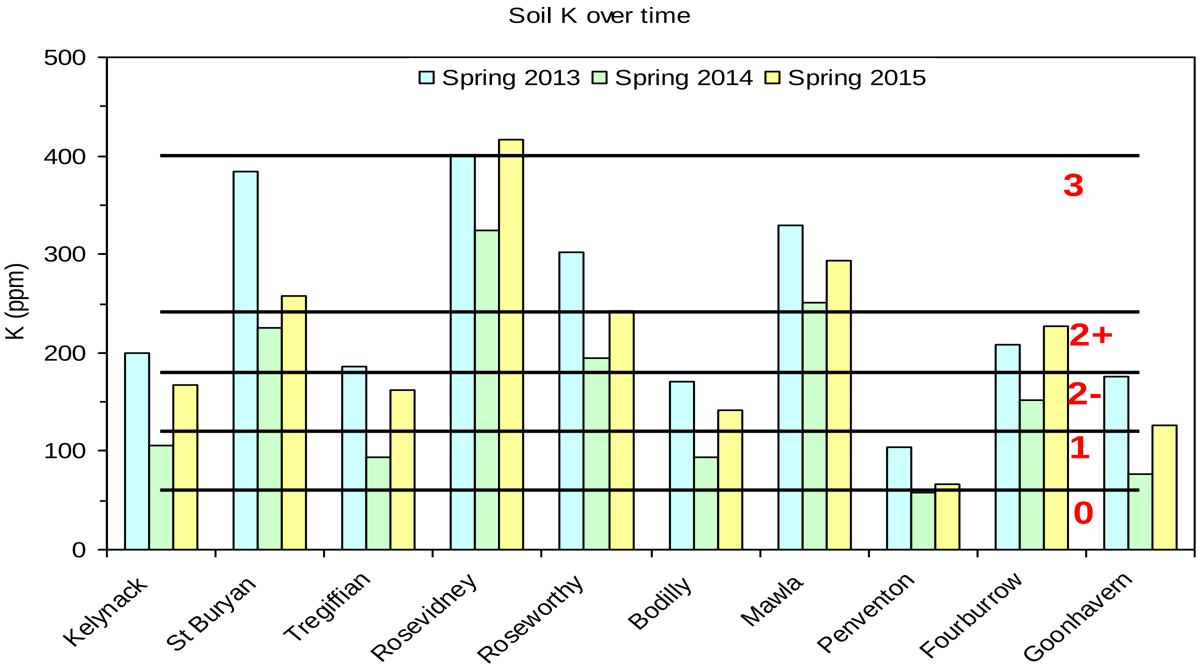


Figure 13. Soil K concentrations in 2013 to 2015; the horizontal lines show the upper boundaries of ADAS K-indices (in red) from index 0 (0–60ppm K) through 1, 2- and 2+ to index 3 (241–400ppm K)

Magnesium

In autumn 2012, after fertiliser had been applied earlier, soil Mg levels initially varied from 56ppm (index 2) at Fourburrow to 233ppm (index 4) at St Buryan, all representing at least adequate levels of Mg (Figure 14). Mg concentrations had fallen by 2014 to between 39ppm (index 1) at Fourburrow and 163ppm (index 3) at St Buryan, and had changed little by 2015. It is unlikely that any of the plants would have been deficient in Mg, though when planting bulbs magnesium fertiliser would not normally be applied to soils with Mg indices above 1. It is possible, in some wet seasons on poorly structured soils with poor rooting, that temporary Mg deficiency symptoms could be seen at the beginning of spring growth, though this is rare.

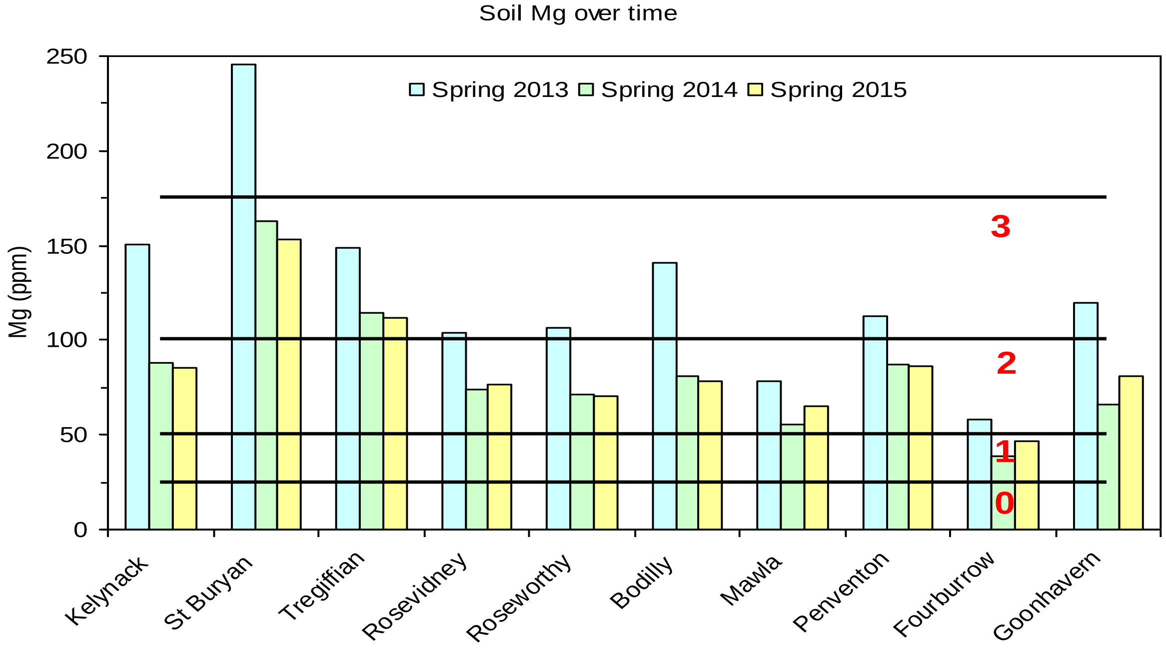


Figure 14. Soil Mg concentrations in 2013 to 2015; the horizontal lines show the upper limits of ADAS Mg-indices (in red) from 0 (25ppm) through 3 (175ppm)

Trace elements and other nutrients

Aluminium (Al) is not an essential trace element but can benefit some crops. Due to a change in the analytical methods used to measure soil Al concentration, comparisons should be made only between sites within years (Figure 15). In 2013 Al levels were low and varied relatively little between sites, from 14 to 35ppm. In 2015, using a different analytical method, much higher concentrations were obtained and the range of values was greater, between *ca* 85ppm at Roseworthy and Goonhavern and *ca* 480ppm at St Buryan. The site to site differences were not consistent between years.

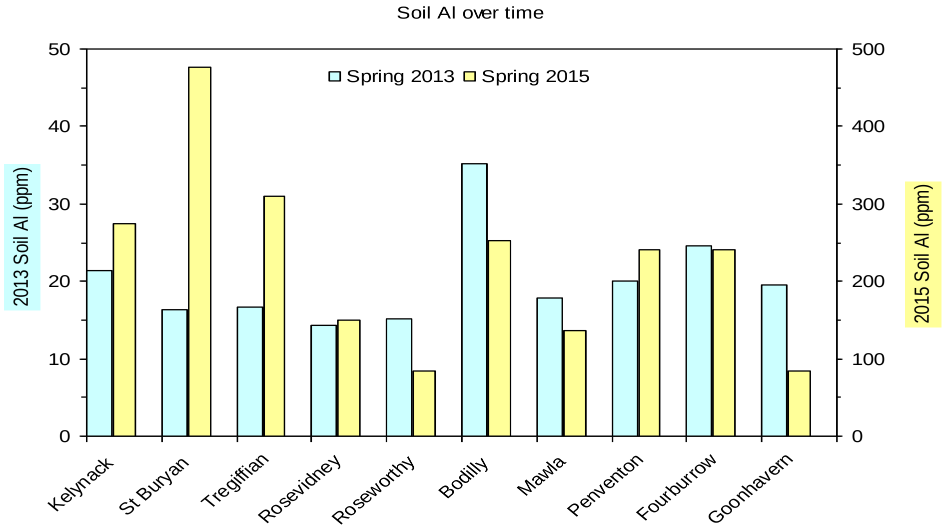


Figure 15. Soil Al concentrations in 2013 (left axis) and 2015 (right axis)

Boron (B) concentrations were relatively consistent, varying between 1.3ppm at Rosevidney, Roseworthy and Mawla and 0.6 to 0.9ppm at the remaining sites in 2014, and over the next year increasing by 0.1–0.3 at each site (Figure 16). For sensitive crops (sugar beet, brassicas and carrots) deficiencies are possible where soil analysis shows B concentrations lower than 0.5 or 0.8ppm. Below 1.0ppm might be regarded as a low level, and between 1.0 and 2.0ppm a satisfactory level on sandy loams and sands. Daffodils are not known as a crop sensitive to trace element deficiencies generally, and no instances of B deficiency appear to have been reported for mainstream daffodil cultivars growing in the UK. There are, however, a few reported instances of B deficiency in daffodils (see ‘Discussion’).

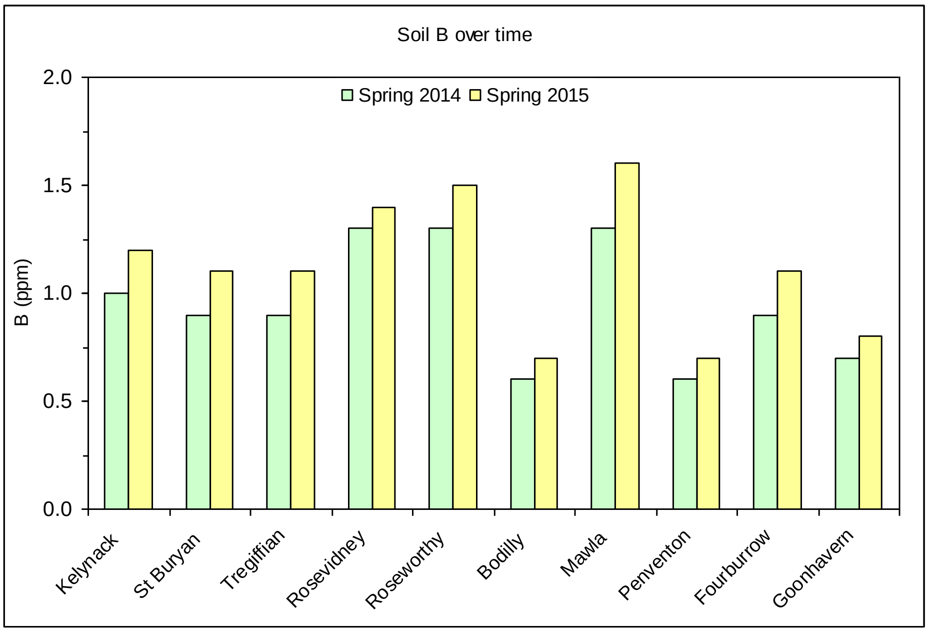


Figure 16. Soil B concentrations in 2014 and 2015

Calcium (Ca): In spring 2013 Kelynack, St Buryan, Roseworthy, Bodilly and Mawla had relatively high Ca levels, around 4,100ppm, while the lowest level occurred at Fourburrow (1,709ppm) (Figure 17). By spring 2014 Ca levels had fallen by nearly a half, perhaps due to the wet weather. Ca concentrations in spring 2015 varied from *ca* 850ppm at Fourburrow to 2615ppm at Roseworthy. Calcium has been reported as important in daffodil growing for root growth and bulb yields.

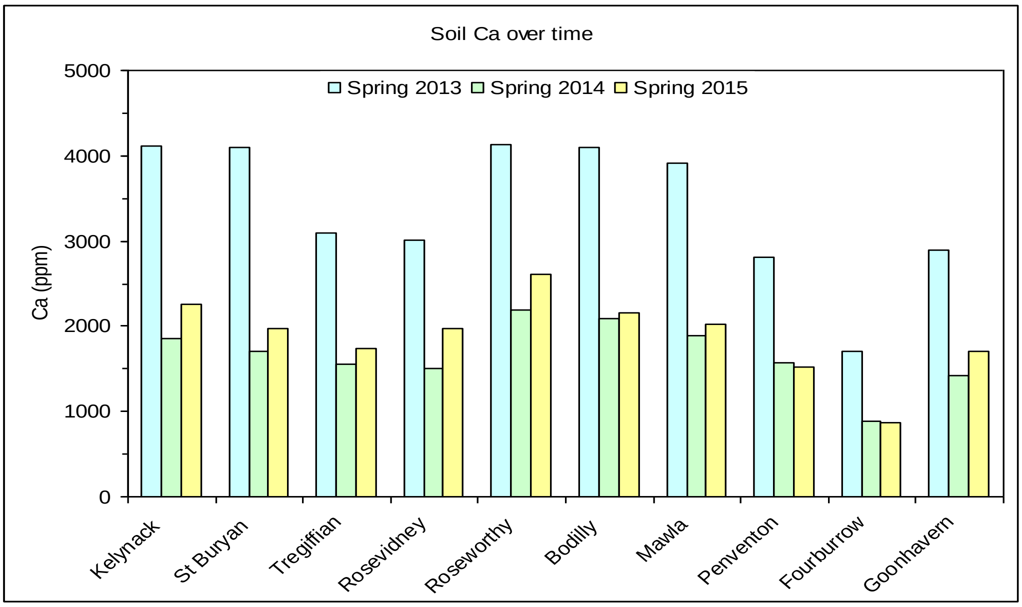


Figure 17. Soil Ca concentrations in 2013 to 2015

Copper (Cu) concentrations in the soil showed very marked differences between sites (Figure 18). For example, in 2014 Mawla and Penventon exhibited high levels of Cu (39 and 49ppm, respectively) and Rosevidney and Roseworthy somewhat lower levels (19 to 24ppm), while at the other sites Cu levels varied from 7.4 (Goonhavern) to 1.3ppm (St Buryan). In spring 2015 Cu concentrations at all sites had increased slightly, taking the levels at Mawla and Penventon to 50 and 63ppm, levels that would be potentially harmful for susceptible crops, especially if associated with a pH<6.5 – which applies to Penventon, but not Mawla. Some crops have been reported to suffer Cu deficiency on lighter soils containing <1.6mg/L, but no symptoms have been reported for daffodils as far as is known.

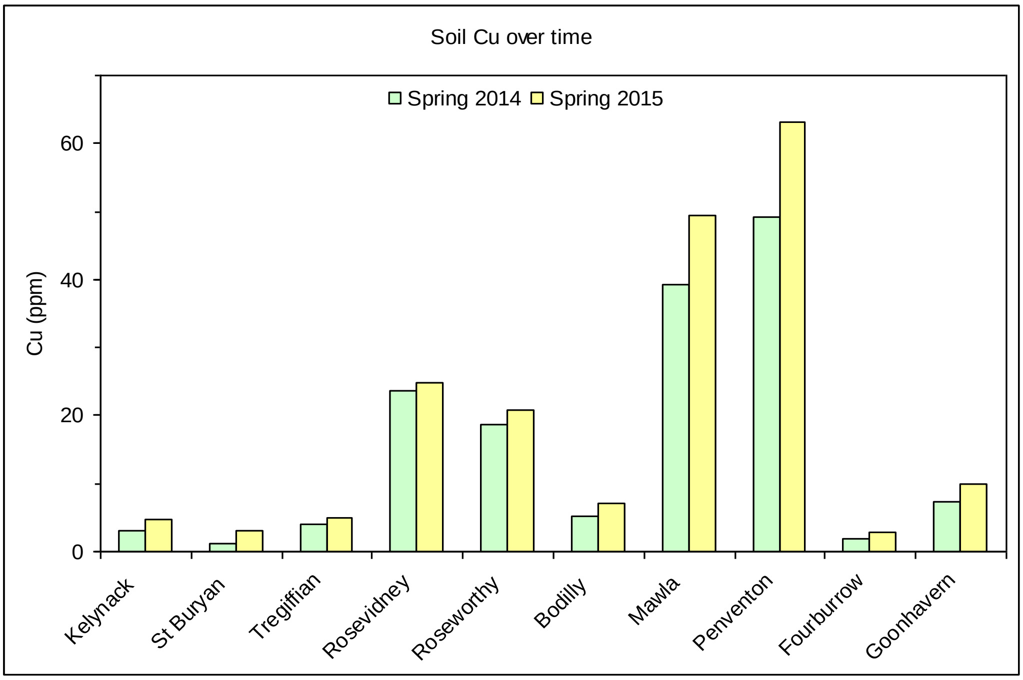


Figure 18. Soil Cu concentrations in 2014 and 2015

Iron (Fe) measurements suffered a change in analytical methods over years, so comparisons should be made only within a year (see 'Materials and methods'). Deficiencies in Fe cannot reliably be diagnosed through soil or plant analysis, as Fe levels depend on pH, water-logging and many other factors. It can be seen from Figure 19 that the method used in 2013 (cation exchange using barium chloride) produced very low concentrations. Subsequently DTPA extraction was used. In 2014 Fe concentrations varied from 23ppm at Roseworthy to 181ppm at St Buryan, and by spring 2015 Fe levels had generally increased. There was no reason to suspect a deficiency at the trial sites.

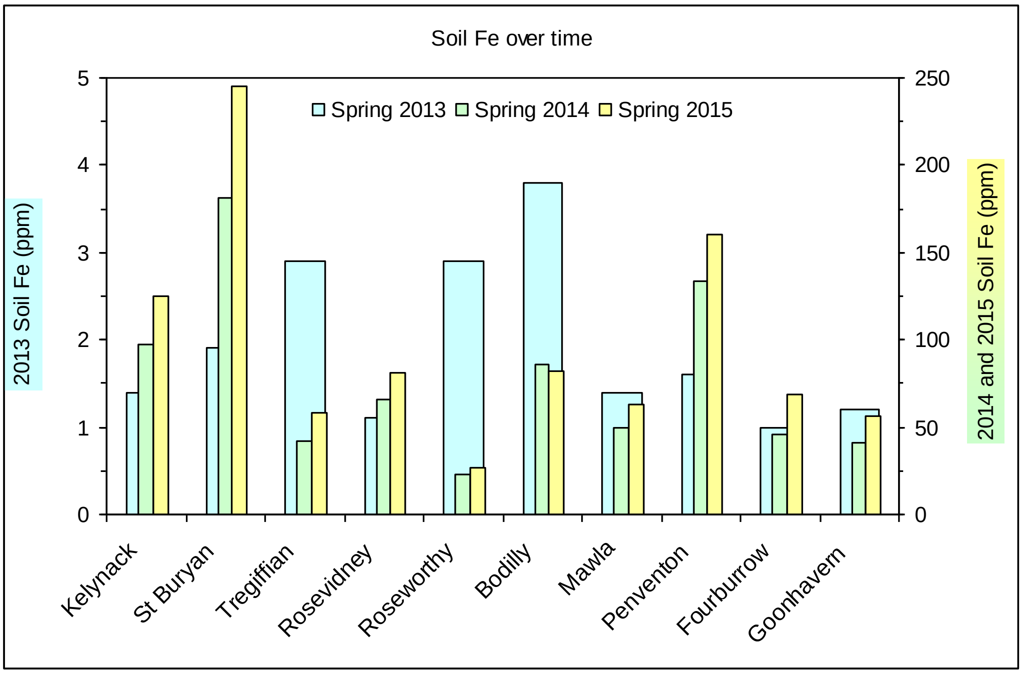


Figure 19. Soil Fe concentrations 2013–2015

Manganese (Mn) concentrations varied considerably between sites (Figure 20). In the 2013 analyses Mn levels at Kelynack, St Buryan, Tregiffian, Roseworthy, Bodilly, Mawla and Goonhavern were relatively low, below about 2ppm, while at the remaining sites they were relatively high (3.5-5.8ppm). Mn concentrations appear to have increased at most sites between 2013 and 2014 or 2015, and only Kelynack, St Buryan and Bodilly remained in the low-Mn group. Soil analysis is unreliable as a diagnostic tool for Mn as it is influenced by pH, soil texture and OM content. No specific Mn-deficiency symptoms have been reported for daffodils, although it is one of the most widely reported deficiencies in field crops, affecting sugar beet, cereals and peas on high-pH organic, peaty, sandy and over-limed soils and resulting in symptoms such as pale, limp foliage, interveinal mottling, in-curling leaf margins and internal discolouration. It is known that some growers have applied Mn to daffodils on peat soils as a precautionary measure, but they have not reported actual symptoms of deficiency (nor harmful effects due to Mn applications).

Molybdenum (Mo) levels in soil over 2014–2015 were consistent, varying between only 0.2 and 0.5ppm (Figure 21). Mo concentrations increase with pH and deficiencies can occur on acid soils or un-limed peats, but symptoms have not been reported for daffodils.

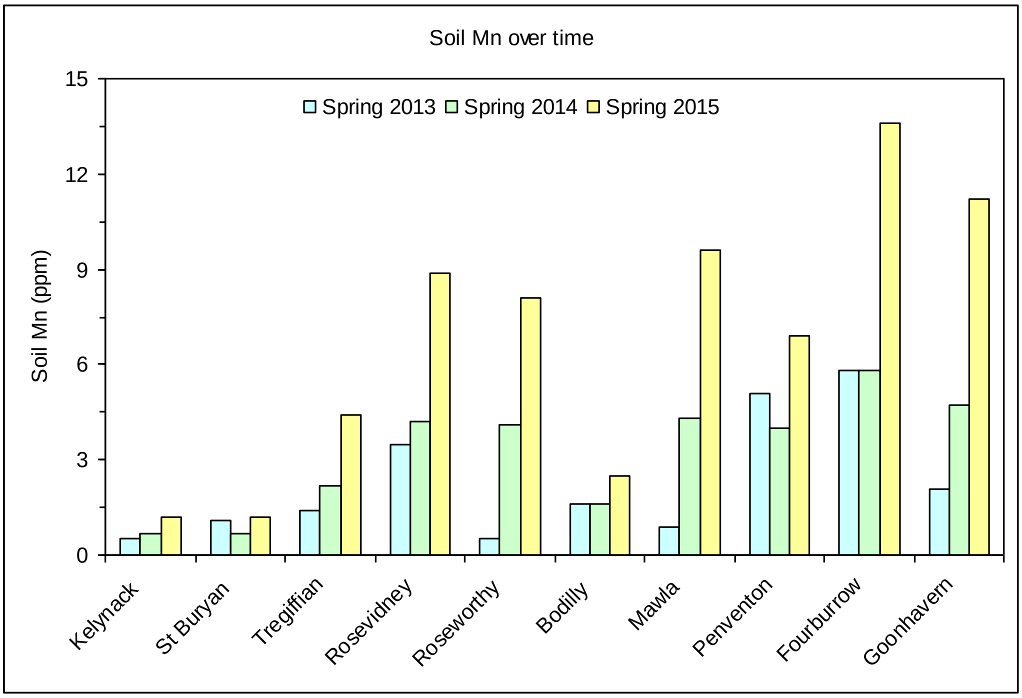


Figure 20. Soil Mn concentrations between 2013 and 2015

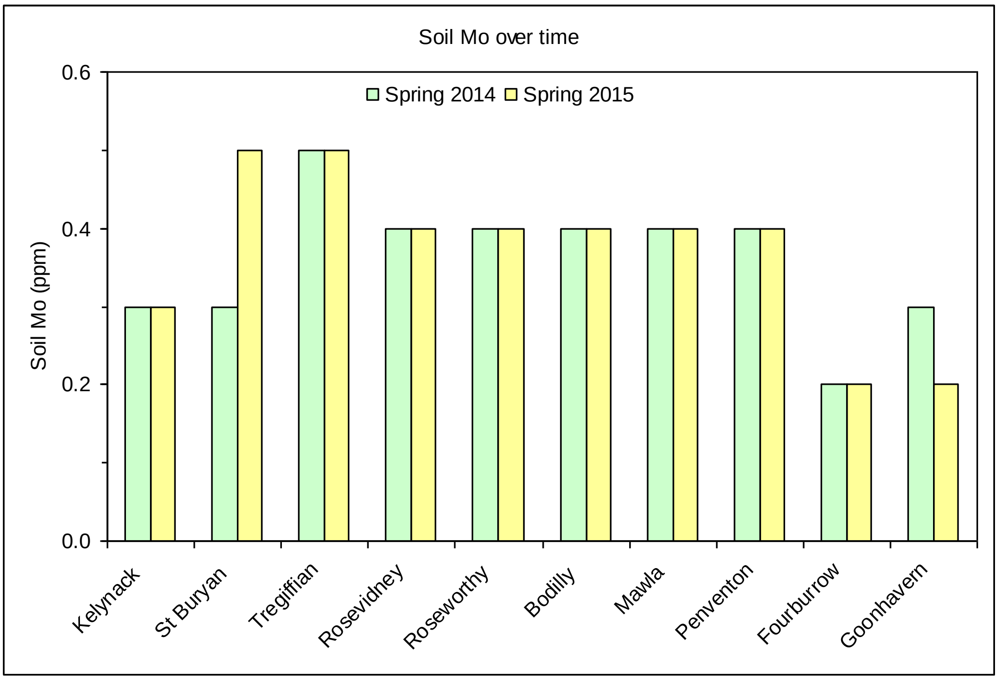


Figure 21. Soil Mo concentrations in 2014 and 2015

Sodium (Na) measurements were subject to a change in analytical methods over the years, so comparisons should be made only within a year (see 'Materials and methods') (Figure 22). It can be seen that the method used in 2013 (cation exchange using barium chloride) produced much higher concentrations than ammonium nitrate extraction used subsequently. In 2013 concentrations ranged between about 750 and 1,100ppm, with the lowest values for Mawla, Penventon and Goonhavern. The 2014 and 2015 analyses gave concentrations <100ppm, with the exception of Tregiffian where the concentration was >200ppm. No responses to Na seem to have been reported for daffodils.

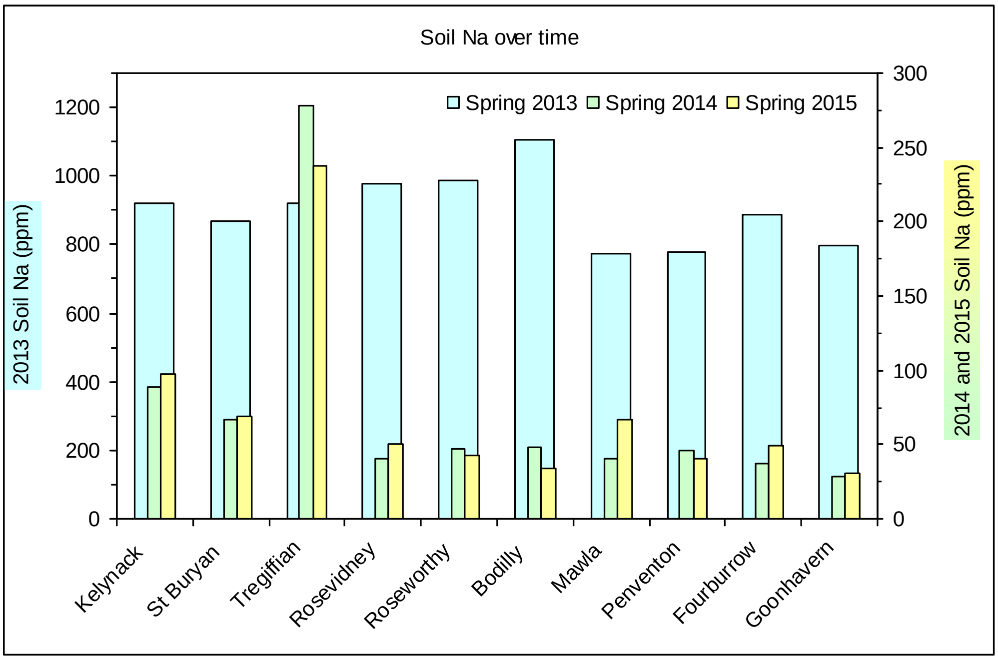


Figure 22. Soil Na concentrations 2013–2015

Sulphate (SO_4) soil concentrations in spring 2014 were relatively low at Rosevidney, Roseworthy, Mawla, Penventon and Fourburrow (18-24ppm) and relatively high at Tregiffian and Bodilly (52 and 60ppm) (Figure 23). However, at Tregiffian and Bodilly SO_4 levels had dropped markedly by spring 2015, while at the sites with initially lower SO_4 any changes in concentration were small and variable. Low sulphate levels are likely only on light soils following very wet winters.

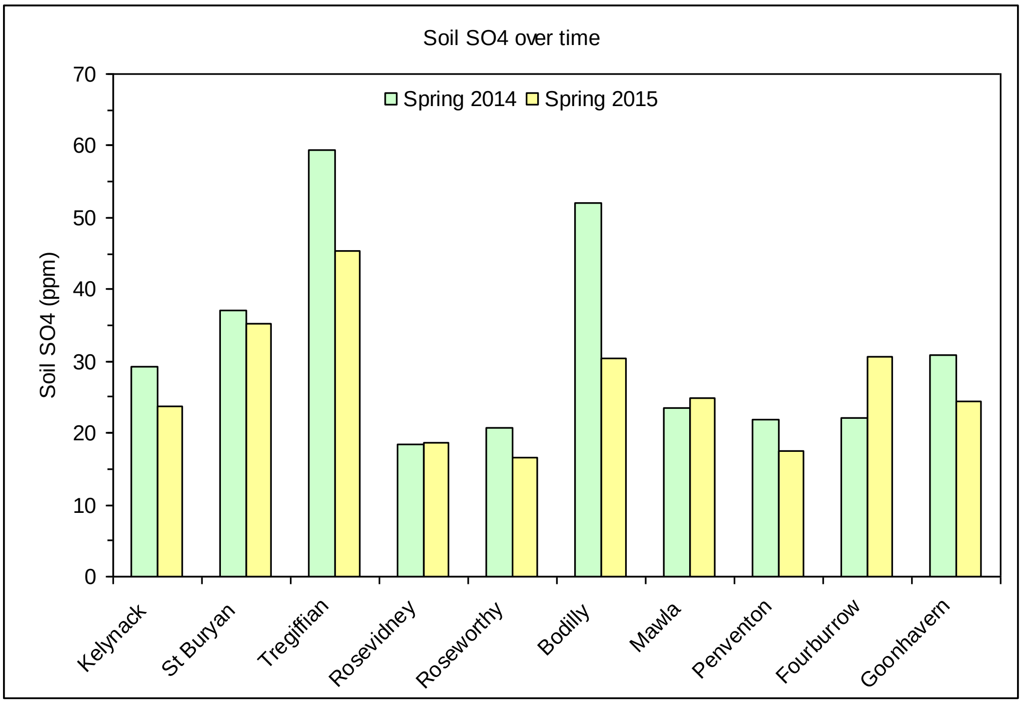


Figure 23. Soil SO_4 concentrations in 2014 and 2015

Zinc (Zn) concentrations varied considerably between sites, being much higher at Goonhavern than elsewhere in both 2014 and 2015 (18–20ppm) (Figure 24). The lowest levels, 1–2ppm, were found at Tregiffian and Fourburrow. As in the case of sulphate, low Zn levels are likely only on light soils following very wet winters. Zn-deficiency occurs most notably on top-fruit and forest nursery stock growing on sandy soils with high pH and high phosphate. No deficiencies have been reported for daffodils. Zn concentrations <2ppm are regarded as very low and 3–5ppm as normal, while up to 130ppm is probably safe. For susceptible crops, soil analysis of <1.5ppm would indicate a possible deficiency and <0.5ppm the risk of a probable deficiency.

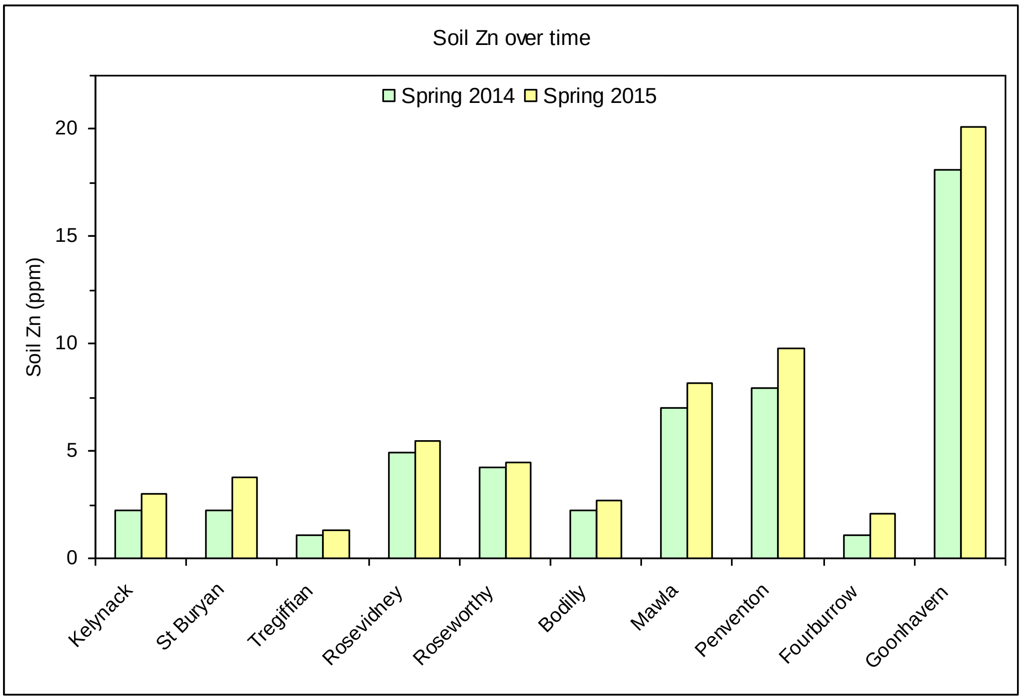


Figure 24. Soil Zn concentrations in 2014 and 2015

Leaf analysis

The results are summarised in Table 15 (major nutrients) and Table 16 (trace elements). Few ‘reference’ nutrient concentrations are available for daffodils, but some published values for the major nutrients and calcium are given in Table 14. There was good agreement between the concentrations found in the present project and these published values.

Table 14. Some examples of reported ‘normal’ nutrient concentrations in daffodil tissues and, for comparison, the range of concentrations found in the present study

Plant part sampled	Tissue nutrient content (% fresh weight)					Literature source[1]
	N	P	K	Mg	Ca	
Leaf	2.30–3.80	0.21–0.42	1.30–3.20	0.11–0.22	n/d[2]	This project
	3.70	0.46	2.75	0.18	n/d	1
	2.60	0.33	1.70	0.15	0.35	2
	0.42	n/d	n/d	n/d	n/d	5
Stem	2.29	0.39	2.83	0.12	0.29	3
Bulb	1.15	0.34	0.81	0.17	1.71	3
	1.10	0.16	1.00[3]	0.06	n/d	1
	1.40	1.40	1.23	0.09	0.39	4 (high)
	0.38	0.30	0.64	0.06	0.21	4 (low)
	n/d	0.24	0.63	n/d	n/d	6
	0.72	n/d	n/d	n/d	n/d	5

[1] 1, Rosewarne EHS data quoted by ADAS (1976); 2, ADAS Starcross data quoted by ADAS (1976); 3, Data quoted in Annual Report of project BOF 76 (2013); 4, Ruamrungsri *et al*. (1997); 5, Vickery (1976); 6, ‘*RB 209*’

[2] Not determined [3] or 0.50% in deficient bulbs

Table 15. Leaf chemical analysis (major nutrients) in April 2014 and 2015

2014	Concentration (% w/w)				
	N	P	K	Mg	S
A Kelynack	3.02	0.3962	2.4400	0.1954	0.1967
B St Buryan	3.15	0.3613	2.9800	0.1811	0.2209
C Tregiffian	3.81	0.3497	1.9300	0.2235	0.2352
D Rosevidney	2.85	0.4100	3.2000	0.1390	0.2041
E Roseworthy	3.14	0.4045	2.5100	0.1302	0.2031
F Bodilly	3.18	0.4189	1.9700	0.1517	0.1962
G Mawla	3.23	0.3250	2.6900	0.1368	0.2078
H Penventon	2.67	0.3714	1.9400	0.1583	0.1732
I Fourburrow	3.16	0.3226	2.7300	0.1424	0.1999
J Goonhavern	3.37	0.3094	2.4200	0.1364	0.2094
2015					
A Kelynack	2.66	0.2840	1.8662	0.2020	0.1374
B St Buryan	2.88	0.2439	2.7562	0.1654	0.1697
C Tregiffian	3.02	0.2072	1.3069	0.1809	0.1546
D Rosevidney	2.34	0.2852	2.7104	0.1090	0.1259
E Roseworthy	2.45	0.3181	2.4748	0.1119	0.1422
F Bodilly	3.16	0.2749	1.3951	0.1355	0.1461
G Mawla	2.50	0.2614	2.3302	0.1096	0.1478
H Penventon	2.60	0.3112	1.5292	0.1765	0.1359
I Fourburrow	3.01	0.2055	2.2491	0.1112	0.1501
J Goonhavern	2.66	0.2273	1.9715	0.1118	0.1357

Leaf nitrogen

In spring 2014 leaf N varied from 2.7% at Penventon to 3.8% at Tregiffian (Table 15). The levels in 2015 were lower, with an average value of 2.7%, perhaps a consequence of the normal increasing plant size over the three years of the crop. There was no loss of foliage colour by 2015, so it is unlikely that there was a deficiency in N.

Leaf phosphorus

In 2014 leaf P was relatively high at Kelynack, Rosevidney, Roseworthy and Bodilly (*ca* 0.40%) and relatively low at Mawla, Fourburrow and Goonhavern (*ca* 0.31%) (Table 15). In 2015 leaf P concentrations were lower at all sites, by an average of 29%.

Leaf potassium

In 2014 the concentration of leaf K was highest at St Buryan and Rosevidney (*ca* 3.0%) and lowest at Tregiffian, Bodilly and Penventon (*ca* 2.0%) (Table 15). By spring 2015 K concentrations were lower at all sites, by an average of 17%. Potassium concentrations remained highest at St Buryan and Rosevidney and lowest at Tregiffian, Bodilly and Penventon.

Leaf magnesium

Leaf Mg concentrations in 2014 was notably higher (*ca* 0.20%) at the three western-most sites, Kelynack, St Buryan and Tregiffian; at the other sites Mg concentrations were *ca* 0.14% (Table 15). By 2015 these values had fallen to 0.18 and 0.12%.

Leaf sulphur

Foliar concentrations of S in 2014 varied from a low of 0.17% at Penventon to a high of 0.24% at Tregiffian (Table 15). By the next year concentrations were lower (0.13-0.17%).

Trace and other nutrients in leaves (Table 16)

Aluminium (Al) levels in 2014 ranged quite widely, from 24ppm at Penventon to 112ppm at Mawla. Kelynack, St Buryan, Bodilly, Penventon and Goonhavern formed a low-Al group (24-52ppm) while Tregiffian, Rosevidney, Roseworthy and Mawla made a high-Al group (92-112ppm). A year later five sites – Kelynack, St Buryan, Tregiffian, Bodilly – showed substantially higher concentrations of leaf Al. More acidic soils would normally be expected to show higher Al concentrations, but in the present case soil pH did not vary widely. Although Al is not a direct trace element, in some circumstances it appears to have a beneficial effect on plant growth.

Table 16. Leaf chemical analysis (trace and other nutrients) in April 2014 and 2015

2014	**Concentration (ppm)**								
	Al	**Ca**	**Fe**	**Mn**	**Na**	**Cu**	**Zn**	**Mo**	**B**
A Kelynack	47	12,400	92	14.7	2,668	6.8	35.6	3.51	19.3
B St Buryan	47	9,000	122	14.7	1,252	7.6	47.0	1.42	20.1
C Tregiffian	99	14,600	204	56.4	2,281	8.1	29.2	0.43	23.4
D Rosevidney	101	9,300	206	38.1	605	8.4	44.3	2.15	20.8
E Roseworthy	92	10,300	198	50.5	856	7.9	29.0	3.99	19.3
F Bodilly	52	10,700	99	22.7	343	7.7	37.9	3.62	17.8
G Mawla	112	9,800	216	45.7	723	8.3	38.4	2.23	22.5
H Penventon	24	10,600	64	45.8	215	6.7	85.1	1.40	27.0
I Fourburrow	77	9,400	173	54.0	653	6.9	29.3	0.13	20.0
J Goonhavern	39	8,800	97	27.0	167	7.8	73.0	0.70	16.4
2015									
A Kelynack	77	12,787	102	9.4	2,470	6.1	21.4	1.97	13.9
B St Buryan	80	8,173	134	8.4	727	5.8	26.1	1.52	14.2
C Tregiffian	133	11,951	203	54.8	987	7.0	15.1	0.29	12.0
D Rosevidney	65	8,685	142	25.3	239	6.6	23.1	2.66	13.4
E Roseworthy	82	10,779	146	41.6	290	7.0	21.4	3.95	12.3
F Bodilly	87	11,335	121	17.8	234	6.6	20.3	3.04	10.2

G	Mawla	86	9,554	160	30.6	749	6.9	22.0	1.51	13.7
H	Penventon	36	10,186	72	37.0	196	6.8	66.9	1.05	16.9
I	Fourburrow	59	8,679	136	64.0	491	5.9	18.1	0.12	12.0
J	Goonhavern	43	8,313	89	18.7	130	6.3	44.0	0.64	10.0

Boron (B) levels in 2014 at the sites varied across a fairly narrow band, from 16ppm at Goonhavern to 27ppm at Penventon. By 2015 levels were lower by a substantial and reasonably consistent amount. Leaf B concentrations <20ppm may be symptomatic of deficiency for susceptible crops on light soils with a high pH. With a soil pH of 7.4 the crop at Goonhavern may have been marginally at risk.

Calcium (Ca) levels measured in spring 2014 were higher at two sites, Kelynack (12,400ppm) and Tregiffian (14,600ppm), than elsewhere. Both Kelynack and Tregiffian are maritime situations, <1km distant from the sea. At the other, non-maritime, sites leaf Ca concentrations were lower, between 8,800 and 10,700ppm. The next year leaf Ca levels were higher at some sites and lower at others, with no overall trend. Other information (see 'Discussion') gives similar daffodil stem and bulb Ca concentrations of 0.21–1.71%.

Copper (Cu) foliar levels were rather consistent across the sites than for other elements. In 2014 levels varied little, from a low of 6.7ppm at Penventon to highs of 8.3-8.4ppm at Rosevidney and Mawla. With one exception – Penventon - leaf Cu levels were consistently lower the next year. Although leaf analysis is not regarded as a reliable indication of Cu deficiency, concentrations below 3ppm are sometimes considered deficient: on this basis no deficiencies would be expected at these sites.

Iron (Fe): analysis in 2014 gave two groups, with high-Fe (173-216mg/kg) at Tregiffian, Rosevidney, Roseworthy, Mawla and Fourburrow and low-Fe (64-122mg/kg) at the remaining sites. In general this distinction was still evident a year later, though Fe concentrations were lower at four of the five high-Fe sites (Rosevidney, Roseworthy, Mawla and Fourburrow). In the case of Fe, deficiencies cannot be reliably diagnosed by soil or plant analysis.

Manganese (Mn) foliar concentrations were highly variable across the sites. Measured in 2014 at Tregiffian, Roseworthy, Mawla, Penventon and Fourburrow, levels were relatively high (46-56ppm), while they were low at Kelynack and St Buryan (each 15ppm). By 2015 foliar Mn levels were somewhat lower at most sites. Leaf analysis is considered useful in determining Mn deficiencies, with concentrations below 20ppm being considered deficient, so the crops at Kelynack and St Buryan may have been at risk.

Molybdenum (Mo) leaf concentrations varied markedly between the sites. Measured in both years they varied from 0.1ppm at Fourburrow to 4.0ppm at Roseworthy. In the case of ‘whiptail’ disorder of cauliflower, caused by Mo deficiency on acidic soils, a leaf/curd Mo content of 2.0ppm is considered normal while 0.35ppm is considered deficient. On this basis the crop at Fourburrow – where the pH was 5.6 – could be at risk of deficiency.

Sodium (Na): leaf Na was found in particularly high concentrations (1,000–3,000ppm) at the three sites close to the sea (Kelynack, St Buryan and Tregiffian) in 2014, and just at Kelynack in 2015, probably as a result of sea spray after storms. At the other sites they were an order of magnitude lower (167-856ppm).

Zinc (Zn) levels in leaves in 2014 were much higher at Penventon (85ppm) and Goonhavern (73ppm) than at the other sites, where concentrations ranged from 29 to 47ppm. By 2015 concentrations were substantially lower at most sites (15–26ppm) but remained relatively high at Penventon and Goonhavern (67 and 44ppm, respectively). Leaf analysis is regarded as a useful indicator in the case of Zn: <15mg/kg is considered deficient, so several of the sites, especially Tregiffian and Fourburrow, might be approaching Zn deficiency – again, possibly an effect due to increasing plant size.

Fungal and bacterial diagnostics

2014 results

During microscopic examination no evidence of bacterial infection was seen in any of the samples. Isolations from lesions with water-soaked margins – found in samples from Kelynack, Tregiffian and Bodilly - resulted in either sterile plates or an inconsistent mix of different bacteria.

In samples from five of the sites the presence of *Ramularia* sporophores and conidia was very obvious, despite the efforts taken to avoid stems with white mould symptoms. However, many of the white mould lesions were actively sporulating during the sampling period and spores would have been spread widely around the field. No other fungi were noted. The microscopic observation of cleared and stained tissues did not provide any insights into the cause of the rust symptoms.

The stem samples from eight sites bore the typical lesions of daffodil rust, while the stems from two sites, Rosevidney and Penventon, had either predominantly (Rosevidney) or exclusively (Penventon) 'streak' symptoms rather than typical rust lesions. When assessing crops in the field, ‘typical’ rust lesions predominated and rust-coloured ‘streak’- or ‘blotch’-like lesions occurred infrequently (see ‘Rust assessments’ under ‘Materials and Methods’). An unidentified *Stemphylium* species – with dark brown/black, solitary, muriform or multi-septate, slightly verrucose conidia born terminally on pale brown conidiophores - was the most consistently isolated fungus from both surface-sterilised and non-surface-sterilised typical rust lesions from eight of the ten sites. *Stemphylium* was not isolated in samples from Rosevidney and Penventon. In agreement with other assessments in the field, in observations on plants recovered from the test sites it did not appear that that discrete rust lesions developed into streaks, suggesting the streak symptoms may have a different origin from the typical rust lesions.

In five of the ten samples *Stemphylium* was also obvious during humid box incubation, and *Ramularia* and *Botrytis* species were also isolated from some samples.

The results are summarised in Table 17.

2015 results

Only fungal isolations were attempted in 2015 (Table 17).

As in the previous year, a *Stemphylium* species – conforming with the description in the previous paragraph – was consistently isolated from surface-sterilised typical rust lesions from eight of the ten sites, but was not found in either the Goonhavern sample (which had typical rust lesions) or the Penventon sample (which showed 'streak' symptoms). The only other fungus found, and at only one site, was *Botrytis*. *Stemphylium*, alone, was found in cultures of typical rust lesions from samples supplied by a Cornish bulb grower, in five out of eight cultures from ‘Watford’ and one out of four cultures from ‘Counsellor’.

Table 17. Summary of fungi seen in lesions under damp incubation and isolated from lesions in 2014 and 2015

Site	**2014**			**2015**	
	Description of lesions on stems examined	**Fungi observed in damp incubation**	**Fungi isolated (no. of cultures out of total)**	**Description of lesions on stems examined**	**Fungi isolated (no. of cultures out of total)**
Kelynack	Typical rust lesions (some with water-soaked areas) Yellow streaks	Stemphylium	Stemphylium (8/10)	Typical rust lesions	Stemphylium (5/8)
St Buryan	Typical rust lesions White mould spots	Fluffy white mycelium (probably *Ramularia*)	Stemphylium (2/12) and Ramularia	Typical rust lesions	Stemphylium (7/8)
Tregiffian	Typical rust lesions and streaks (some with water-soaked areas) White mould spots	Fluffy white mycelium (probably *Ramularia*)	Stemphylium (5/8)	Typical rust lesions	Stemphylium (2/8)
Rosevidney	Streaks predominantly	Mixed fungi including *Stemphylium*	None	Typical rust lesions	Stemphylium (2/8) Botrytis (3/8)
Roseworthy	Typical rust lesions White mould spots	Stemphylium	Stemphylium (3/8)	Typical rust lesions White mould spots	Stemphylium (6/8)
Bodilly	Typical rust lesions (some with water-soaked areas) White mould spots	Botrytis	Stemphylium (2/12) and Botrytis	Typical rust lesions	Stemphylium (3/8)
Mawla	Typical rust lesions and streaks	Botrytis, Stemphylium and others	Stemphylium (2/8)	Typical rust lesions	Stemphylium (5/8)
Penventon	Streaks only	None	None	Streaks only	None
Fourburrow	Rust lesions (hard to find among severe white mould spotting)	Stemphylium	Stemphylium (3/8)	Typical rust lesions White mould spots	Stemphylium (3/8)
Goonhavern	Infrequent small rust lesions	None	Stemphylium (1/8) and Botrytis	Typical rust lesions	None

Identification of fungal isolates by sequencing

Eight fungal isolates were obtained, covering five of the sites. Five isolates were identified as a *Stemphylium* species and three were identified as *Alternaria infectoria* (Table 18 and Figure 25). The *Stemphylium* isolates all had identical sequences, whereas the *Alternaria* isolates differed by two base pairs.

Table 18. Identity of fungi isolated from rust lesions

Site	Isolate ref.	Identity
Kelynack	1-1	Stemphylium sp.
Kelynack	1-2	Alternaria infectoria
Tregiffian	3-1	Stemphylium sp.
Rosevidney	4-1	Stemphylium sp.
Roseworthy	5-1	Alternaria infectoria
Roseworthy	5-2	Stemphylium sp.
Mawla	7-1	Stemphylium sp.
Mawla	7-3	Alternaria infectoria

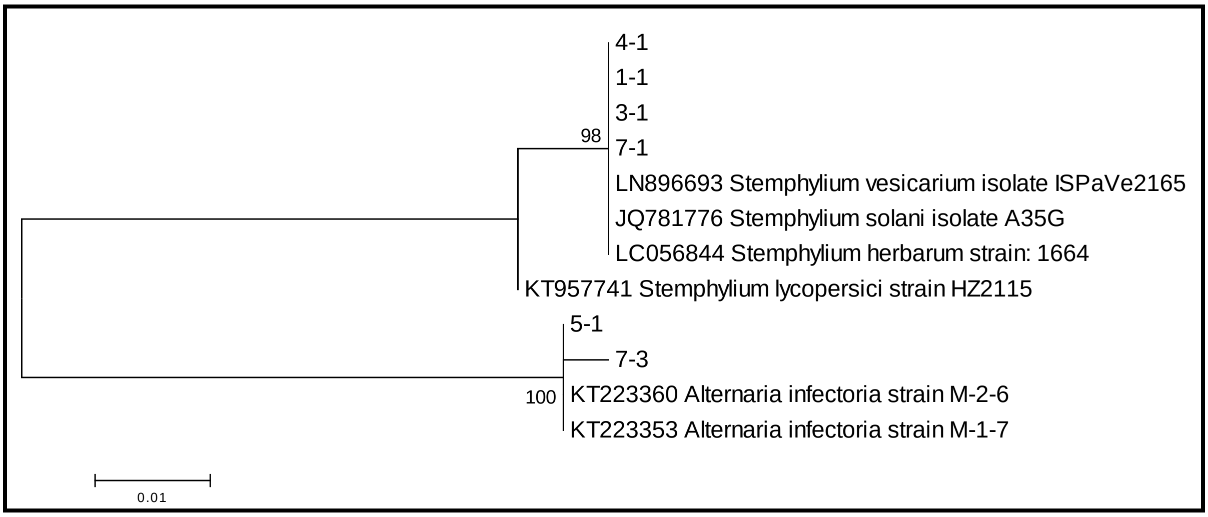


Figure 25. Maximum likelihood tree of fungi isolated from rust lesions based on ITS; the numbers represent bootstrap values from 1000 replicates and the scale bar indicates 0.01 substitutions per site

Analysis of viral RNA in stems

Keys to the sample numbers are given in Table 19. Samples were tested for the virus genera important to daffodils:

- Potyviruses, the group including many of the more serious, aphid-borne viruses of daffodils, such as Narcissus Degeneration Virus, Narcissus Latent Virus, Narcissus Late Season Yellows Virus (NLSYV) and Narcissus Yellow Stripe Virus;
- Nepoviruses, the group including nematode-borne viruses that attack daffodils, such as Arabis Mosaic Virus (AMV), Tomato Black Ring Virus and several ring-spot viruses;
- Carlaviruses, which include the aphid-transmitted daffodil virus Carnation Latent Virus;
- Tospoviruses, which include the thrips-borne Tomato Spotted Wilt Virus that can attack daffodils).

Table 19. Details of samples (green, lesion-free; black, with lesions; red, positive controls and blue, 'virus-free' samples) used in testing for viral RNA

Sample	Site	Rust lesions	Notes	Primers
1	Kelynack	No	Lesion-free	Potyviruses
2	Mawla	No	Lesion-free	
3	Penventon	No	Lesion-free	
4	Tregiffian	Yes		
5	Mawla	Yes		
6	Kelynack	Yes		
7	Penventon	Yes		
8	St Buryan	Yes		
9	Roseworthy	Yes		
10	Fourburrow	Yes		
11	Goonhavern	Yes		
12	Bodilly	Yes		
13	Rosevidney	Yes		
14	Wellesbourne	No	Lesion-free	
15	Wellesbourne	No	Lesion-free	
16	TuMV	Yes	Positive control	
17	Kelynack	No	Lesion-free	Potyviruses, carlaviruses and nepoviruses
18	Mawla	No	Lesion-free	
19	Penventon	No	Lesion-free	
20	Tregiffian	Yes		
21	Mawla	Yes		
22	Kelynack	Yes		
23	Penventon	Yes		
24	St Buryan	Yes		
25	Roseworthy	Yes		
26	Fourburrow	Yes		
27	Goonhavern	Yes		
28	Bodilly	Yes		

29	Rosevidney	Yes		
30	Wellesbourne	No	Lesion-free	
31	TuMV	Yes	Positive control	
32	PVM	Yes	Positive control	
33	TSWV	Yes	Positive control	
34	‘Virus-free’ 1	No	’Virus-free’	
35	‘Virus-free’ 2	No	’Virus-free’	
36	Kelynack	No	Lesion-free	Tospoviruses
37	Mawla	No	Lesion-free	
38	Penventon	No	Lesion-free	
39	Tregiffian	Yes		
40	Mawla	Yes		
41	Kelynack	Yes		
42	Penventon	Yes		
43	St Buryan	Yes		
44	Roseworthy	Yes		
45	Fourburrow	Yes		
46	Goonhavern	Yes		
47	Bodilly	Yes		
48	Rosevidney	Yes		
49	Wellesbourne	No	Lesion-free	

Potyvirus detection

Potyviruses were detected in daffodil samples from all ten sites in both lesion-free stems (samples 1-3 and 17-19) and stems with rust lesions (4-13 and 20-29), and as expected in the TuMV samples (16 and 31) (Figure 26). Other than a potentially very low level detected using OligodTV cDNA and shown by weak bands, no potyviruses were detected in the lesion-free samples from Wellesbourne (14, 15 and 30). Potyviruses were not present in the Scottish ‘virus-free’ samples (34 and 35).

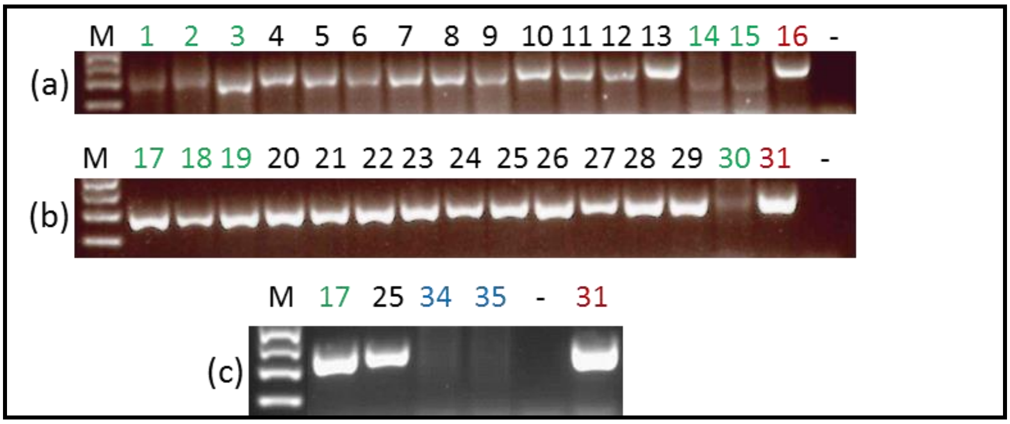


Figure 26. PCR results using Potyvirus primers on cDNA synthesised using (a) a virus-specific primer or (b and c) OligodTV; for sample codes see text and Table 19

On examination of the PCR product sequences, a mixed sequence was observed, the dominant sequence having a 90% match to Narcissus Late Season Yellows Virus (NLSYV). Of the 63 cloned products, eight were identified as NLSYV (96% identity) and eight as an unknown virus with 76% identity to Artichoke Latent Virus (ALV). The remaining sequences were from plant genes. Both NLSYV and ALV were present at the three sites which were examined by cloning the PCR product and there were some small differences between the sequences of individual clones (Figure 27). The phylogenetic tree confirmed the identity of eight clones as NLSYV whilst the identity of the other virus group is unknown.

Specific primers were designed for the unknown Potyvirus and these produced a 100bp product (Figure 28). This virus was present in all samples from all sites, including healthy stems (samples 17-29) but was not detected in the Wellesbourne sample (30) or the 'virus-free' samples (34 and 35)..

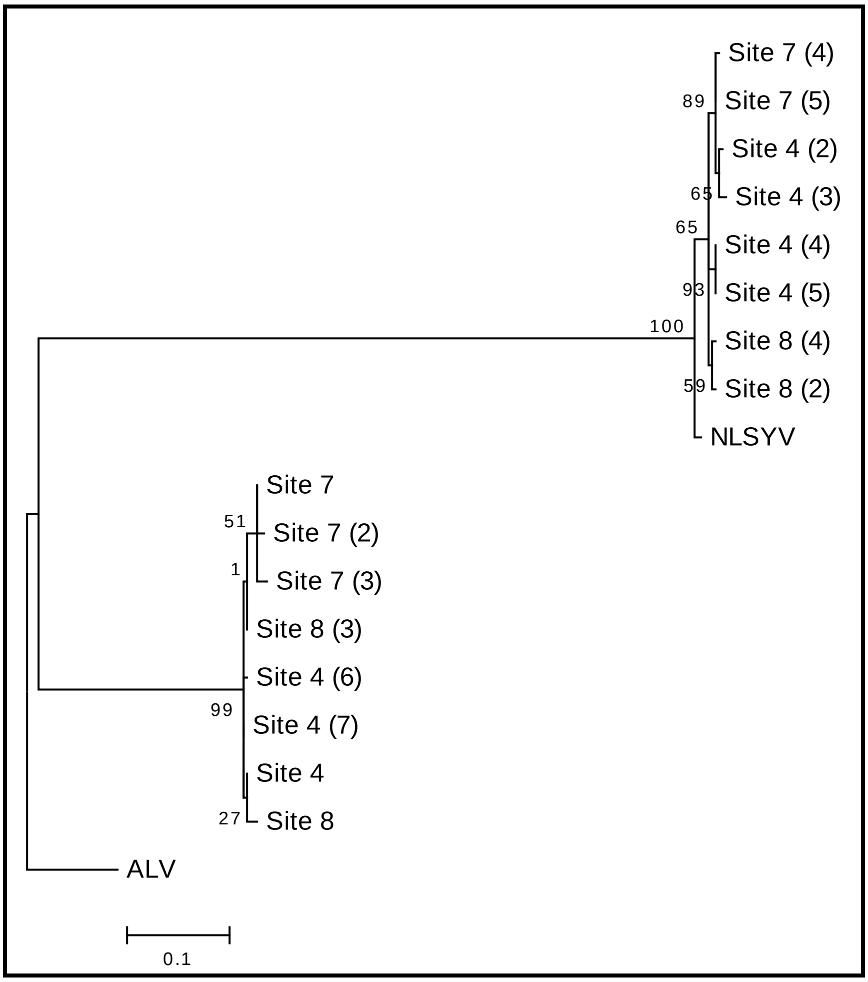


Figure 27. Maximum likelihood tree of virus sequences obtained using Potyvirus primers; the numbers represent bootstrap values from 1,000 replicates, the scale bar indicates 0.01 substitutions per site, NLSYV is GenBank accession number KP125508 and ALV is KP405233; the tree is rooted through ALV

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Figure 28. PCR results using primers designed for the unknown Potyvirus: samples from ten sites (with or without rust lesions) were tested as well as the lesion-free sample from Wellesbourne and the 'virus-free' samples, and sample 31 was included to show that these primers do not detect TuMV; for sample codes see text and Table 19

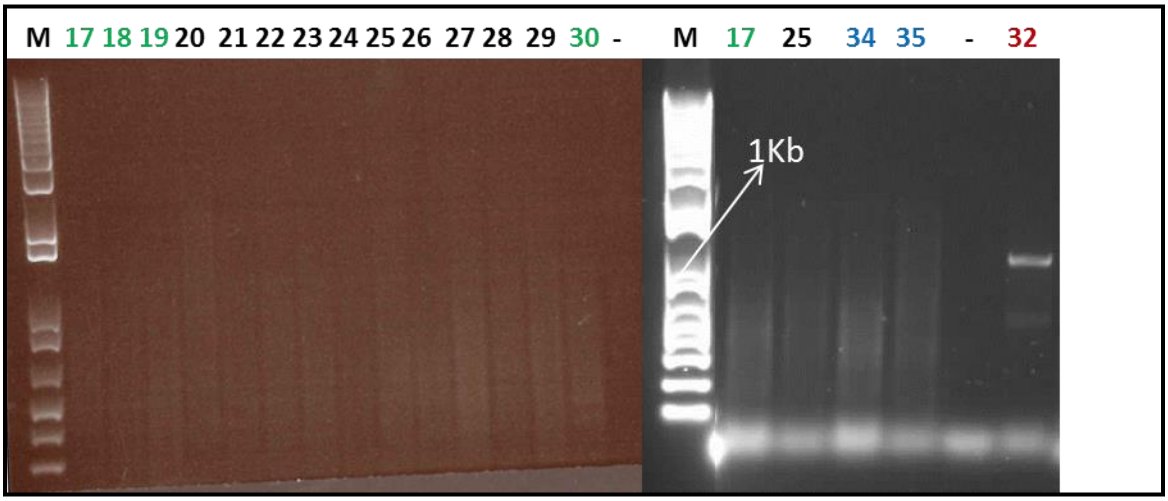


Figure 29. PCR results using Carlavirus primers against samples from the 10 sites (left) and 'virus-free' samples (right), the positive result was for the PVM sample (32); for sample codes see text and Table 19

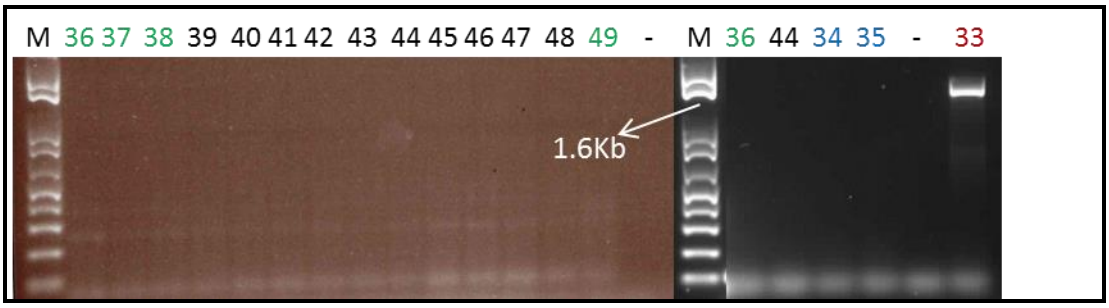


Figure 30. PCR results using Tospovirus primers against samples from the 10 sites (left) and 'virus-free' samples (right), the positive result was for the TSWV sample (33); for sample codes see text and Table 19

Tospovirus, Carlavirus and Nepovirus detection

None of the daffodil samples (samples 17-29) tested positive for Carlaviruses and negative PCR results were also seen for the lesion-free Wellesbourne sample (30) and the 'virus-free' samples (34 and 35) (Figure 29). None of the daffodil samples (36-48) tested positive for Tospoviruses and negative PCR results were also seen for the lesion-free Wellesbourne sample (49) and the 'virus-free' samples (34 and 35) (Figure 30).

PCRs for Nepovirus subgroups B and C were also negative for samples from the ten sites (17-29), the lesion-free Wellesbourne sample (30) and the 'virus-free' samples (34 and 35). However, daffodil samples from all sites tested positive for the presence of Nepovirus subgroup A, while the Wellesbourne and 'virus-free' samples were negative (Figure 31). All these sequences were identified as Arabis Mosaic Virus (91% identical to GQ369526, an isolate from Switzerland).

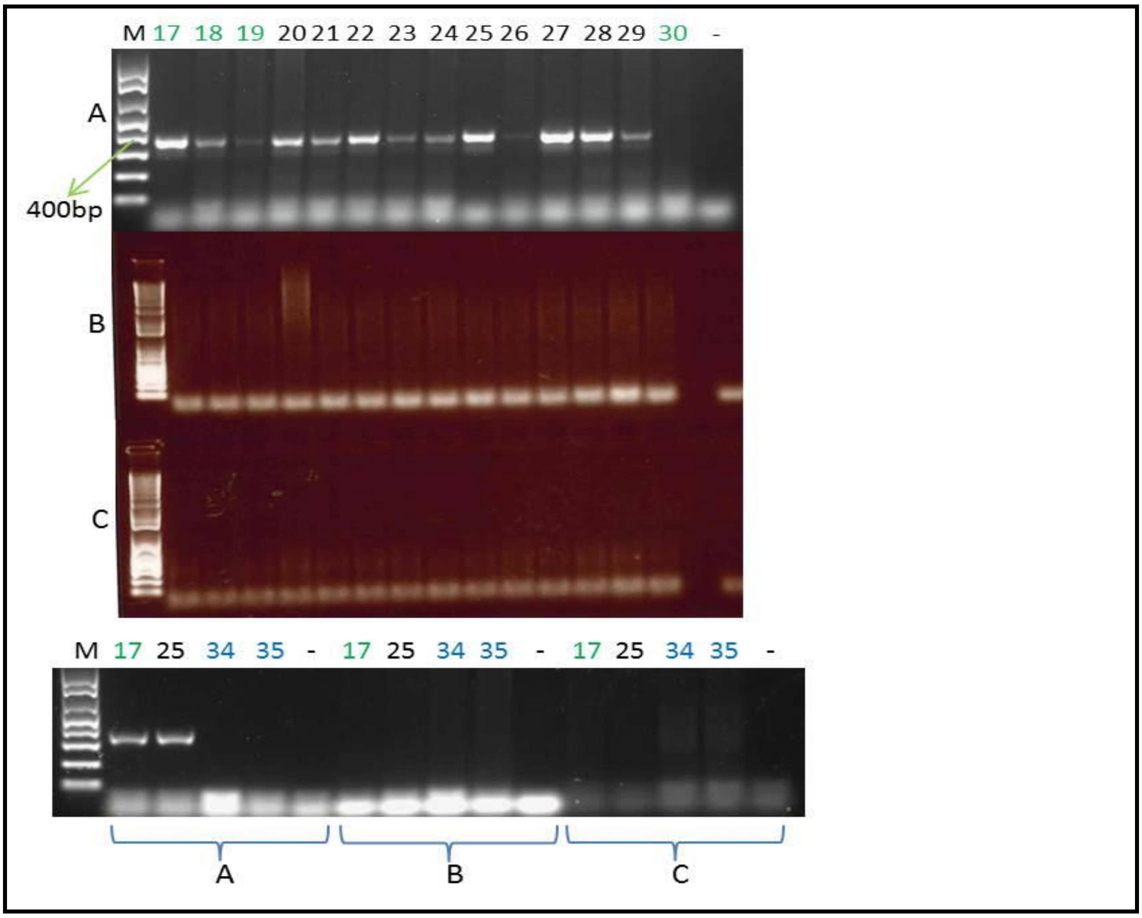


Figure 31. PCR results using Nepovirus subgroup A, B and C primers against samples from the 10 sites and the lesion-free Wellesbourne sample (top) and representative samples from the ten sites and 'virus-free' samples (bottom), expected product sizes A 340bp, B 280bp, C 640bp; for sample codes see text and Table 19

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 an overview of all three years' data are presented here. In addition, some observations were made on the earliest date rust symptoms can be seen and on the incidence of rust lesions on leaves. .

2015: Pre-picking stage

The first crop and rust assessment of 2015 was carried out on 14–16 February (Figure 33). As in previous years the plants at most sites were at an early stem extension stage with many buds visible (GS 2.3); 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 these crops was already close to an early picking stage. The delay in planting at Mawla and Goonhavern in 2012 resulted in delayed emergence and cropping at these sites in 2013, but this effect was no longer evident. Overall, a low level of rust, similar to the previous year, was found (Figure **32**). The sites where rust first appeared had been different in each year: for example, rust incidence was higher at Penventon (>40 stems/plot with rust) than at the other sites this year or in previous years. Sporulating white mould lesions were seen on the Rosevidney, Bodilly and Fourburrow plots and the disease appeared to have been under control at this time.

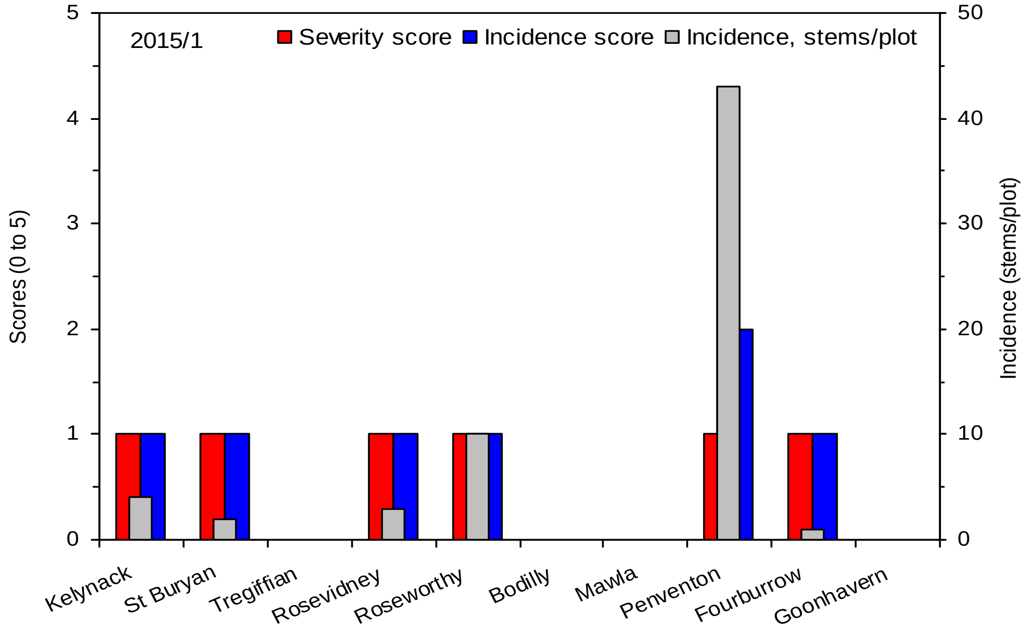


Figure 32. Severity and incidence scores for rust assessed in 2015 at the pre-picking stage; incidence is also shown as the number of stems per plot with rust

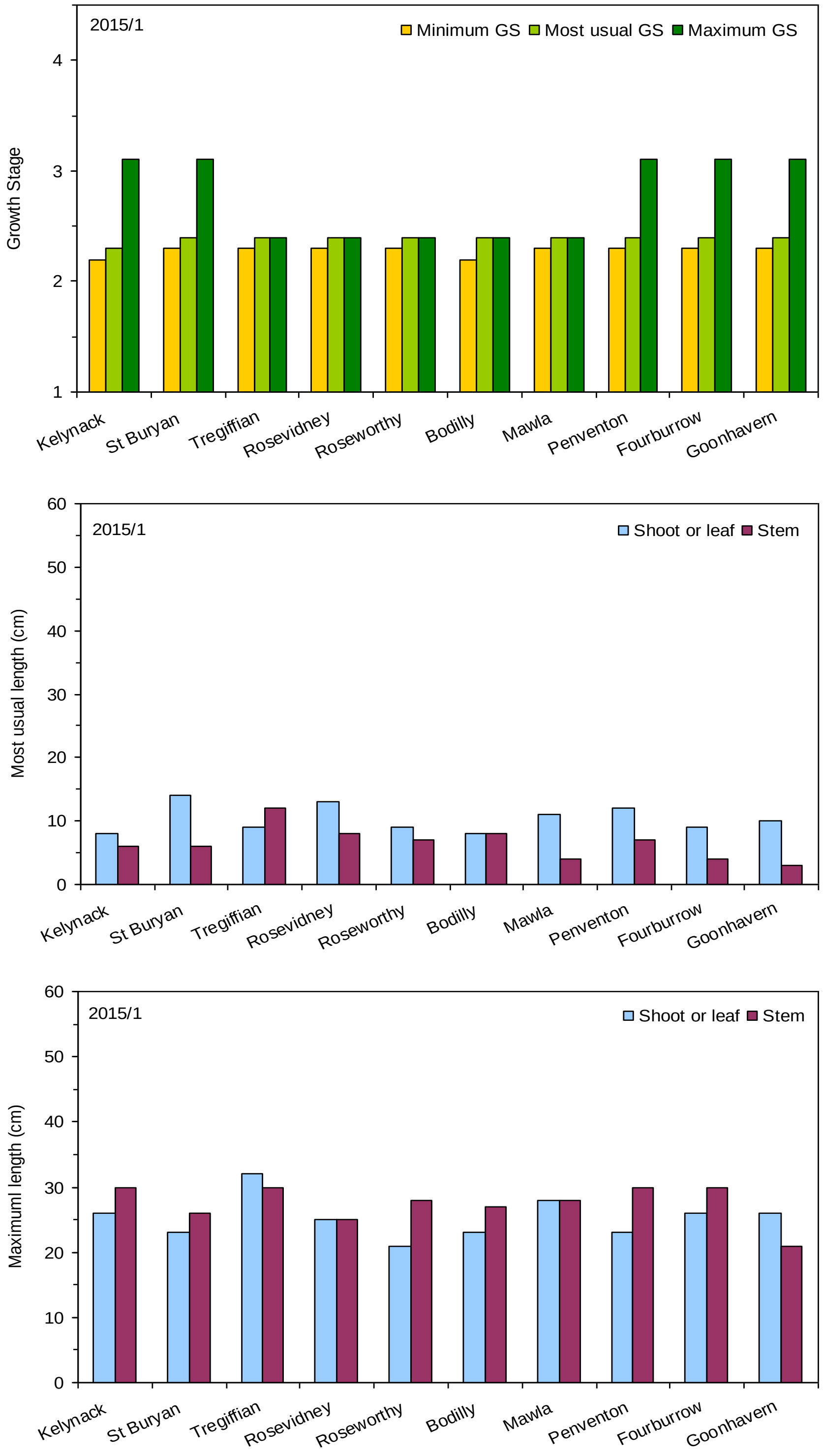


Figure 33. Crop development assessed at pre-picking stage in 2015: (top) minimum, most usual and maximum GS, (middle) most usual shoot/leaf and stem lengths and (bottom) maximum shoot/leaf and stem lengths

2015: Picking stage

The second assessment 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 35). Shoot/leaf and stem lengths varied across the sites – for example, plants were taller at Tregiffian and Penventon. The plots at all sites were vigorous, but those at Penventon were particularly so, as noted in previous years. There were no indications of nitrogen deficiency or other issues, other than white mould. White mould lesions were noted at St Buryan, Roseworthy, Goonhavern and, particularly, at Fourburrow, which had been seriously affected in 2014 and where leaves and the upper stems were becoming more seriously affected, though not so seriously as to affect the assessment of rust lesions.

Rust levels had again increased markedly since the pre-picking assessment (Figure 34). 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.

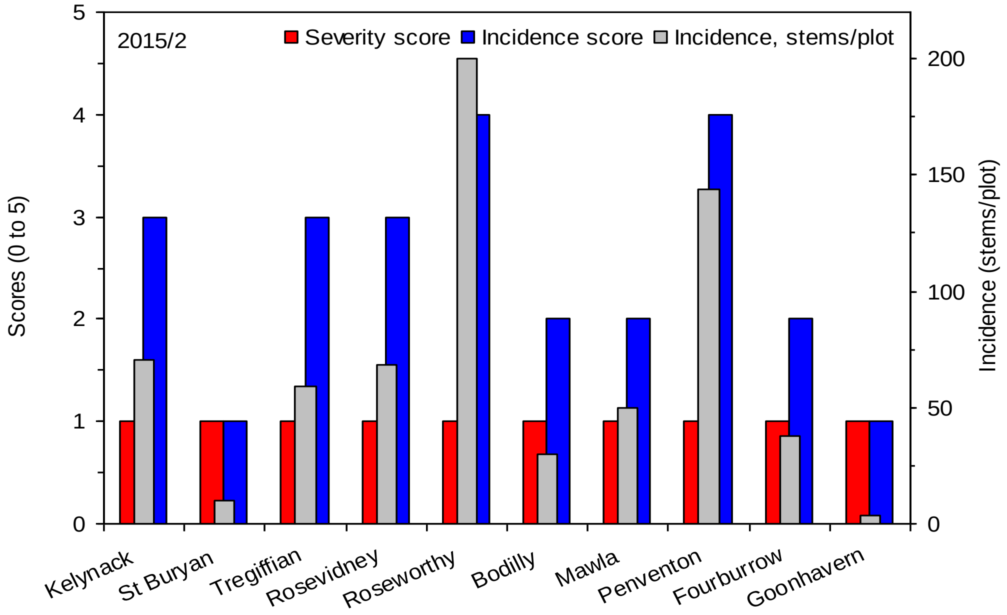


Figure 34. Severity and incidence scores for rust at ten sites assessed in 2015 at the picking stage; incidence is shown as both a 0–5 score and as the number of stems per plot with rust

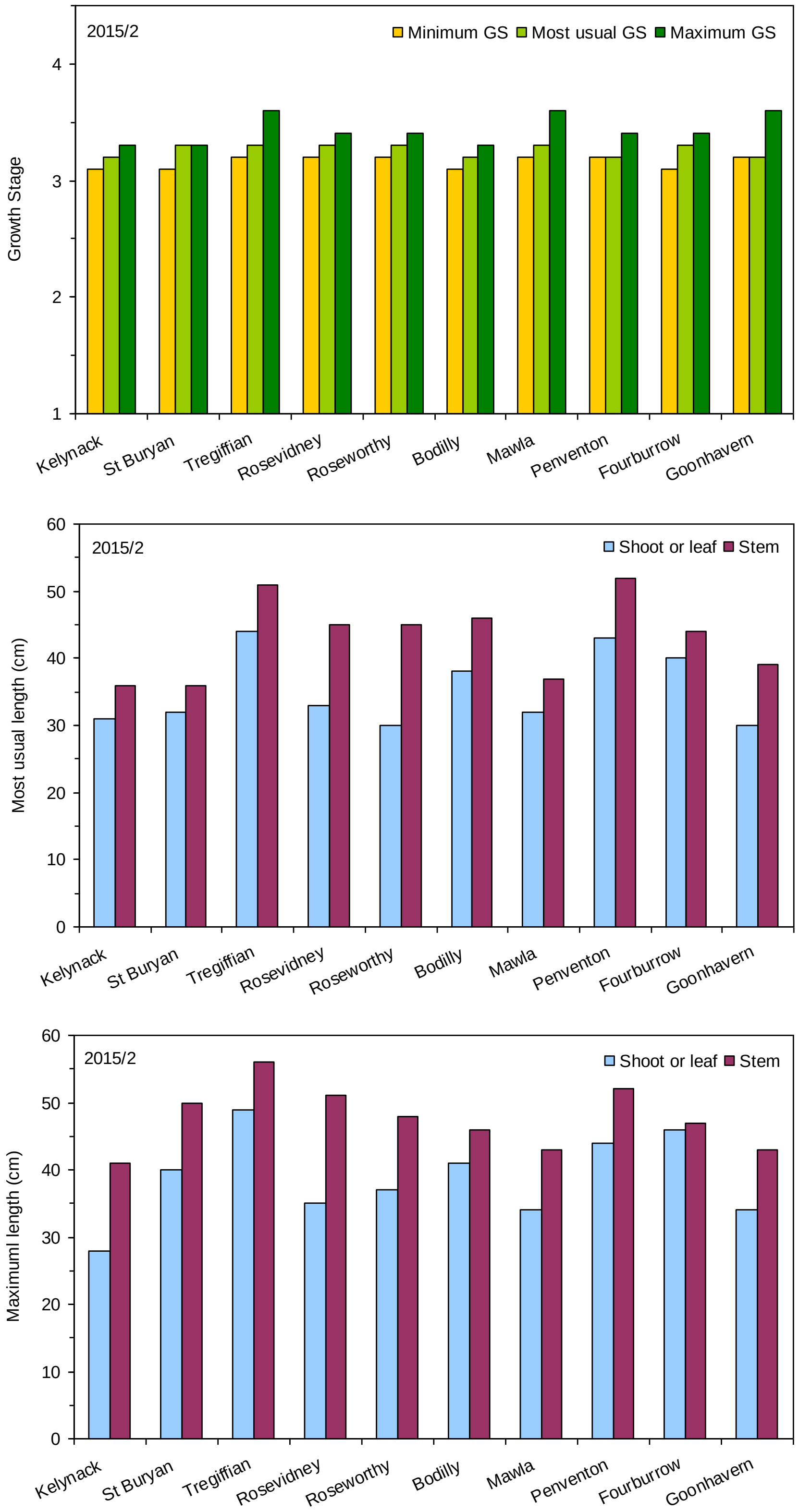


Figure 35. Crop development at ten sites assessed at picking stage in 2015: (top) minimum, most usual and maximum GS, (middle) most usual shoot/leaf and stem lengths and (bottom) maximum shoot/leaf and stem lengths

2015: Post-picking stage

The third assessment of 2015 was carried out on 10–12 April. The developmental stage of crops was uniform, GS 3.7. At this time root growth was also checked: the usual rather meagre daffodil root system was evident at each site, with no site having obviously better or poorer root systems. The upper 10 to 30cm of soil appeared to have remained friable in all cases, with no obvious compaction.

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 36). 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.

Five of the sites – Kelynack, Tregiffian, Rosevidney, Penventon and Goonhavern – appeared to be free of white mould or had it in 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 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, most or all of the stems having rust lesions.

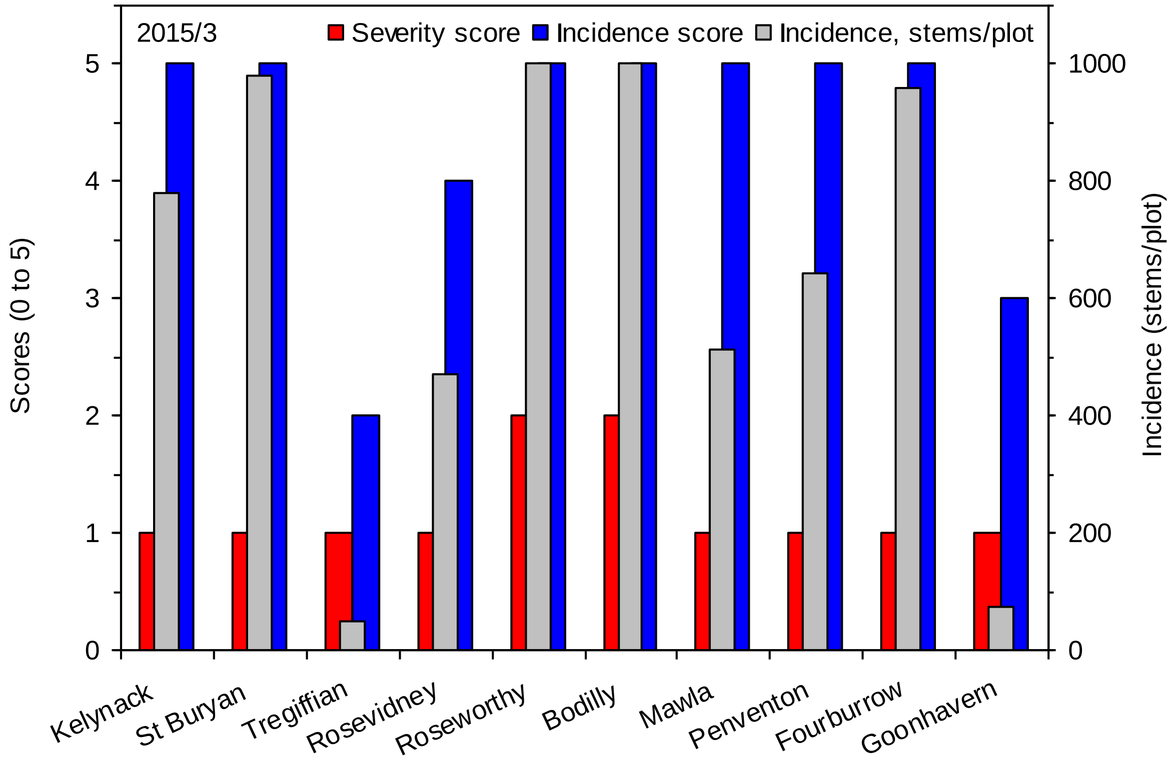


Figure 36. Severity and incidence scores for rust at ten sites assessed in 2015 at the post-picking stage; incidence is shown as both a 0–5 score and as the number of stems per plot with rust

Rust levels 2013 to 2015

The severity and incidence scores, and the incidence of rust as the number of stems with rust per plot, are shown for the three years in Figure 37 to Figure 39. 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.

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 be an indication of an underlying problem with the stock or location. In 2015 the final incidence of rust was lower overall than in 2014, with Tregiffian and Goonhavern having a low incidence of rust. Figure 39 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.

Other pests, diseases and disorders

With the exception of white mould (noted elsewhere) and extensive predation of stems, leaves and flowers by snails and slugs, other pests and diseases were observed only at low levels. Leaves were occasionally damaged by leaf scorch (*Stagonospora curtisii*) (leaf-tip lesions) and smoulder (*Botrytis narcissicola*) (primaries, leaf lesions and flower spotting) and by foliar viral symptoms. Damage by bulb-scale mite (*Steneotarsonemus laticeps*) in combination with smoulder was also seen. There were occasional flower-opening disorders (buds failing to develop or failing to open normally), mainly in 2013 and probably largely resulting from prior hot-water treatment. None of these issues was considered site-specific and were typical of daffodil crops in the South-West. As expected, some damage due to wind (broken stems), frost (scorched leaf-tips) and hail (white flecking) was seen, and at Tregiffian in 2014 hail damage to the surrounding early-flowering daffodil crop was extensive (buds and leaf-tips killed), though damage to the later-flowering 'Golden Ducat' plots was avoided.

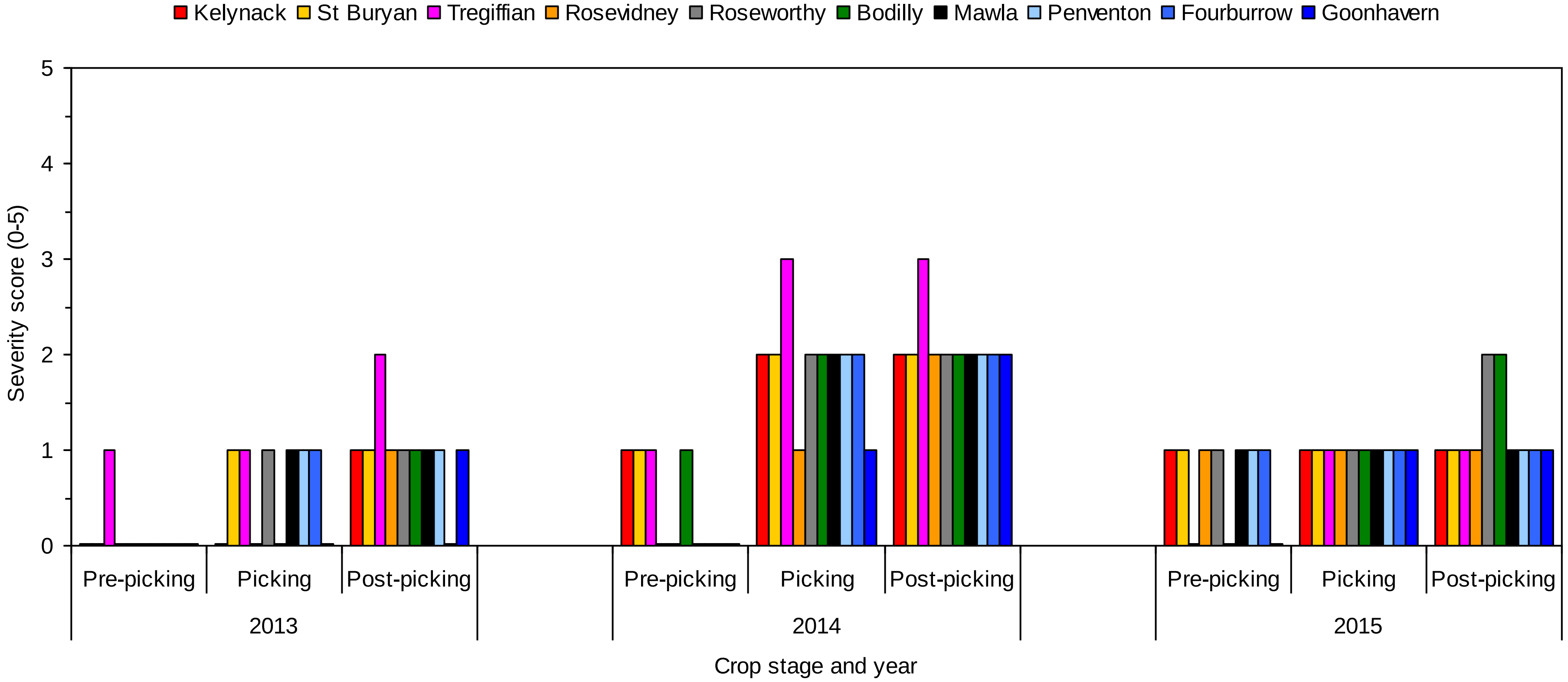


Figure 37. Severity scores for rust at the ten sites assessed in 2013, 2014 and 2015 at pre-picking, picking and post-picking stages

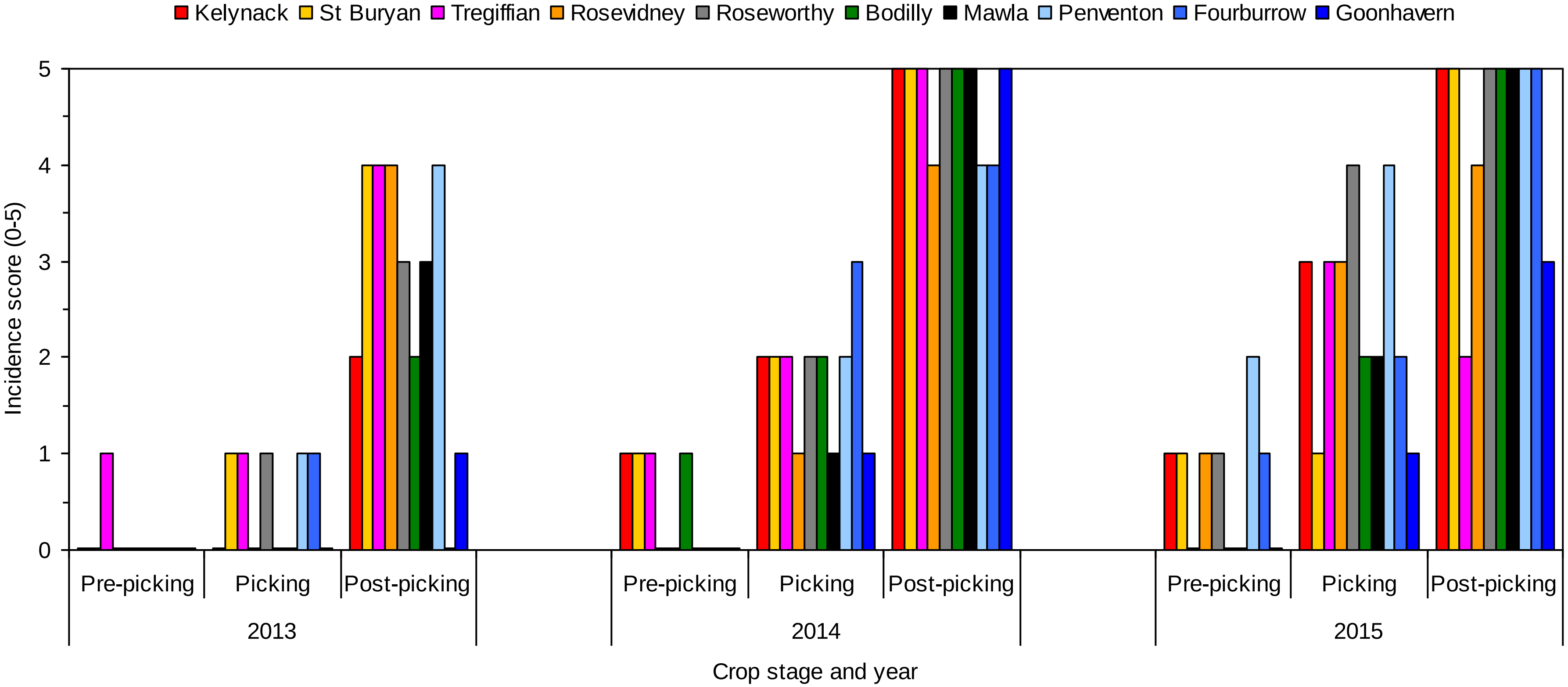


Figure 38. Incidence scores for rust at the ten sites assessed in 2013, 2014 and 2015 at pre-picking, picking and post-picking stages

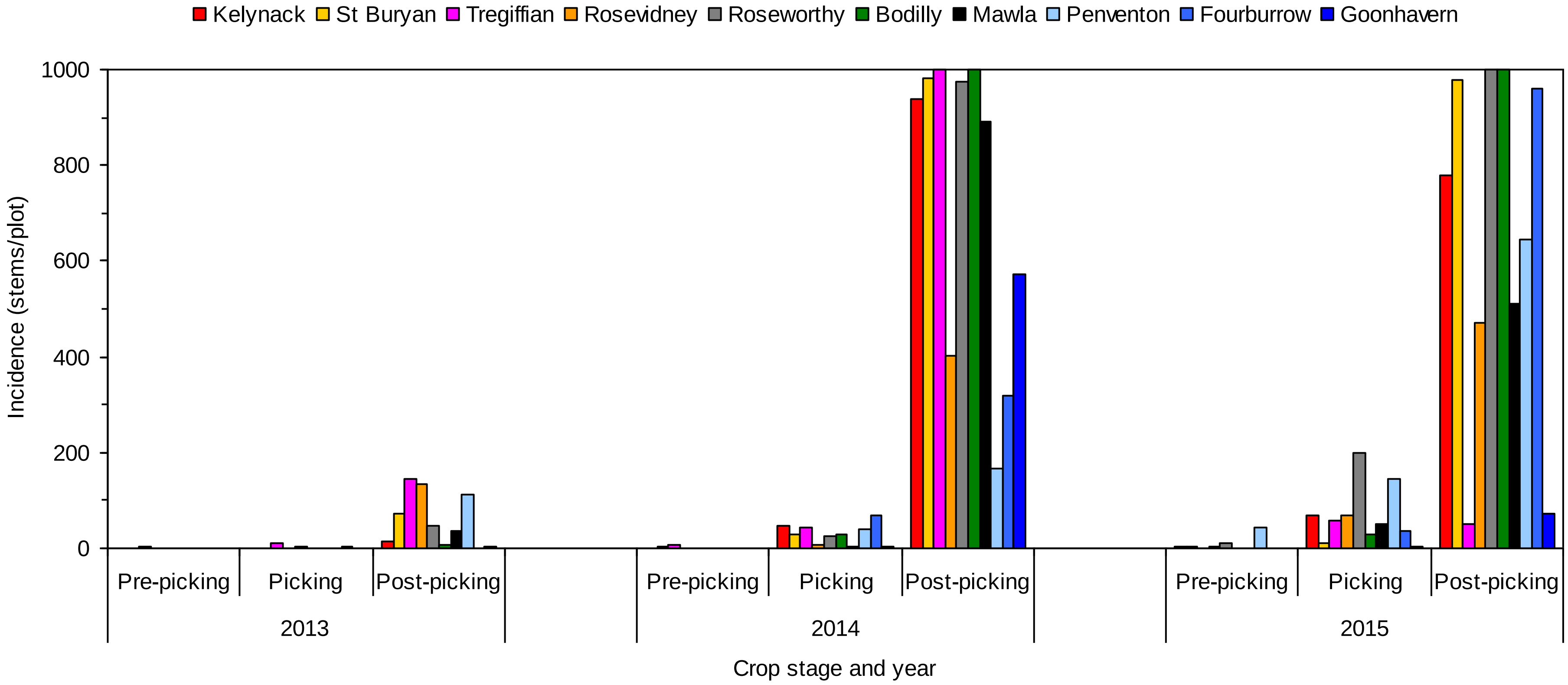


Figure 39. Incidence (stems per plot) of rust at the ten sites assessed in 2013, 2014 and 2015 at pre-picking, picking and post-picking stages

Observations on rust lesions

Although not specifically assessed, presumptive ‘early-stage lesions’ were often noted during regular assessments, consisting 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 (Figure 5). To discover whether these small, sparse lesions might develop to more damaging levels after picking, on 1 March 2013 a sample of ten stems with ‘early-stage lesions’ was picked and the pattern and size of lesions recorded. The stems were transported and stored (dry for 2d at ambient temperatures) before placing in vases of plain water in ambient home conditions for 5d. During this time the lesions showed no further development. The impression that rust lesions do not develop further following picking was confirmed by comments from growers and merchants.

Normally rust assessments involved only the green, above-ground part of the stem. To determine whether rust lesions could be found on the underground part of the stem – the middle, yellow part passing through the soil or the bottom, white part within the bulb - a random sample of about ten plants was taken on 12–14 February 2014 from each of the sites. The plants were placed in loosely closed polythene bags, transported under ambient conditions and kept in an un-heated room until 19 February, when entire stems were dissected out. Stems were carefully wiped clean with paper tissue and examined. Of 89 stems examined, only five had a single small rust lesion. However, 20 stems (22%) had numerous groups of paler yellow marks or pitting – presumptive early-stage lesions - in the yellow sections of the stems. The stems were placed in vases of plain water in ambient home conditions and examined again after 10d. Twenty-two stems now had one or two typical rust lesions on the green or yellow sections of the stem, while all stems had early-stage lesions, mainly on the yellow section but occasionally on the green part. Neither type of lesion was found on the white part of the stem, though some patches of roughened surface were observed there.

Regular sampling was carried out the following winter/spring. Ten whole stems dissected out from six plant samples dug from Roseworthy between 12 November 2014 and 10 March 2015 were examined. Few putative lesions were found, and in only two of the six samples (26 January and 10 March 2015). In the first of these, two out of ten stems had two or three small groups of more or less rounded, up to 1mm diameter, dull flat lesions mid-way along the green part of the stem. In the second positive sample typical rust lesions were found on the green part of the stems, with a single stem showing a zone of blistery irregularities about

2 x 1cm in extent part-way along the white part of the stem (Figure 40). Obviously there are some contradictions in these preliminary observations; ideally the early development of rust lesions should be examined more formally, under a range of conditions.

Figure 40. Putative rust lesions on the underground part of a stem of ‘Golden Ducat’

Rust lesions on leaves

Rust lesions also occur on leaves, though their commercial significance is minimal as they have no obvious effect on the flower crop and the extent of loss of GLA they cause does not appear to approach the level of die-back caused by other factors. The levels of leaf lesions were recorded as incidence scores (Table 7) and severity scores (Table 8, score a). Occasionally, rust-like lesions were also observed on leaf sheaths and flower spathes.

In 2013 leaf rust lesions were assessed at the post-picking stage. Leaf rust was most serious at Kelynack, St Buryan and Tregiffian, the three westernmost sites, with an incidence score of 5 (>50% of leaves were affected), and at Kelynack and St Buryan the lesions were also extensive giving a severity score of 3. Leaf rust was least serious at Rosevidney, Fourburrow and Goonhavern, where only very few and inconspicuous rust lesions were seen (incidence and severity scores of 1).

In 2014 leaf rust was recorded at an early senescence stage, when the rust score were 5 at all sites (54–88% of leaves affected). The lesions varied from a few small lesions per leaf to larger groups of lesions (severity scores of 2 or 3). In 2015 leaf samples were assessed at a post-flowering, pre-senescence stage. The incidence score was 3 or 4 at all sites (5–50% of leaves affected) and rust severity was mild throughout with only small groups of lesions (severity score, 2). Leaf rust develops largely after flowering and by the onset of general leaf die-back appeared to be widespread. It is not known whether its predisposing factors are the same as for rust lesions on stems.

Relationships between levels of rust, SWC and other factors

Rust, SWC, temperature and RH

The preliminary data analysis of SWC, temperature, RH and precipitation was based on box-and-whisker plots. These provided a convenient visualisation of which factors varied between sites and might, therefore, have some association with the level of rust, which had already been shown to vary from site to site. Striking between-site differences were seen in the plots of SWC, whereas the plots of temperature, RH and precipitation did not show so much difference between sites. Hence, further analysis was centred around exploring any association between rust levels and SWC. As an example, Figure **41** shows the box-and-whisker plot covering the year to April 2015 for SWC at the ten sites. In the figure the boxes representing each site are colour-coded for rust levels in spring 2015: note the large differences in SWC between sites. (The rust levels will be discussed later, but can also be seen to vary widely.) Figure 42 shows the equivalent data for soil temperature, air temperature and RH; these data were uniform across the ten sites and were therefore deemed less likely to be of use in understanding the development of rust. Subsequently only the suggested association of rust and SWC was considered in detail.

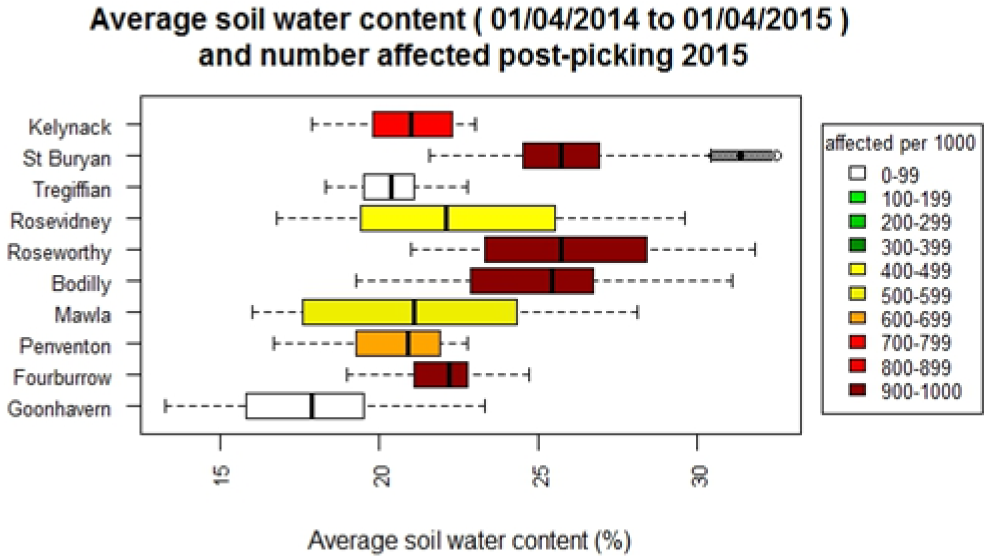
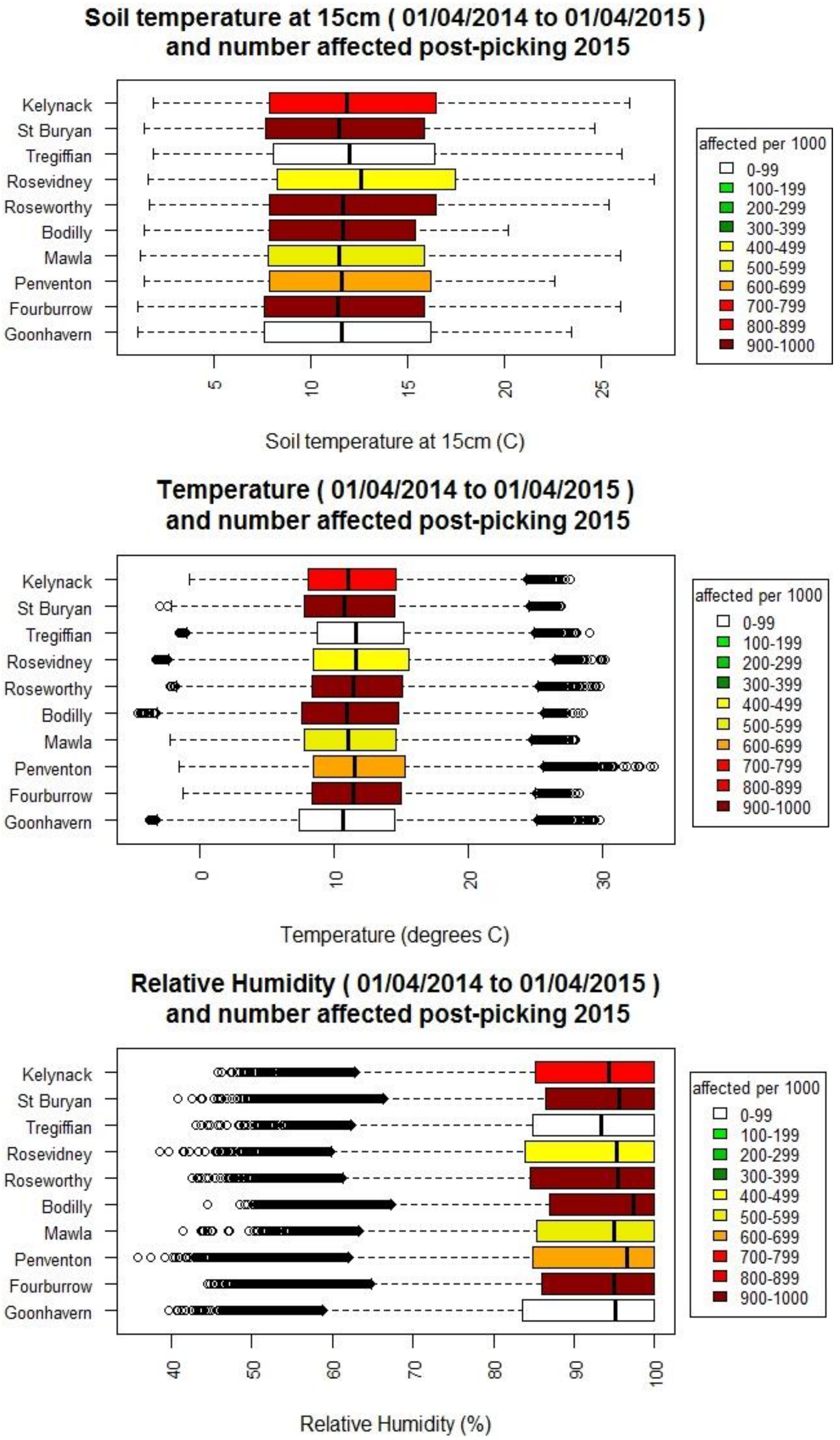


Figure 41. Box-and-whisker plot showing SWC data at the ten sites for the year to 1 April 2015: note the large differences in SWC between sites

Note 1: SWC expressed as the average of readings 10, 20 and 30cm-deep, with each site's box colour-coded (see legend) for rust incidence in 2015 (expressed as the number of stems per plot with rust at the post-picking stage)

Note 2: The box for each site shows the median value of SWC (the central black bar), the range of values that fall in the second and third quartiles (the box itself), the minimum and maximum values excluding any 'outliers' (i.e. extreme values, the extent of the whiskers) and the extent of the outliers (defined as those falling outside a value 1.5 × the inter-quartile range, the circles); this pattern is followed in all subsequent box-and-whisker plots



Soil temperature at 15cm (01/04/2014 to 01/04/2015) and number affected post-picking 2015

Temperature (01/04/2014 to 01/04/2015) and number affected post-picking 2015

Relative Humidity (01/04/2014 to 01/04/2015) and number affected post-picking 2015

Figure 42. Box-and-whisker plots showing (top) soil temperature, (middle) air temperature and (bottom) RH at the ten sites for the year to 1 April 2015; note that the differences (in temperature, etc.) between sites are small
Note: Each site's box is colour-coded (see legend) for rust incidence in 2015 (expressed as the number of stems per plot with rust at the post-picking stage)

Rust and SWC, 2012-2013

In 2013 rust levels in the plots were generally low, but there were nevertheless marked differences between sites, justifying the search for associations between rust and SWC. The five months' of available data, starting November 2012, were explored for any association of average SWC with rust levels in spring 2013. The results' most noticeable feature is that three of the four sites with a higher incidence of rust – St Buryan, Tregiffian and Rosevidney - were associated with a higher SWC, but this pattern was broken at Penventon where there was also relatively high rust incidence but a low SWC (Figure 43). Sites with higher SWC (averaging >25mm/100mm) appeared to be associated with higher rust levels (>100 stems with rust per plot), but higher rust levels also occurred at one site with low SWC (Penventon, averaging *ca* 21mm/100mm). If this result were borne out by further data it would suggest a positive association between SWC and rust level, albeit an association with which some other, unknown, factor was interacting.

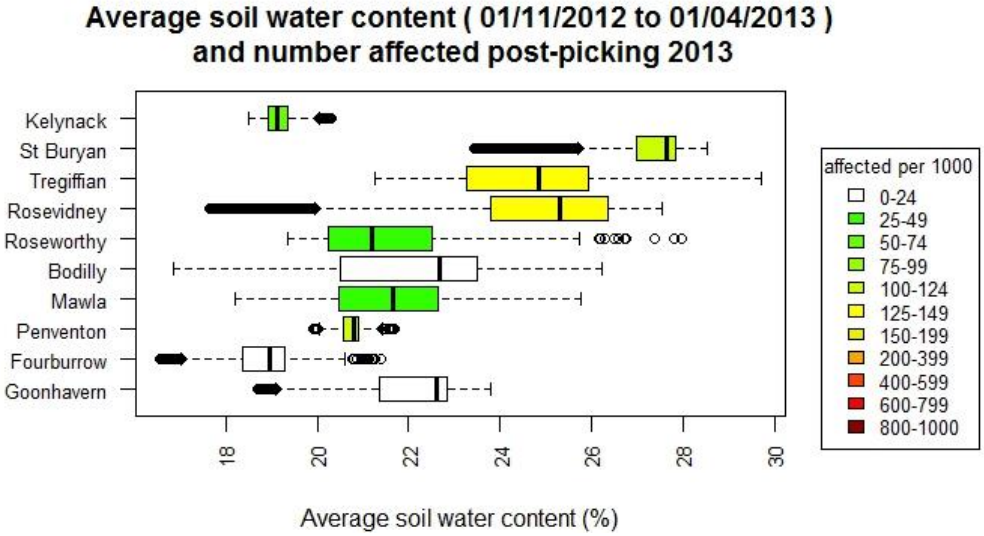


Figure 43. Box-and-whisker plot showing rust incidence in 2013 in relation to average SWC over the 5 months to 01 April 2013, with each site's box colour-coded (see legend) for rust incidence in 2013 (expressed as the number of stems per plot with rust at the post-picking stage); compare with Figure 45

To determine if there is a critical time for the effect of high SWC within the November–March period, the data were broken down to shorter periods. Figure 45 shows that the response pattern seen in Figure 43 was progressively lessened over the 5, 4, 3, 2 and 1-months to April; if only the March data were considered, St Buryan alone would show the link between SWC and rust. SWC during the winter months tended to be 'predictive' of subsequent high rust levels. When the data for individual months (Figure 46) or weeks (Figure 44) were considered the pattern was clearer: SWC during November and December seems to play an important role, but the effect is weaker in January and February and almost lost in March.

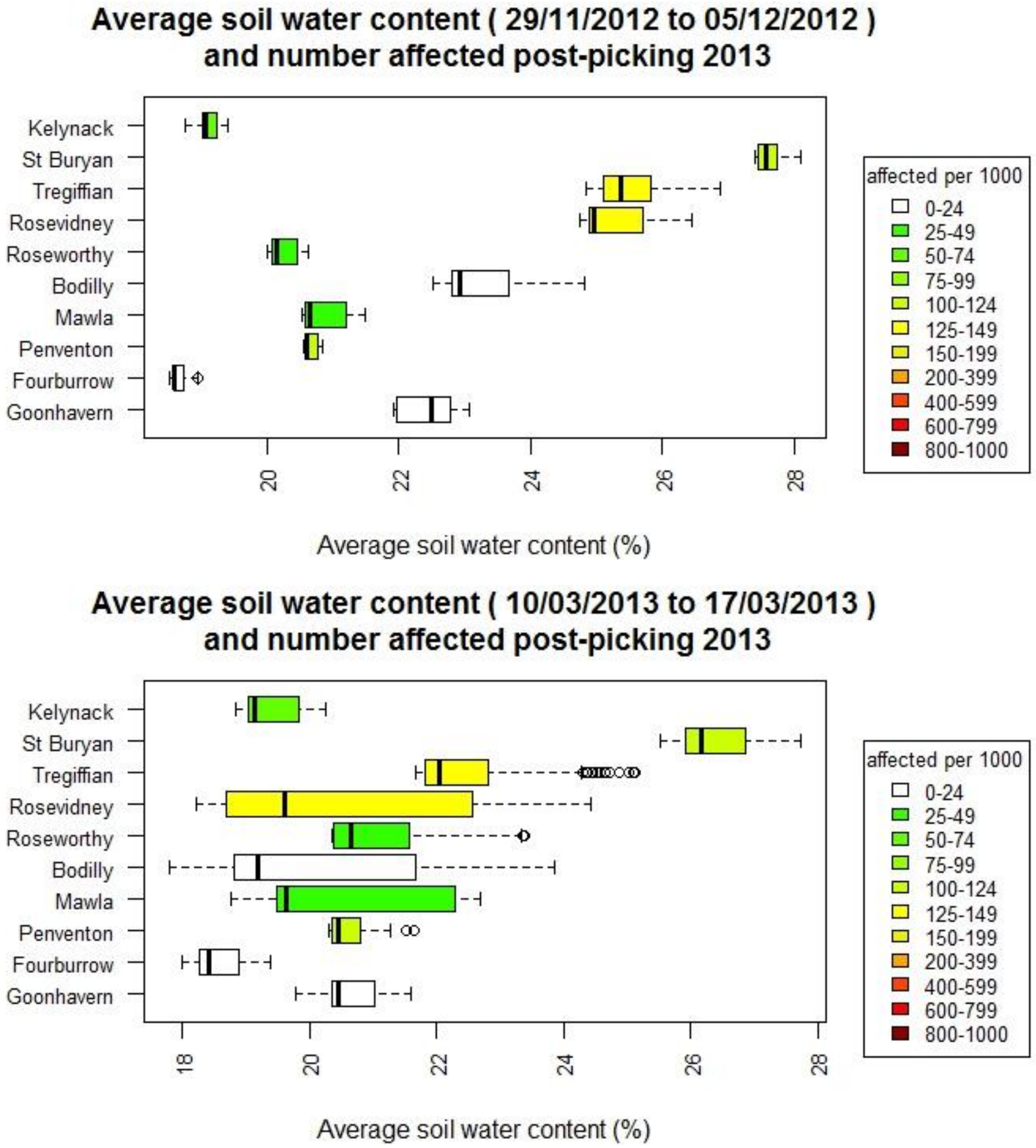


Figure 44. Box-and-whisker plots showing rust incidence in 2013 in relation to average SWC during the weeks beginning (top) 29 November 2012 and (bottom) 10 March 2013; each site’s box is colour-coded (see legend) for rust incidence in 2013 (expressed as the number of stems per plot with rust at the post-picking stage)

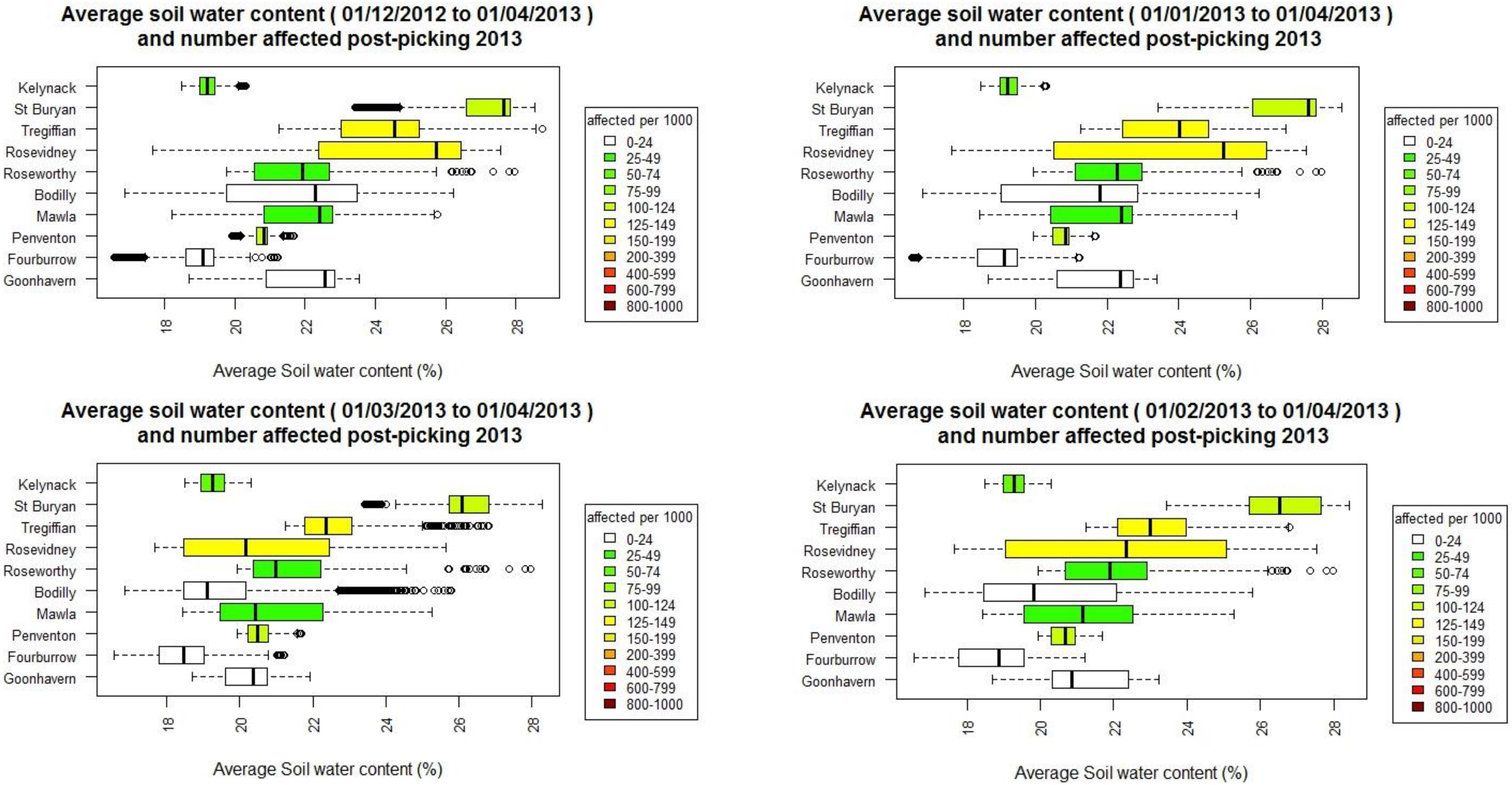


Figure 45. Box-and-whisker plots showing rust incidence in 2013 in relation to average SWC over the (top-left then clockwise) 4 months, 3 months, 2 months and 1 month to 01 April 2013 (for the 5 months data, see Figure 43); each site's box is colour-coded (see legend) for rust incidence in 2013 (expressed as the number of stems per plot with rust at the post-picking stage)

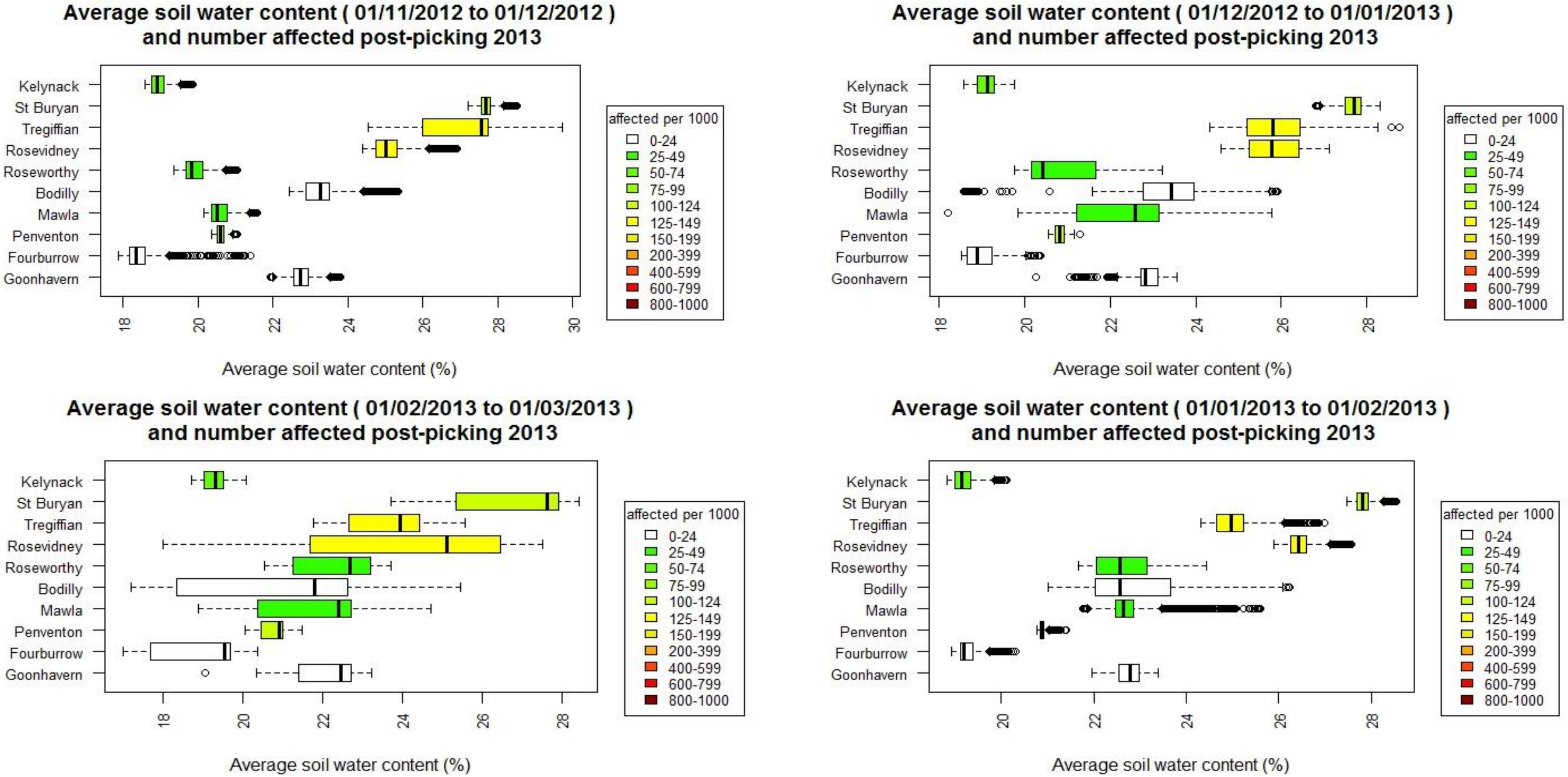


Figure 46. Box-and-whisker plots showing rust incidence in 2013 in relation to average SWC during (top-left then clockwise) November 2012, December 2012, January 2013 and February 2013 (for SWC in March 2013, see Figure 45 bottom-left); each site's box is colour-coded (see legend) for rust incidence in 2013 (expressed as the number of stems per plot with rust at the post-picking stage)

SWC in the soil profile

In the previous examples SWC was expressed as the average of the sensors located 10, 20 and 30cm below the soil surface, but SWC can vary widely between the surface and deeper layers of the soil, depending on precipitation, flooding, drainage, soil-type, nature of the sub-soil, and so on. It has been reported for daffodils that 77% of the root dry-weight occurred in a 20cm-deep layer immediately under the bulbs within the planting ridge, with little horizontal or vertical spread (Price, 1977a). Is the ‘average’ SWC the best indicator to use in this study? Figure 47 shows the SWC at each depth over the November to March period. The 10cm, 30cm and average SWC results were broadly consistent, with the St Buryan, Tregiffian and Rosevidney sites again showing relatively high SWC and relatively high subsequent rust levels. The other sites, including Penventon, had a consistently lower SWC. The SWC measured at 20cm often did not agree with the SWC measured in the layers above or below, perhaps because of some differences between sites in the amount of water penetrating the ground or running-off.

SWC and method of scoring rust levels

In the field stem rust ‘levels’ were always recorded as 0–5 incidence and severity scores as well as the number of stems with rust per plot, the last of these usually being preferred since it provides a finer scale and more experimental variation. But as scores are considerably quicker to record, it is of interest to know whether they give results comparable to those obtained as stem counts. Figure 48 shows rust levels recorded in all three ways (using the same set of SWC figures used in Figure 47). None of three recording methods was effective when used on the low rust levels found at the pre-picking and picking stages (data not presented). The post-picking stage is also the most useful as rust will be fully expressed (and most relevant to commercial cut-flower production) by that time.

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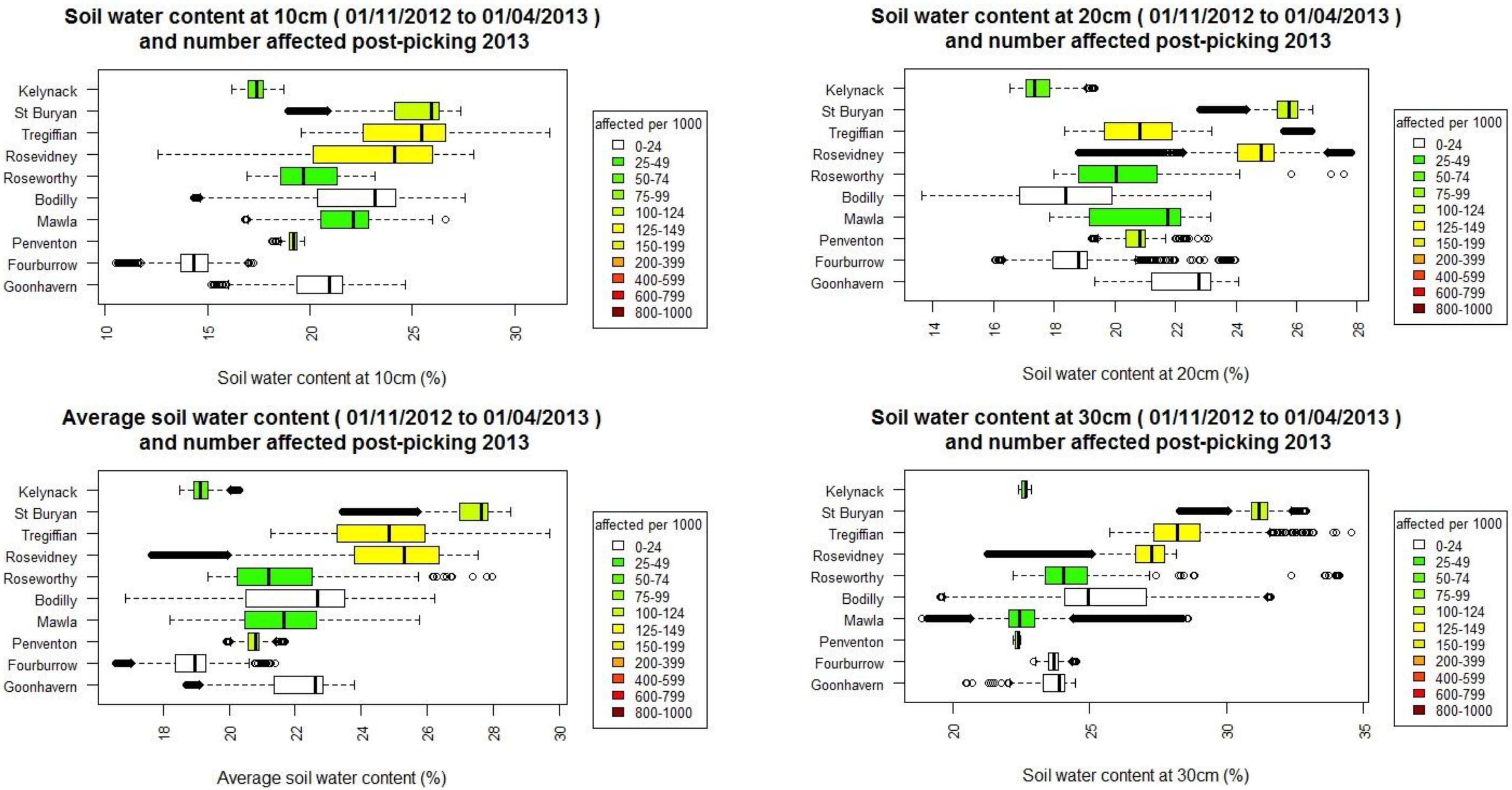


Figure 47. Box-and-whisker plots showing rust incidence in 2013 in relation to SWC over the 5-month period to 01 April 2013: (top-left then clockwise) SWC at 10cm, 20cm, 30cm and average SWC; each site's box is colour-coded (see legend) for rust incidence in 2013 (expressed as the number of stems per plot with rust at the post-picking stage)

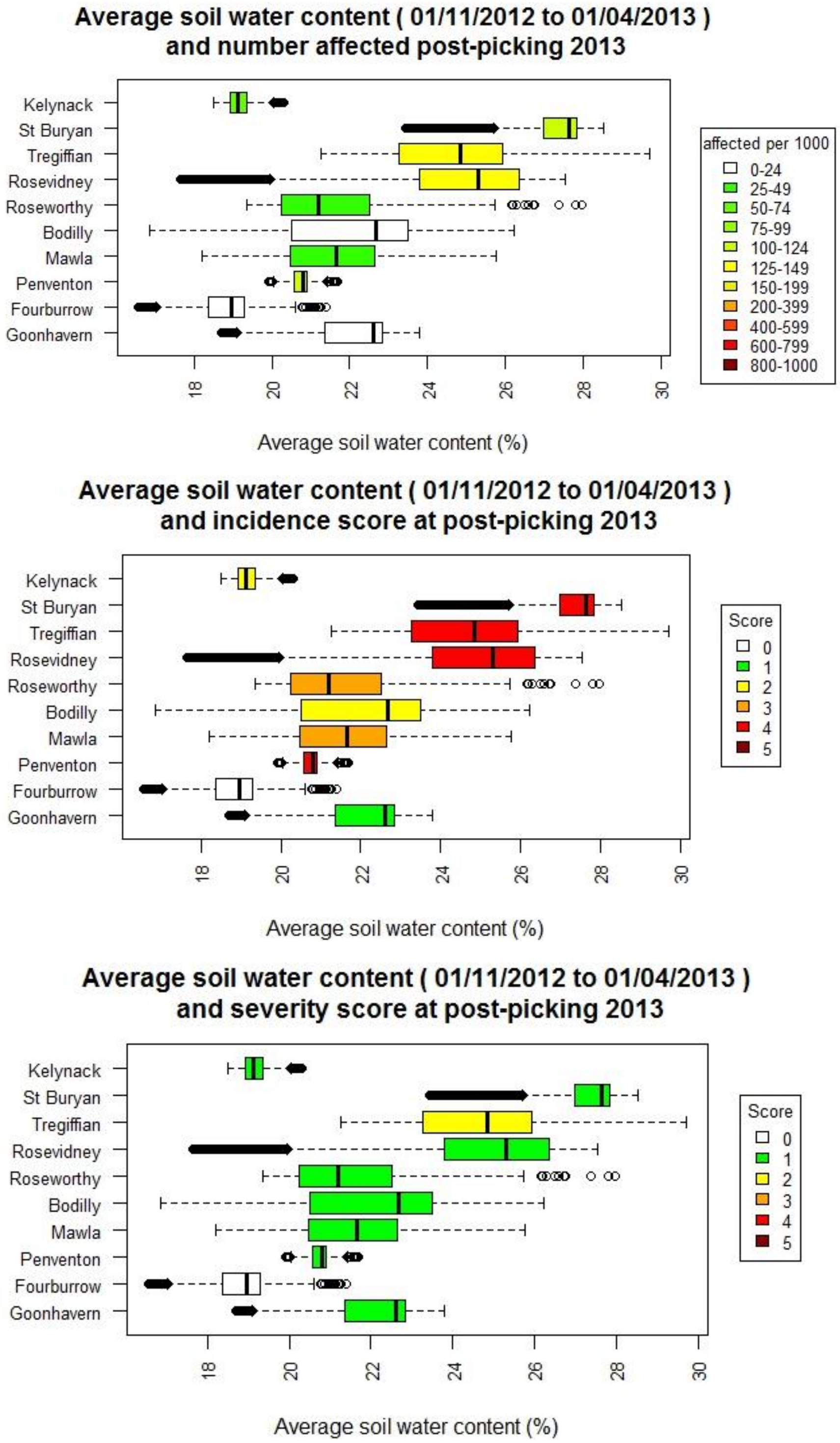


Figure 48. Box-and-whisker plots showing rust levels in 2013 in relation to average SWC over the 5-month period to 01 April 2013, with rust level expressed as (top) the number of stems per plot with rust, (middle) incidence score and (bottom) severity score, all at the post-picking stage

Rust and SWC, 2013-2014

The results obtained in 2013 suggested an association between higher winter SWC and higher subsequent levels of rust, and this was investigated over the next two years with the advantage of having longer runs of data. Rust again showed marked differences between the locations, and by the post-picking stage rust levels were considerably higher than in 2013. The sites most affected by rust or high SWC were not the same as in the previous year.

To examine the effects of SWC over the year to flowering in 2014, the numbers of stems per plot with rust at the post-picking stage were plotted against SWC for the 12-, 11-, 10-…1-month periods before flowering. The plots were remarkably consistent over the whole period and some examples are shown in Figure 49. The highest rust levels (>900 stems with rust per plot) were found at St Buryan, Tregiffian, Roseworthy and Bodilly, corresponding with high SWC (*ca* 27–30mm/100mm). Kelynack and Mawla had rust levels only slightly less severe (>700 stems/plot) but their SWC was lower (*ca* 20–25mm/100mm) and about the same as at the remaining sites which had relatively low rust levels. Only the shortest period of averaging SWC, for March 2014, failed to show this pattern: by this time the soil at Tregiffian was getting drier. All longer periods of averaging SWC appeared reasonably ‘predictive’ of the highest levels of rust in April 2014 - remembering that high rust levels *can* sometimes occur in soils with a lower SWC, as at Penventon in 2013 and at Kelynack and Mawla in 2014.

There is certainly no indication that a short period of high SWC ‘triggers’ the development of rust, it seems more likely to be a result of wet soil persisting over a longer term.

The SWC figures used in the preceding section and Figure 49 were those at 10cm depth, having established previously that average SWC and SWC at 10 and 30cm depth were in generally good agreement (Figure 47). Figure 50 shows the comparable average SWC data and SWC for 10, 20 and 30cm depths for the period 1 April 2013 to 1 April 2014. This confirmed the previous result that the 10 and 30cm measurements and the average SWC were in close agreement.

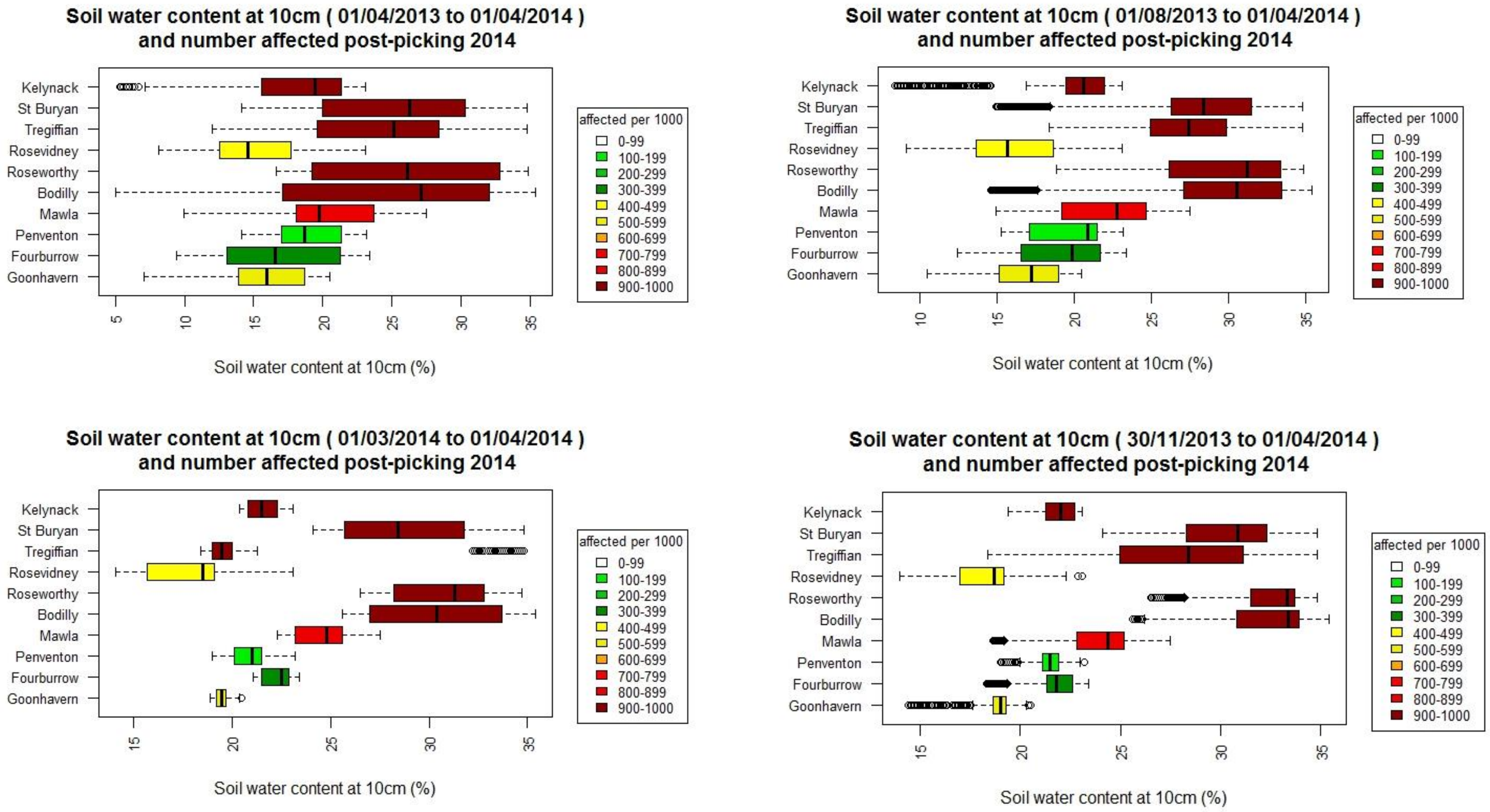


Figure 49. Box-and-whisker plots showing rust incidence in 2014 in relation to SWC at 10cm-depth over the (top-left, then clockwise) 12-month, 8-month, 4-month and 1-month periods to 01 April 2014; each site's box is colour-coded (see legend) for rust incidence in 2014 (expressed as the number of stems per plot with rust at the post-picking stage)

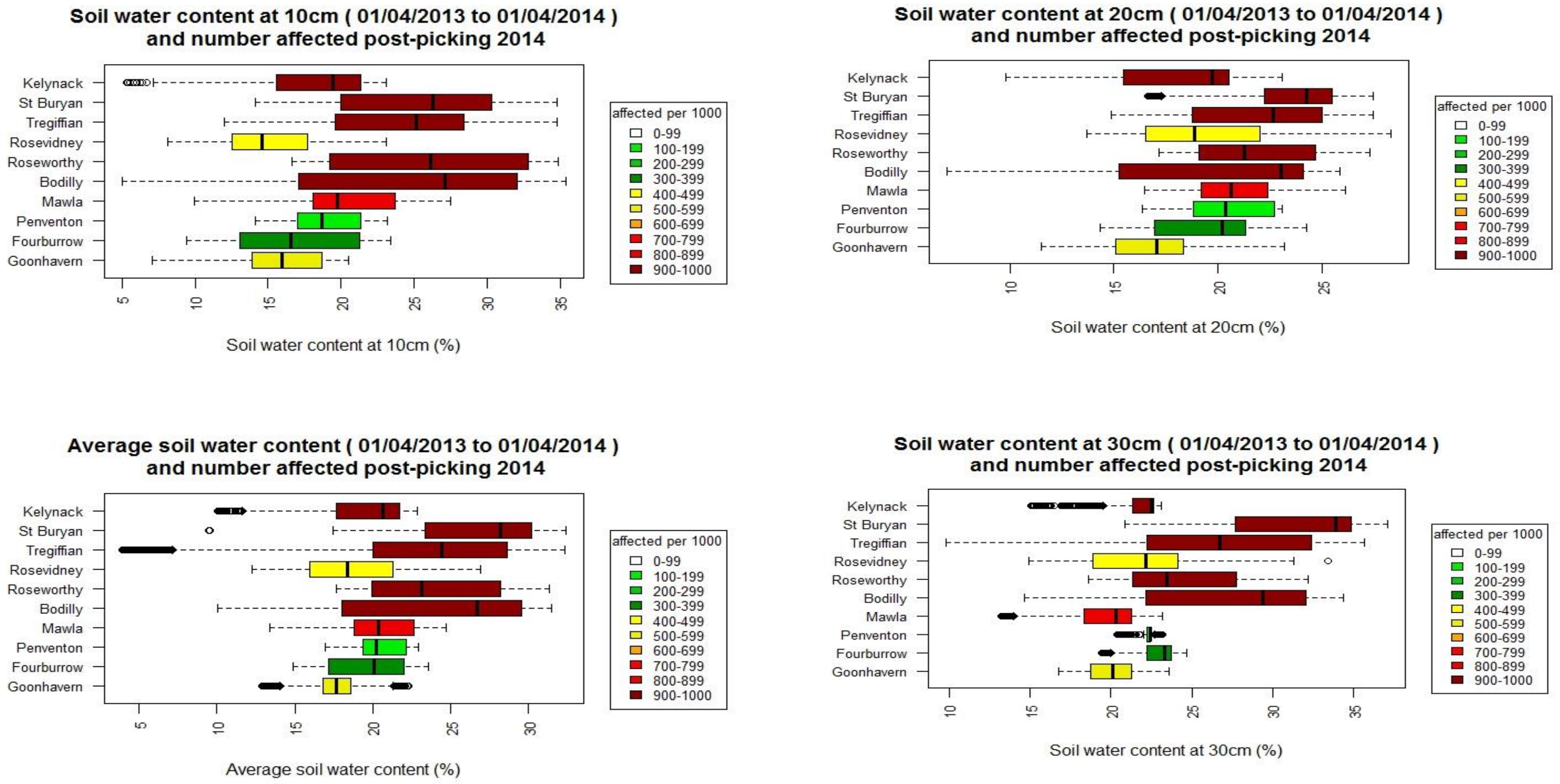


Figure 50. Box-and-whisker plots showing the number of stems per plot with rust at the post-picking stage 2014 in relation to (top-left, then clockwise) SWC at 10, 20 and 30cm deep and average SWC between 01 April 2013 and 01 April 2014 (note that the scale of the x-axes vary between depths)

Rust and SWC, 2014-2015

In the final year of the project rust incidence and severity were generally high and similar to the previous year. To examine the effects of SWC over the year to flowering in 2015, the numbers of stems per plot with rust at the post-picking stage were again plotted against SWC for the 1-, 2-, 3-…12-month periods before flowering. Some examples are presented in Figure 51. The highest rust levels (>900 stems with rust per plot) were found at St Buryan, Roseworthy and Bodilly, which also had high SWC (*ca* 27–28mm/100mm) but also at Fourburrow, where the SWC was lower (*ca* 23mm/100mm) – a comparable pattern to that in 2014. The remaining sites had lower (<800 stems/plot) or very low (<100 stems/plot) rust levels and SWC of 20–25mm/100mm.

For the longer runs of data, 8 to 12 months before April 2015, the box-and-whisker plots showed a clear distinction between the three high rust, high SWC plots and the others. But at Rosevidney and Mawla, which would eventually have rust levels of *ca* 500 stems/plot at the post-picking stage, SWC was increasing steadily and reached *ca* 28mm/100mm by December 2014. Hence only the longer periods of averaging SWC appeared ‘predictive’ of the highest levels of rust in April 2014, a similar result to that of the previous year.

The SWC figures used in the preceding section and Figure 51 were those at 10cm depth. Figure 52 shows the comparable average SWC data and SWC for 10, 20 and 30cm depths for the period 1 April 2014 to 1 April 2015. As found in the previous two years the 10 and 30cm measurements and the average SWC were in good agreement. The middle layer is relatively drier.

A convenient way of expressing the long-term effect of SWC was needed for statistical analysis to take place, and this is discussed in the next section.

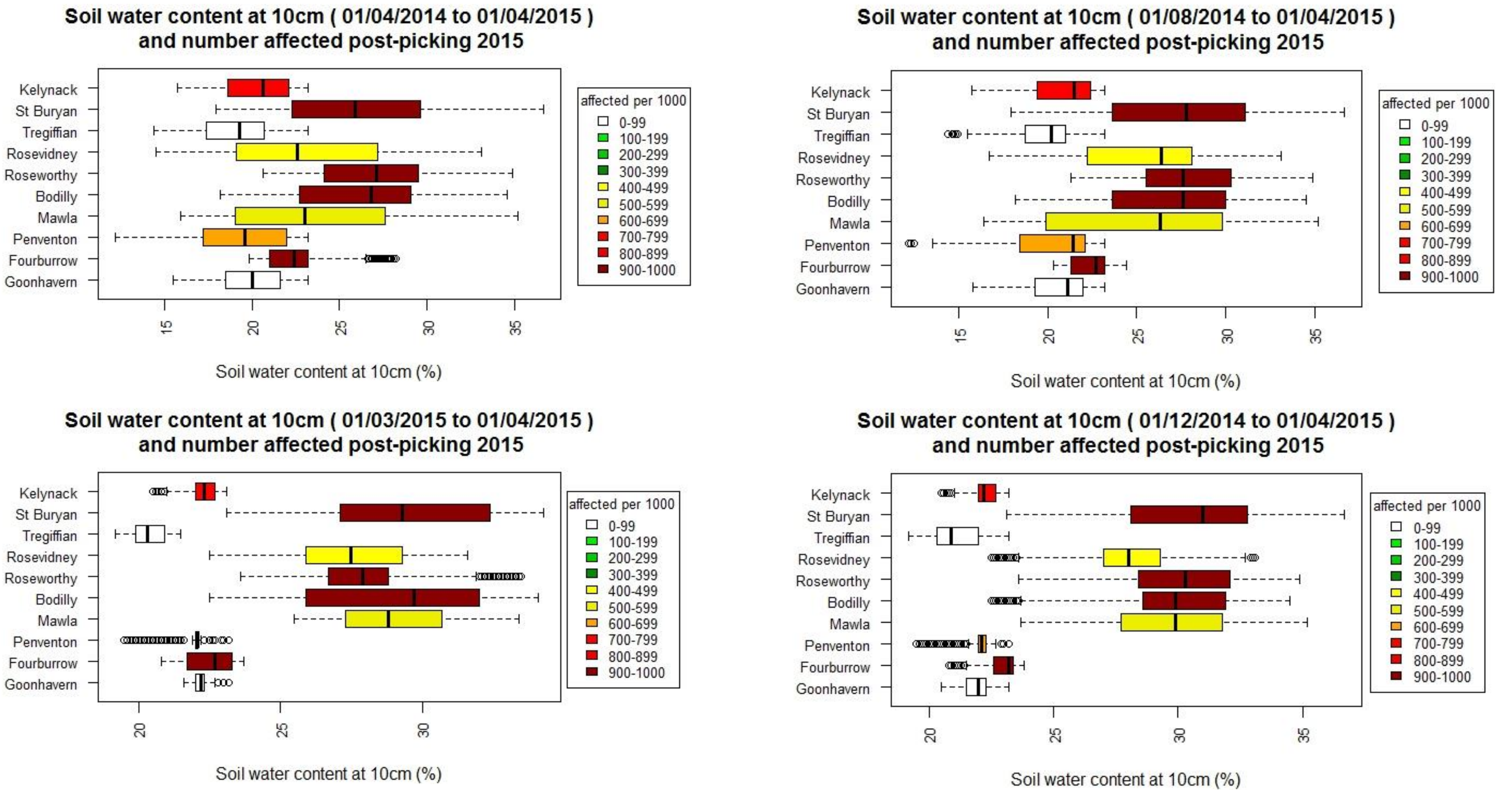


Figure 51. Box-and-whisker plots showing rust incidence in 2015 in relation to SWC at 10cm-depth over the (top-left, then clockwise) 12-month, 8-month, 4-month and 1-month periods to April 2015; each site's box is colour-coded (see legend) for rust incidence in 2015 (expressed as the number of stems per plot with rust at the post-picking stage)

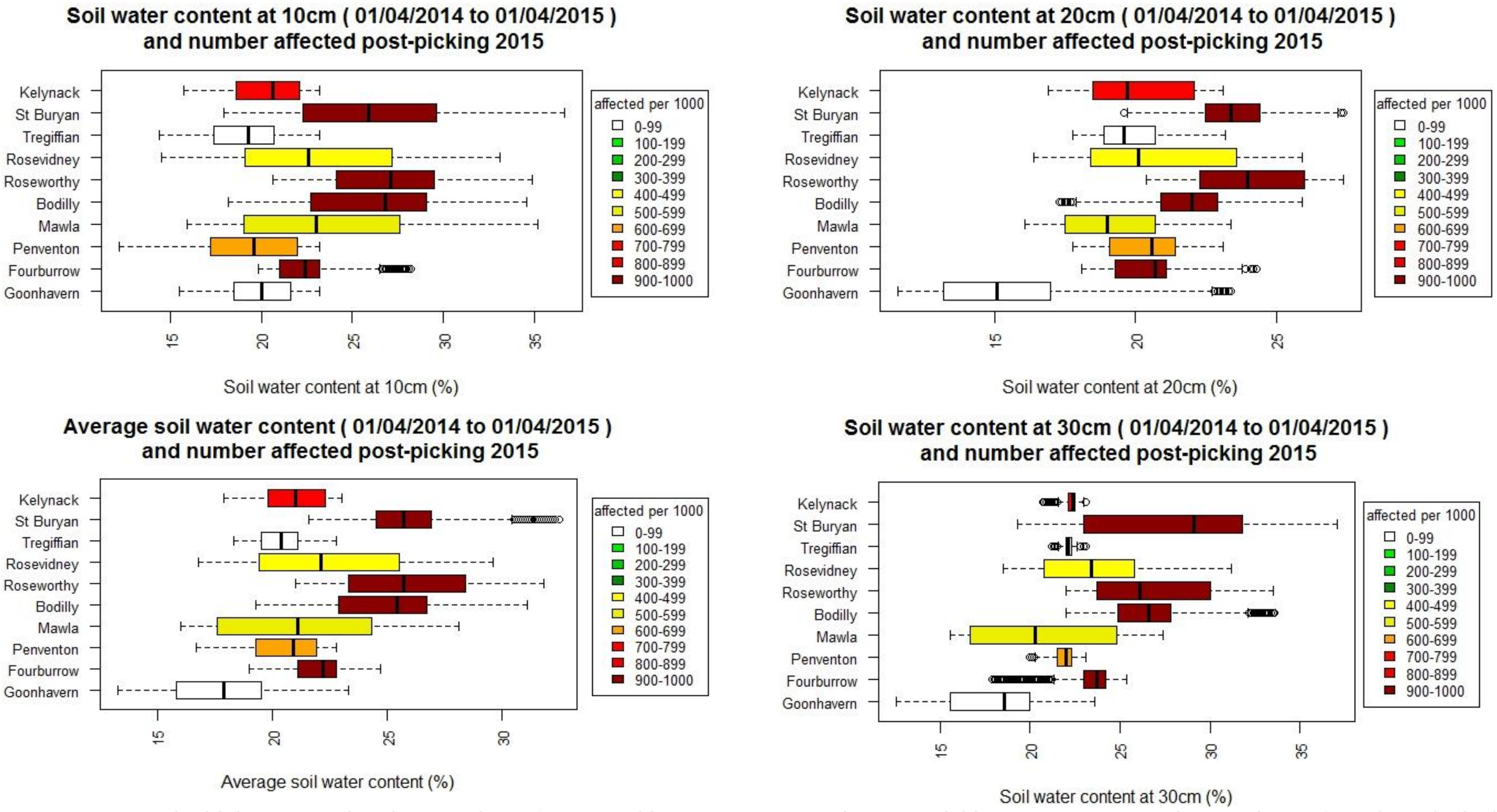
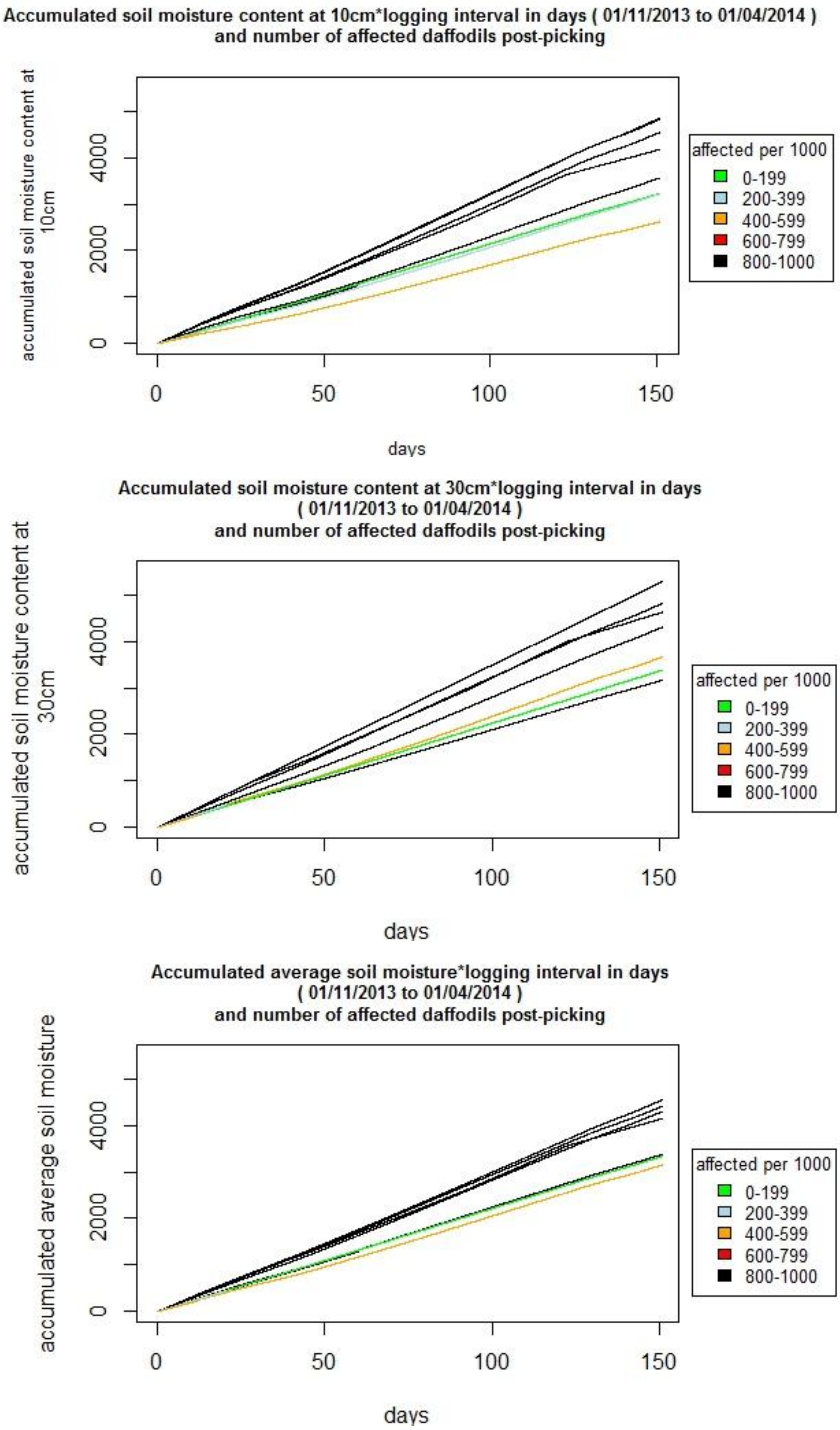


Figure 52. Box-and-whisker plots showing number of stems with rust per plot at the post-picking stage 2015 and SWC (top-left and clockwise) at 10, 20 and 30cm depth and as the average of the three depths between 1 April 2014 and 1 April 2015; note that the scale of the SWC-axes varies between depths

Accumulated SWC

Various expressions of SWC were tested. The accumulated daily SWC over the 5-month period November–March was found to be useful, and is illustrated for 2013–2014 in Figure 53. Only the 10 and 20cm SWC and the average SWC are included as it was shown above that the 20cm measurements could be difficult to interpret. This statistic and format seems to provide a useful way of distinguishing high- and low-rust sites, and the data for all years and soil depths (Table 20) were subjected to regression analysis. The regression lines are shown as examples for the 2013–2014 data in Figure 54, the 10cm and average accumulated SWC data give reasonable linear relationships. The regression analysis is summarised in Table 21 which shows the regression between the average accumulated SWC (and some individual depths) and the level of rust were statistically significant at P<0.1 or P<0.05.



Accumulated soil moisture content at 10cm*logging interval in days (01/11/2013 to 01/04/2014) and number of affected daffodils post-picking

Accumulated soil moisture content at 30cm*logging interval in days (01/11/2013 to 01/04/2014) and number of affected daffodils post-picking

Accumulated average soil moisture*logging interval in days (01/11/2013 to 01/04/2014) and number of affected daffodils post-picking

Figure 53. The effect of accumulated daily SWC over the 5-month period 1 November 2013 to 1 April 2014 on the number of stems with rust per plot at the post-picking stage 2014: SWC measured (top) at 10cm depth, (middle) at 30cm depth and (bottom) as the average of readings at 10, 20 and 30cm depth; although the sites do not need to be identified, the level of rust at each site is indicated by the colour key (black represents the highest rust level)

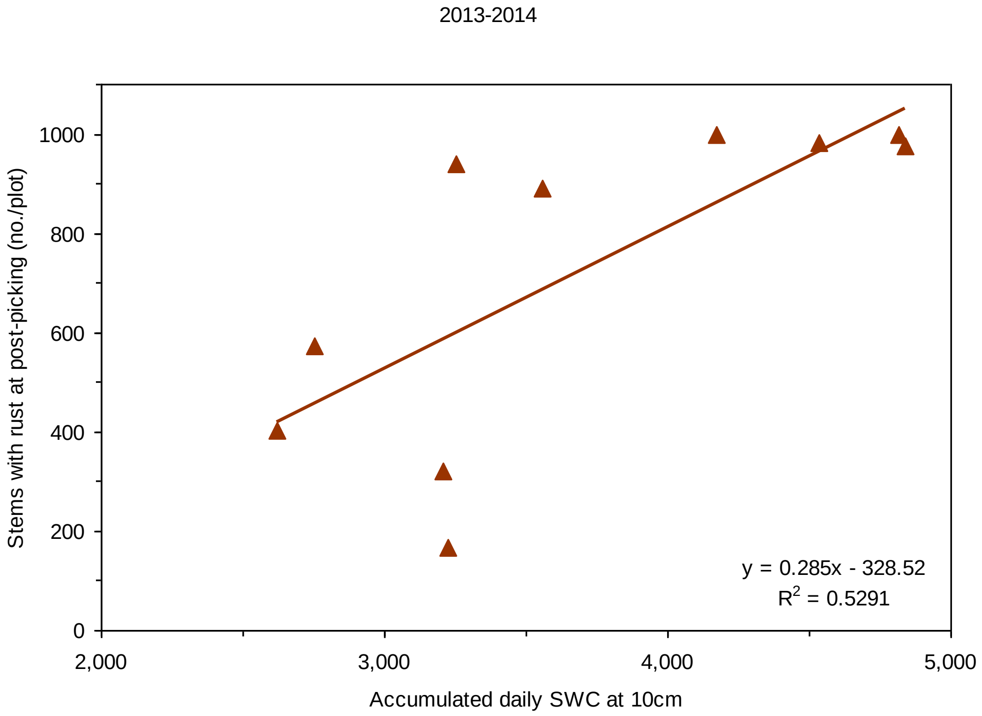
Table 20. The accumulated daily SWC for November–March measured at the indicated soil depth (or the average of these) for the ten sites and three years

	Accumulated SWC			
2012–2013	**10cm**	**20cm**	**30cm**	**Average**
Kelynack	2624	2638	3416	2893
St Buryan	3764	3855	4688	4102
Tregiffian	3743	3207	4278	3743
Rosevidney	3400	3628	4022	3683
Roseworthy	3005	3046	3638	3230
Bodilly	3346	2737	3867	3317
Mawla	3262	3139	3386	3262
Penventon	2890	3112	3379	3127
Fourburrow	2136	2794	3584	2838
Goonhavern	3098	3353	3565	3353
2013–2014				
Kelynack	3250	3112	3404	3256
St Buryan	4534	3817	5282	4544
Tregiffian	4170	3644	4624	4146
Rosevidney	2620	3174	3658	3151
Roseworthy	4839	3748	4298	4295
Bodilly	4815	3600	4811	4408
Mawla	3560	3409	3165	3378
Penventon	3224	3392	3379	3332
Fourburrow	3209	3200	3585	3331
Goonhavern	2754	2359	2995	2704
2014–2015				
Kelynack	3346	3364	3390	3367
St Buryan	4538	3569	3589	3898
Tregiffian	3162	3128	3340	3210
Rosevidney	4174	3566	3904	3881
Roseworthy	4478	3924	4564	4322
Bodilly	4456	3351	4112	3973
Mawla	4370	3151	3718	3746
Penventon	3316	3275	3340	3311
Fourburrow	3461	3194	3653	3436
Goonhavern	3292	2606	3081	2993

Table 21. Summary of linear regression analysis for accumulated SWC and rust levels (the number of stems per plot with rust at the post-picking stage in 2013, 2014 and 2015), showing the regression coefficient (R^2) and its probability level (P)

	2012–2013		2013–2014		2014–2015	
Depth	**R^2**	**P**	**R^2**	**P**	**R^2**	**P**
10cm	0.301	0.100 (*)	0.529	0.017 *	0.314	0.092 (*)
20cm	0.279	0.117 ns	0.211	0.181 ns	0.462	0.031 *
30cm	0.193	0.204 ns	0.329	0.083 (*)	0.385	0.056 (*)
Average	0.319	0.089 (*)	0.429	0.040 *	0.453	0.033 *

(*), *, ** and *** indicate significance at the 0.1, 0.05, 0.01 or 0.001 levels of probability; ns, not significant



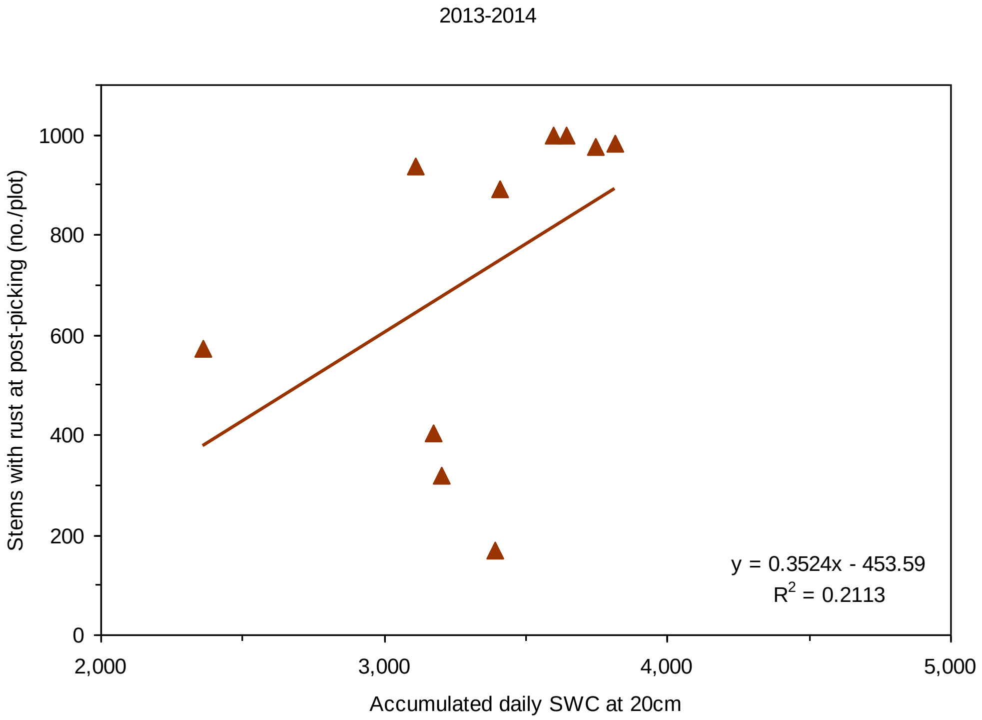


Figure 54. Regression of accumulated daily SWC over the 5-month period 1 November 2013 to 1 April 2014 and the number of stems with rust per plot at the post-picking stage 2014: SWC measured (top) at 10cm depth, (middle) at 20cm depth, (next page, top) at 30cm depth and (next page, bottom) as the average of readings at 10, 20 and 30cm depth

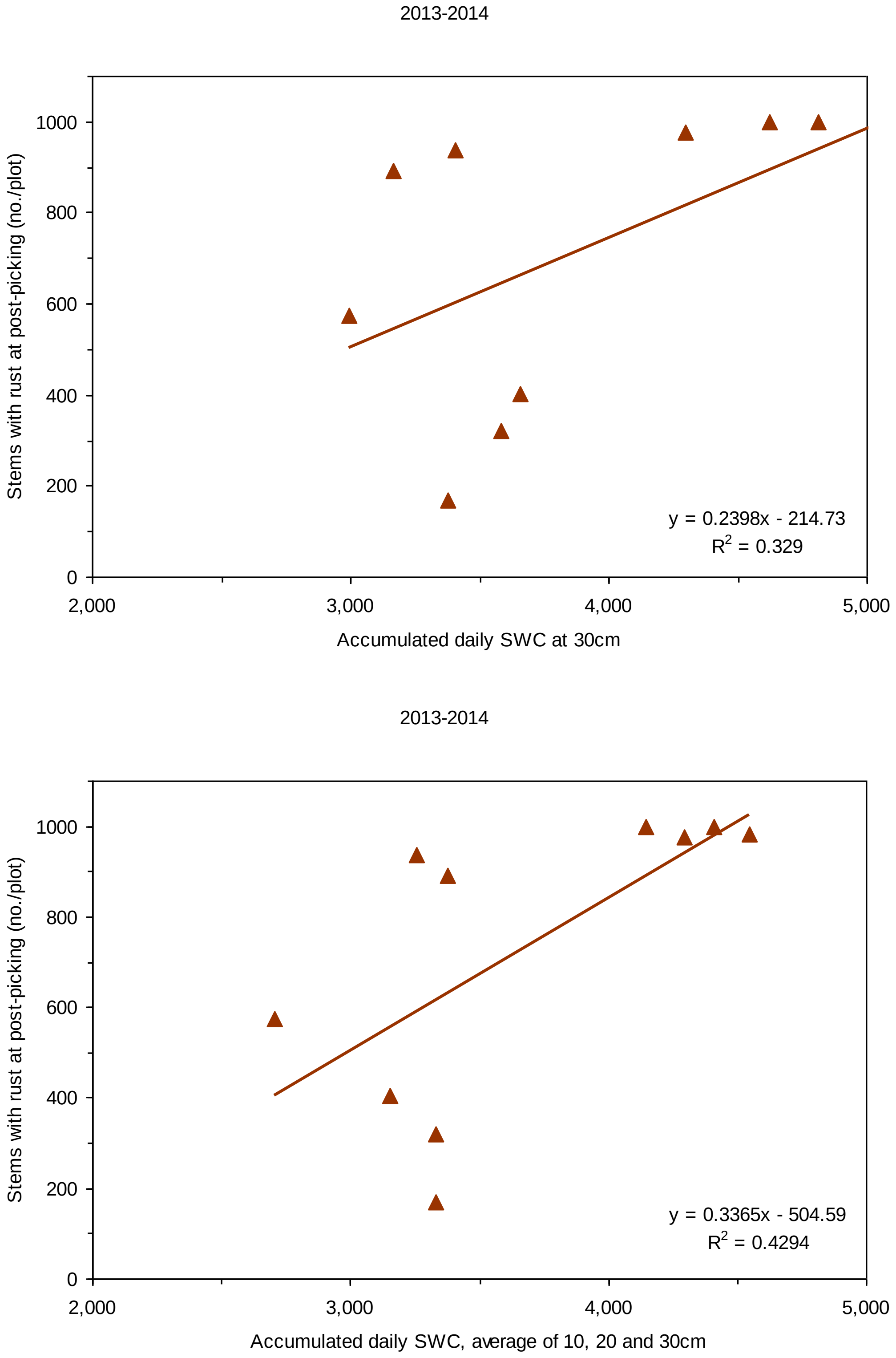


Figure 54 (continued)

Rust and geographical factors

It is likely that some effects of climate on plants could be represented by proxy variables such as latitude (data in Table 1). For example, in the previous sections on ‘Rust and SWC’ there were several examples of higher levels of rust occurring at the more westerly sites where the climate would be more maritime (e.g. milder, wetter and windier). However, regression analysis of longitude, latitude, altitude and distance from the sea against each year’s rust level failed to show any significant associations, indicated by low values of R^2 (≤ 0.36, often much lower).

Rust and soil structure

Regression analysis of the proportion of sand, silt, clay, etc. from the particle size analysis (data in Figure 7) and of the depth of top- and sub-soil (data in Figure 8) against each year’s rust level failed to show any significant associations, indicated by low values of R2 (≤0.24, often very much lower).

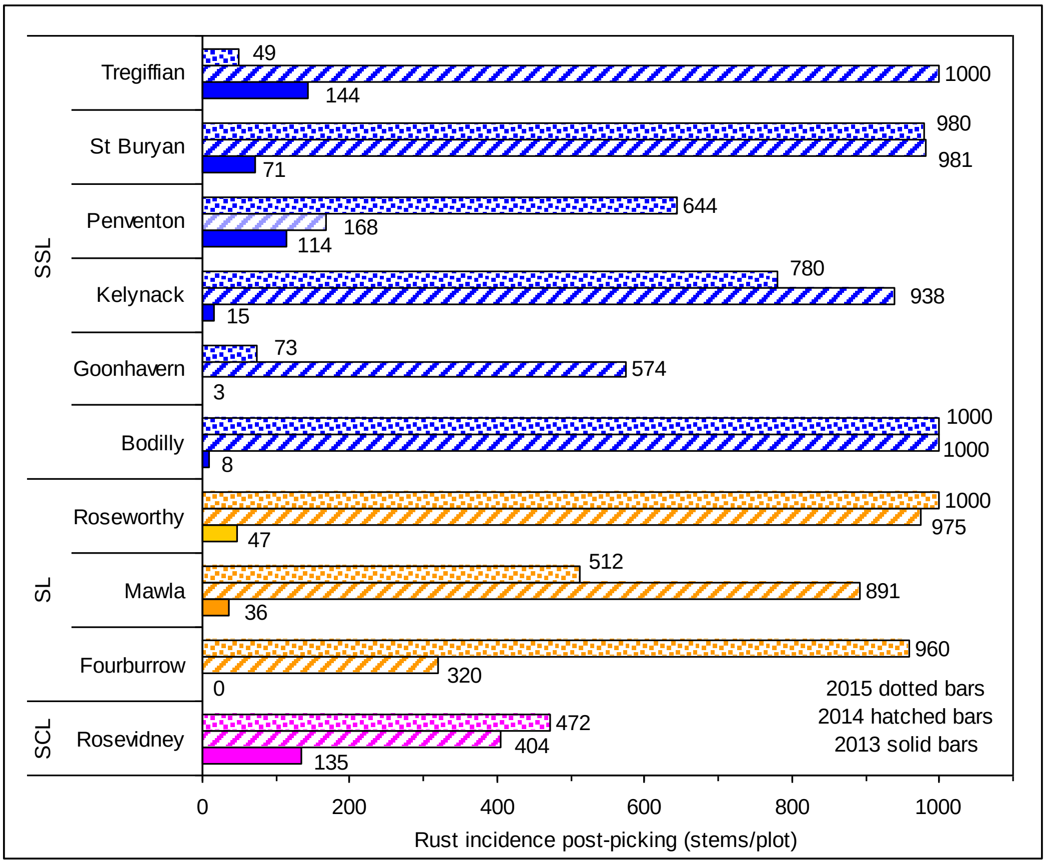


Figure 55. Rust incidence (number of stems per plot with rust post-picking) for the ten sites in 2013–2015, classified by ADAS soil texture (SSL, SL or SCL); the results for each soil texture are grouped together and colour-coded; note the lack of any consistency in the rust levels occurring within the SSL or SL groups

Other aspects of soil structure were not easily quantifiable, and so could not be related to rust levels using regression analysis. Instead, the effects were visualised as simple bar-charts that grouped the sites into categories. Soils at the test sites were predominantly friable (VSSQA score 1) sandy silt loams (SSL in the ADAS classification) of the Denbigh 2 soil series, sub-group 541k (Soil Survey of England & Wales, 1983) (data in Table 4). A wide range of rust levels was present within the six sites having SSL soils (

Figure 55), the six sites having a friable texture (Figure 56) and the six sites within soil group 541k (Figure 57). It appears unlikely that there was any relationship between rust levels (in any year) and these factors. These figures also serve to emphasise that rust levels often vary greatly from one year to another at the same site, suggesting that between-year factors are more important in determining rust levels than between-site factors.

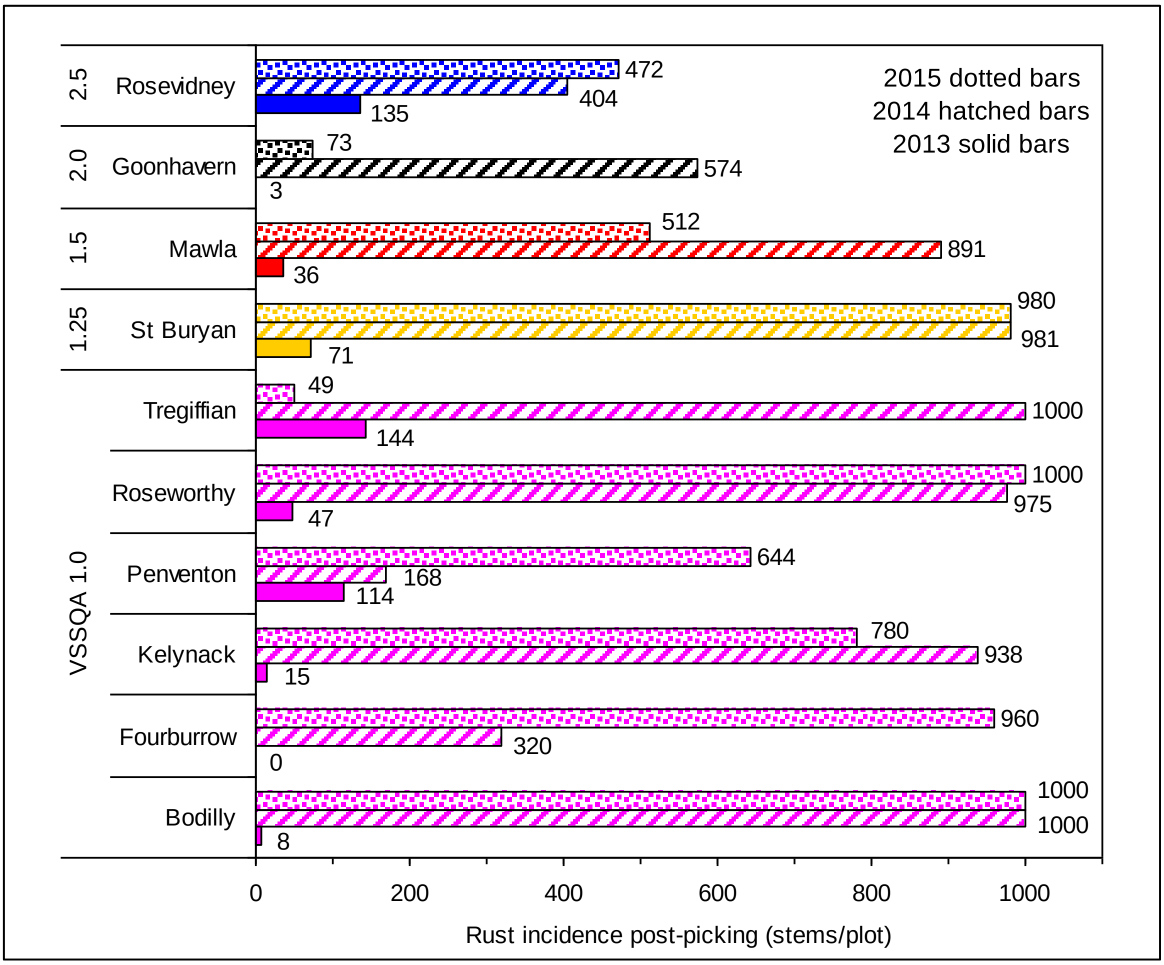


Figure 56. Rust incidence (number of stems per plot with rust post-picking) for the ten sites in 2013–2015, classified by VSSQA score (1 to 2.5); the results for each score are grouped together and colour-coded; note the lack of any consistency in the rust levels occurring within the main group (VSSQA 1.0)

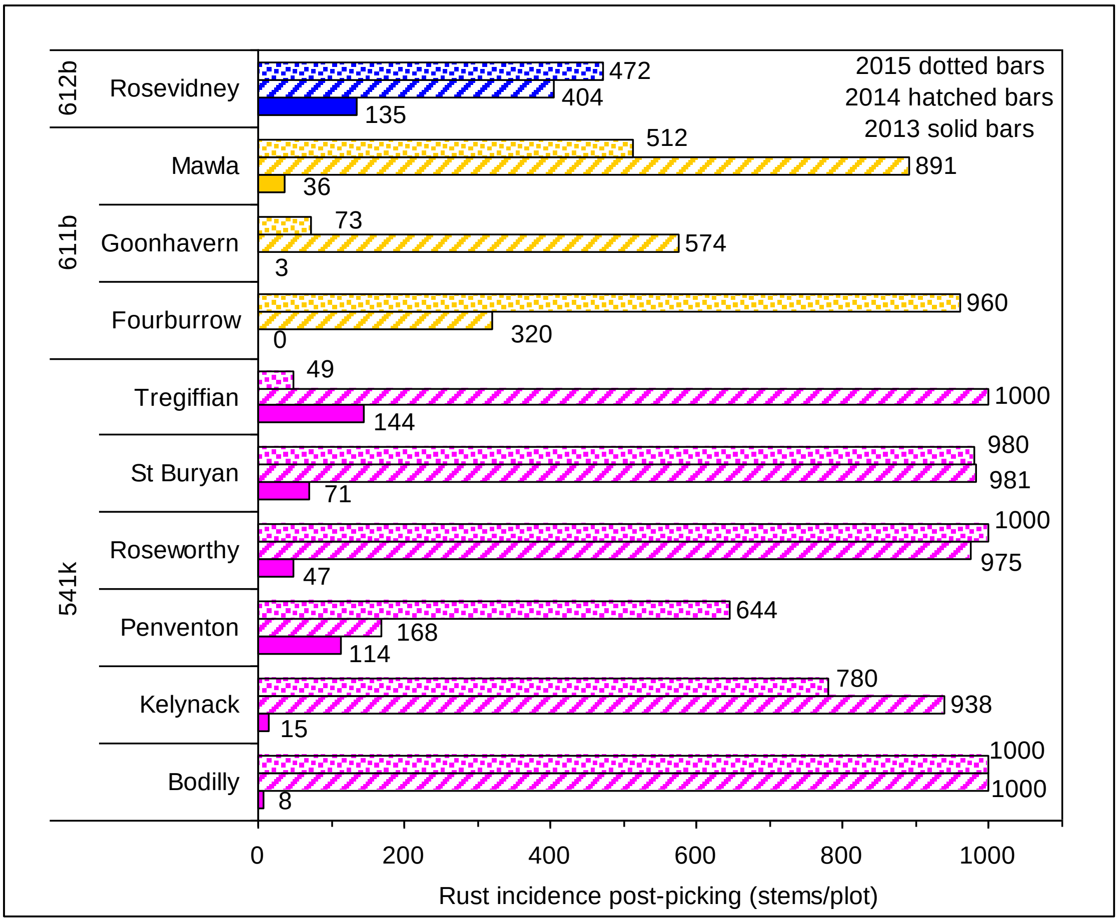


Figure 57. Rust incidence (number of stems per plot with rust post-picking) for the ten sites in 2013–2015, classified by Soil Survey of England & Wales soil sub-group (612b, 611b or 541k); the results for each sub-group are grouped together and colour-coded: note the lack of any consistency in the rust levels occurring within the main groups (541k and 611b)

Rust and husbandry factors

Simple bar-charts showing previous cropping, type of fertiliser applied in advance of bulb planting and the date of bulb planting (data in Table 3) gave no suggestion of any relationship with subsequent rust incidence in any year. For example, a wide range of rust levels was seen each year following either cereals, ley/meadow or brassicas, which would leave the soil in quite different states of nutrient depletion or enrichment (Figure 58), or following the application of different fertilisers (Figure 59). Bulb planting had to be delayed at some sites due to the very wet autumn of 2012, and although this might be expected to have an effect on crop growth and development, there was no trend towards higher or lower rust levels with progressively later planting (Figure 60).

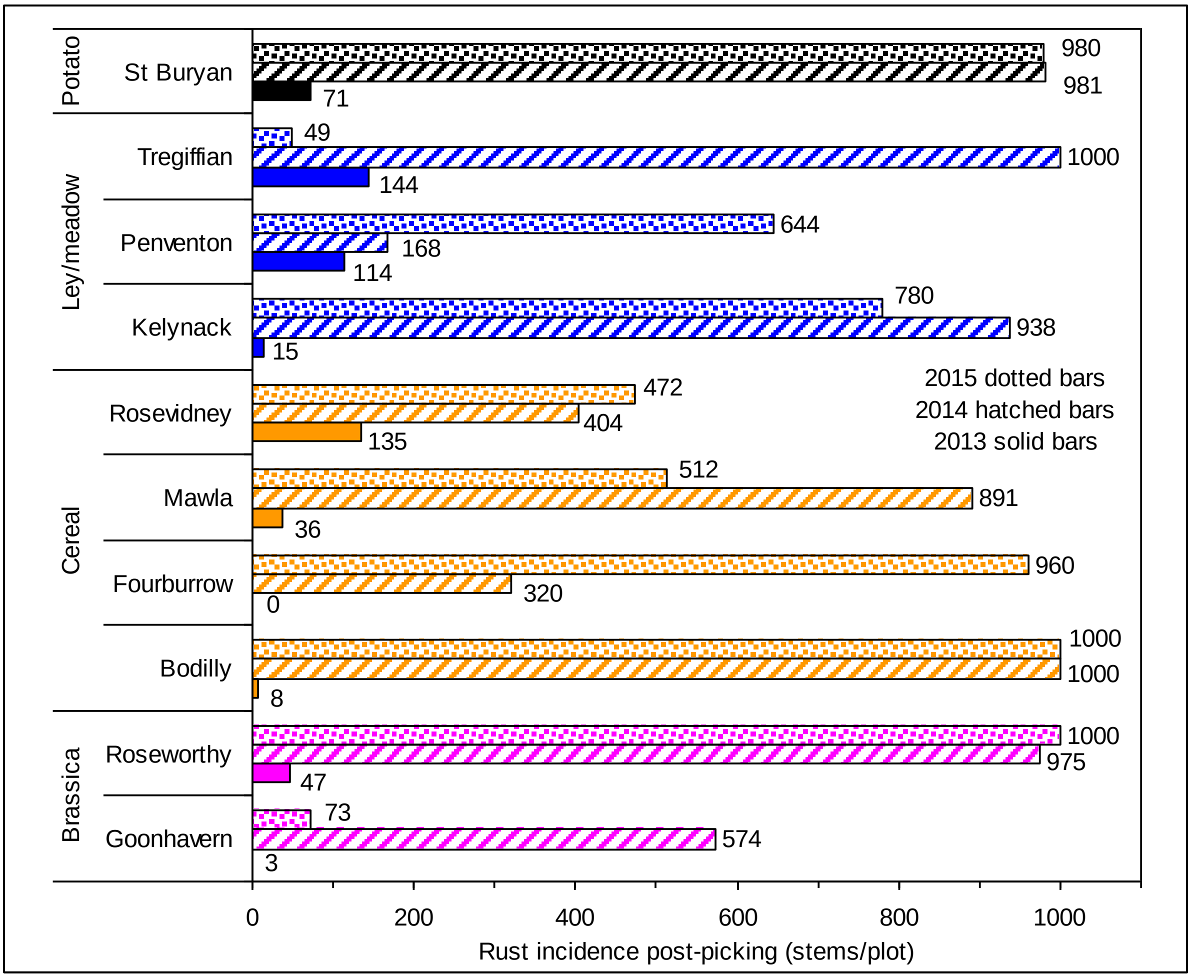


Figure 58. Rust incidence (number of stems per plot with rust post-picking) for the ten sites in 2013–2015, classified by previous cropping (brassicas, cereals, ley or meadow and potatoes). The results for each crop type are grouped together and colour-coded. Note the lack of consistency in rust levels within the main groups

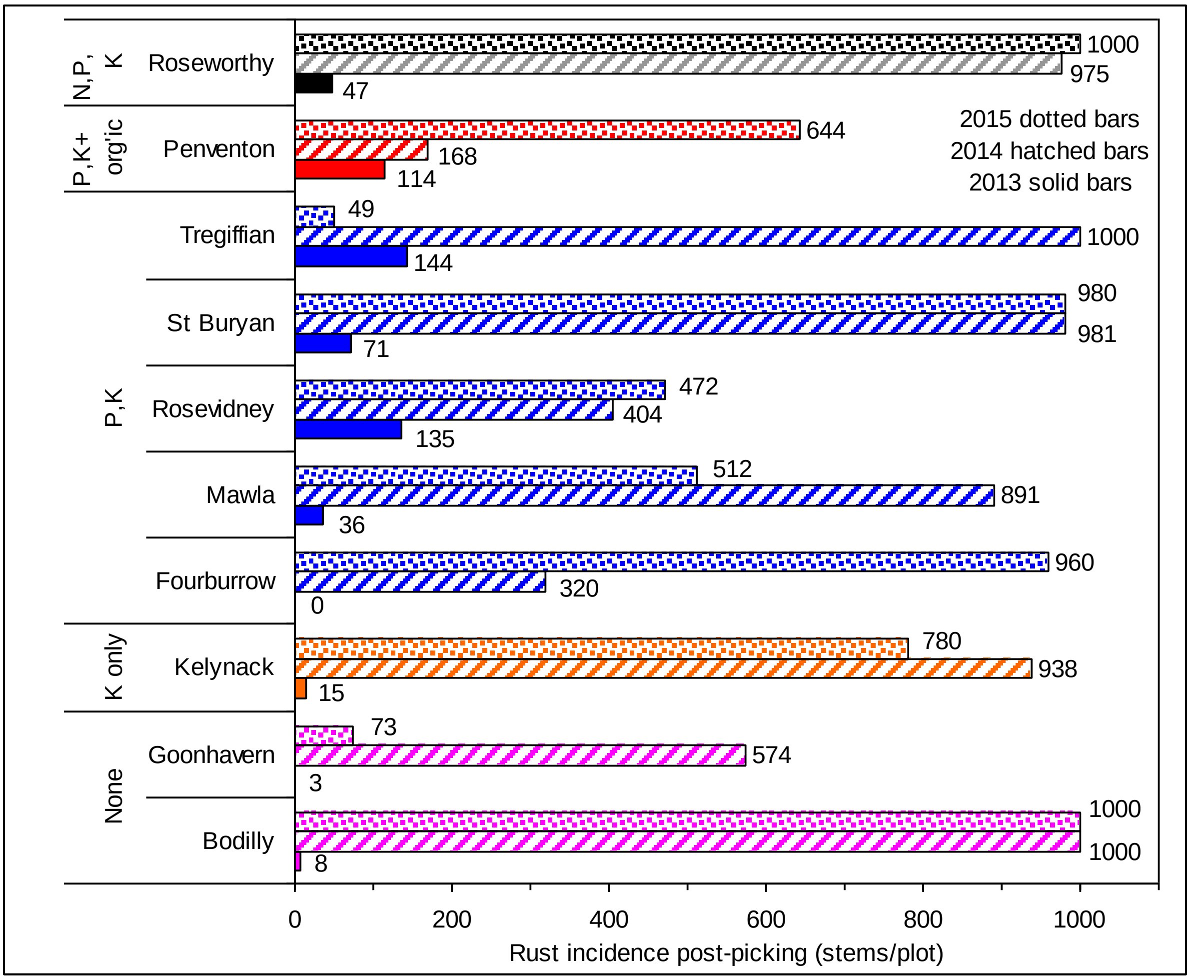


Figure 59. Rust incidence (number of stems per plot with rust post-picking) for the ten sites in 2013–2015, classified by fertiliser type previously applied (none, K only, P and K, P, K and organic fertiliser or N, P and K). The results for each fertiliser type are grouped together and colour-coded. Note the lack of consistency in rust levels within the main P,K group

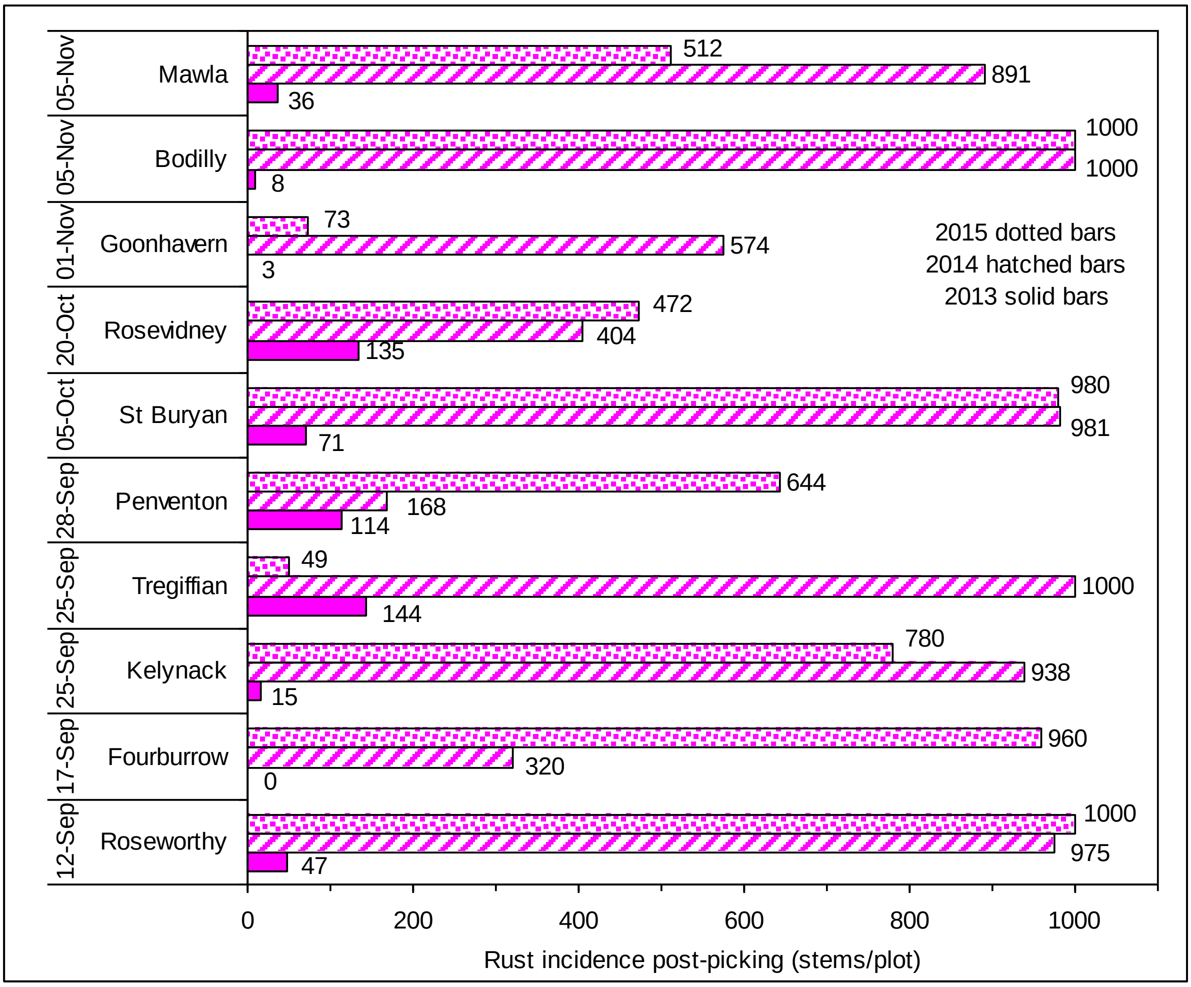


Figure 60. Rust incidence (number of stems per plot with rust post-picking) for the ten sites in 2013–2015, classified by planting date (ordered with last-planted at top). Note the lack of any consistent trend across the planting dates

Rust and nutrient concentrations in soil and leaves

Linear regression analysis was used to seek associations between rust levels and the current year's soil nutrient concentrations. Rust incidence in 2013 was examined using the soil analyses of both autumn 2012 and spring 2013, and rust incidence in 2014 and 2015 was examined using the current soil analyses. The findings are shown in Table 22: in this instance, to demonstrate a significant association ($P<0.05$) between nutrient concentration and rust levels the value of the regression coefficient (R^2) should approach 1 and the F-statistic should exceed 5.6; this occurs in only two instances out of the 53 available; it is clear that rust levels were unrelated to the concentrations of soil nutrients. Rust incidence in 2014 and 2015 was also examined in relation to leaf nutrient concentrations: regression analysis showed that rust levels were unrelated to the concentrations of nutrients in leaves.

Table 22. Summary of linear regression analysis for soil nutrient concentrations (measured in 2012–2015) and rust levels (the number of stems per plot with rust at the post-picking stage in 2013, 2014 and 2015)

Nutrient, soil OM or pH	**R^2 coefficient and (in brackets) the F-statistic[1] with its probability level[2] for regression of soil nutrient concentration and rust incidence (year of nutrient analysis followed by year of rust assessment)**							
	2012-2013		**2013-2013**		**2014-2014**		**2015-2015**	
Aluminium	-		0.314	(0.217)ns	0.220	(1.973)ns	0.072	(0.546) ns
Boron	-		-		0.043	(0.313)ns	0.003	(0.020) ns
Calcium	-		0.001	(0.003)ns	0.470	(6.218)*	0.022	(0.157) ns
Copper	-		-		0.168	(1.415)ns	0.006	(0.045) ns
Iron	-		0.001	(0.001)ns	0.004	(0.031)ns	0.095	(0.734) ns
Magnesium	0.029	(0.211)ns	0.060	(0.446)ns	0.188	(1.619)ns	0.005	(0.035) ns
Manganese	-		0.003	(0.018)ns	0.440	(5.492)(*)	0.041	(0.302) ns
Molybdenum	-		-		0.153	(0.298)ns	0.005	(0.032) ns
Nitrogen	-		0.032	(0.229)ns	0.037	(0.268)ns	0.006	(0.043) ns
nitrate-N	0.039	(0.282)ns	0.049	(0.364)ns	0.035	(0.256)ns	0.059	(0.439) ns
ammonium-N	-		0.019	(0.138)ns	0.245	(2.272)ns	0.328	(3.411) ns
Phosphorus	0.235	(2.153)ns	0.270	(2.595)ns	0.001	(0.007)ns	0.249	(2.319) ns
Potassium	0.018	(0.131)ns	0.026	(0.188)ns	0.010	(0.070)ns	0.014	(0.102) ns
Sodium	-		0.001	(0.007)ns	0.151	(1.244)ns	0.270	(2.587) ns
Sulphate	-		-		0.370	(4.121)(*)	0.049	(0.358) ns
Zinc	-		-		0.091	(0.432)ns	0.255	(2.398) ns
Topsoil OM	-		-		0.477	(6.394)*	0.043	(0.316) ns
Soil pH	0.031	(0.111)ns	0.016	(0.111) ns	0.259	(2.444)ns	0.012	(0.082) ns

[1] with df (regression) =1 and df (residual) =7 an F-value >5.6 is needed for statistical significance at the 5% level of probability

[2] (*), *, ** and ** indicate significance at the 0.1, 0.05, 0.01 or 0.001 levels of probability; ns, not significant

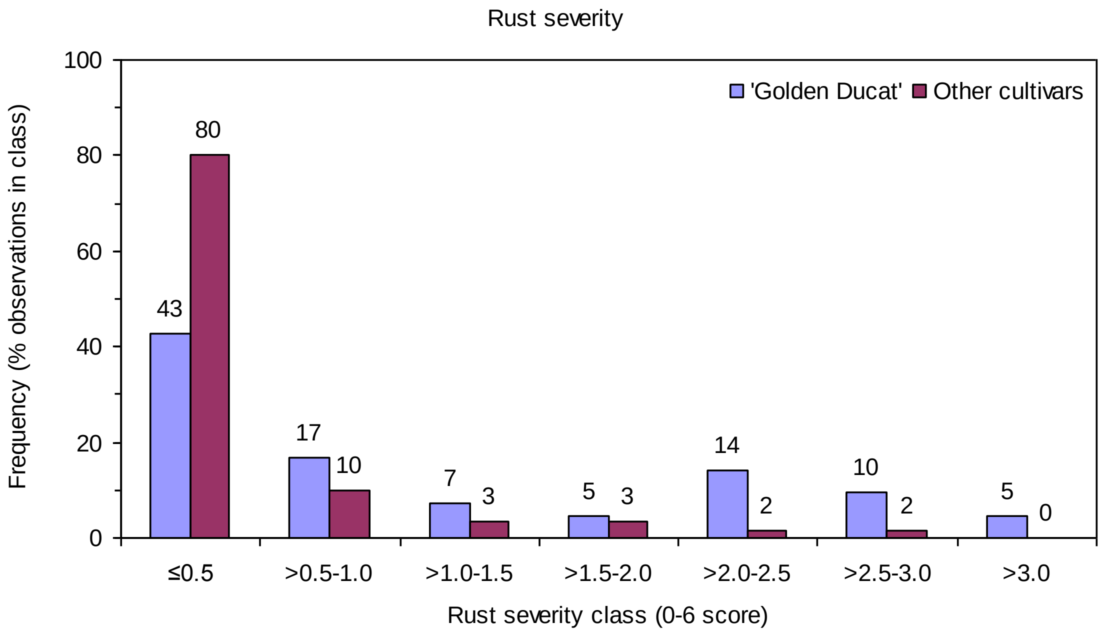
Survey of commercial cut-flowers

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). 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 and 4 a greater level of rust that may cause commercial concerns (in this survey no stems reached a score of 5 or 6).

For rust incidence, ‘Golden Ducat’ averaged just over 50% of stems with any rust (27.4 stems/50 stems) and the other cultivars a much lower 21% (10.7 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 61) 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.

For the ‘Golden Ducat’ samples the severity and incidence of rust in relation to sampling (picking) dates, running from 9 March to 26 April, are shown as scatter plots for severity scores and rust incidence (stems/50 stems) in Figure 62. 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. While some of these examples may still have passed as marketable, they would not have given a good impression of the product. Some examples are shown in Figure 63.



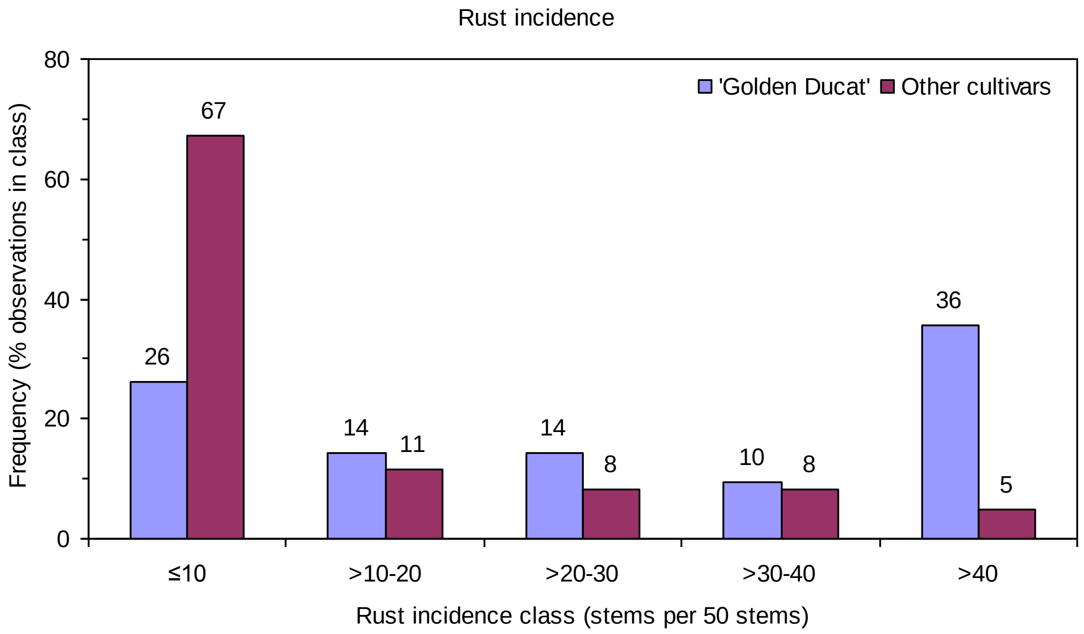
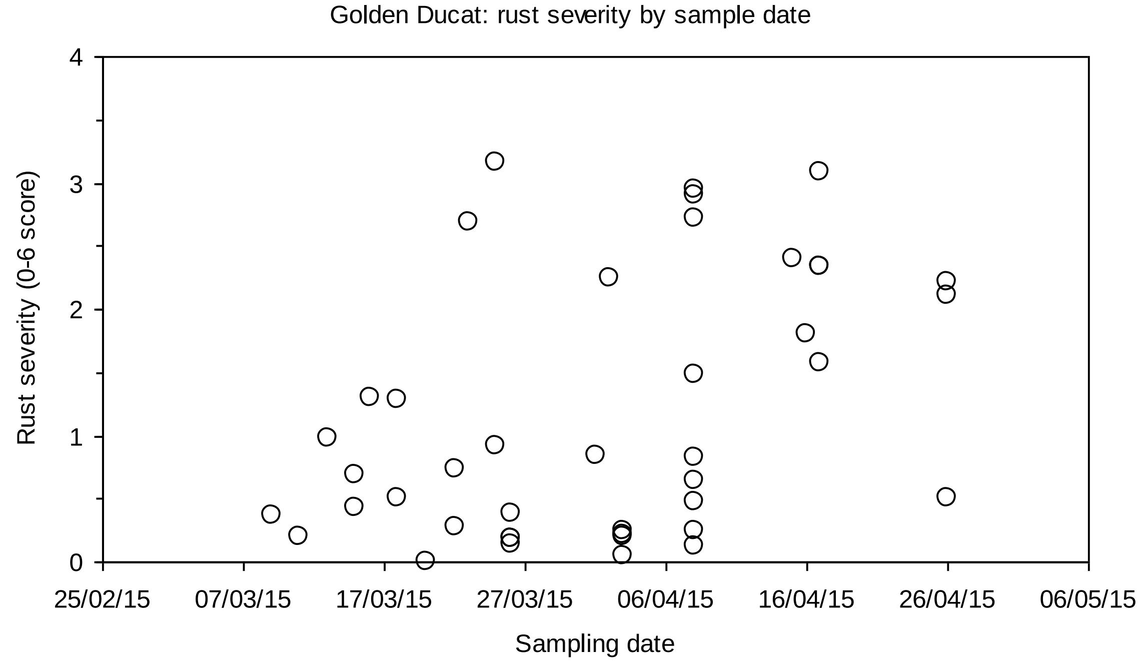


Figure 61. The frequency of rust severity scores (from 0 to 6, top) and of rust incidence (stems with rust per 50 stems, bottom) for samples of ‘Golden Ducat’ and other cultivars. The numbers above the bars are the percentage of observations in each of the classes along the horizontal axis



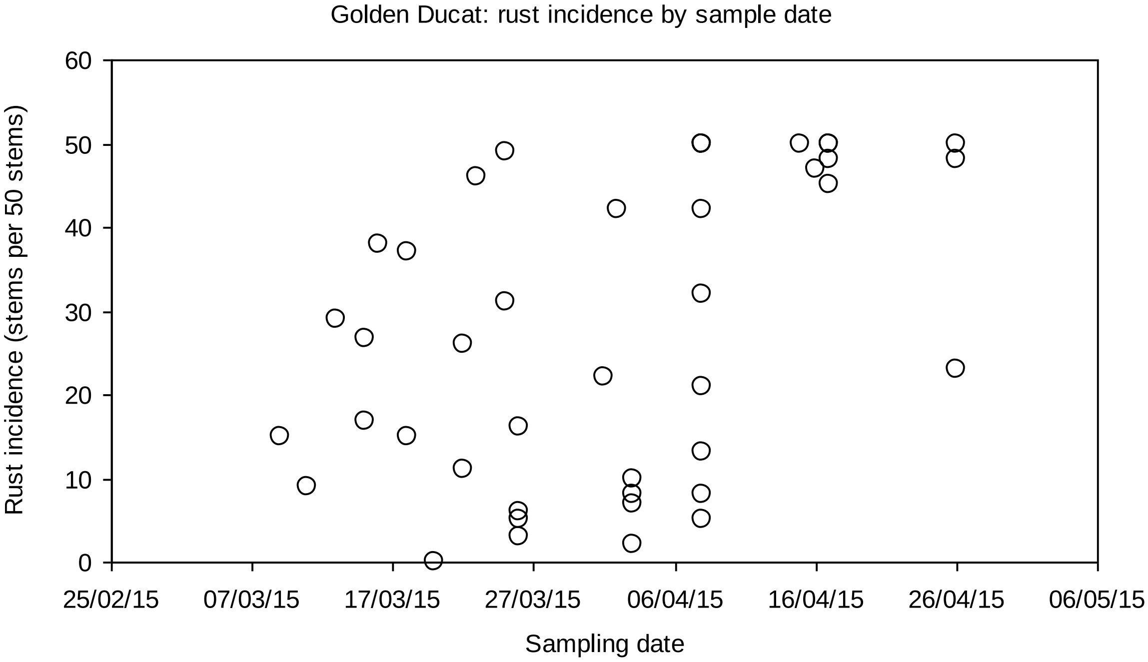


Figure 62. Scatter plots of rust severity scores (top) and rust incidence (stems with rust per 50 stems, bottom) for 42 samples of commercial ‘Golden Ducat’ stems picked between 9 March and 26 April 2015; the lack of any pattern in the data-points indicates a random distribution of rust levels across all picking dates

Figure 63. Unidentified examples of popular cultivars (unidentified, but not ‘Golden Ducat’) from the survey of commercial bunches

Discussion

Rust: pathological, nutritional or environmental?

When daffodil rust came to the attention of growers in the UK during the early-1980s, its disease-like lesions and spread suggested a pathogen as the likely cause. No results of diagnostic investigations appear to have been published, but it is known that samples of affected plants were sent by advisors and growers to plant clinics. Investigations failed to show any pathogen associated with the lesions and rust was thereafter considered a physiological disorder (or abiotic disease). Since some physiological disorders in crops are associated with nutritional deficiencies, the next step was to send soil and plant samples to analytical labs so that nutrient levels in affected and unaffected samples could be compared. Analysis failed to reveal any helpful differences in nutrient concentrations between the samples. Some of these results were made available to the then-HDC and summarised in an earlier report (Annual Report on BOF 76, 2013).

Some physiological disorders with dark lesions have been attributed to adverse environmental conditions. For example, dry hot summer weather and a sudden change in temperature were implicated in the appearance of apple leaf spot and drop, and water stress when transpiration exceeds water uptake, promoted by sudden checks in growth such as low temperatures, in lettuce dry (marginal) tip-burn (Swain, 1985). The general term oedema would cover this type of physiological damage, the dark lesions found in many plants when wet, relatively warm soil and falling air temperatures result in faster water uptake by the roots than can be balanced by transpiration from the leaves, the raised internal water pressures swelling and damaging vulnerable groups of cells and resulting in necrotic areas. Adverse temperatures and water relations have also been implicated in other, little studied physiological disorders of daffodils, such as chocolate spot and *Narcissus poeticus* ‘Flore Pleno’ bud death. Incorporating the findings of the present study, pathogenic, nutritional and environmental explanations of daffodil rust will now be considered in more detail.

Rust: a fungal pathogen?

The name daffodil rust was coined for this physiological disorder because of its rust-coloured lesions and their resemblance to the lesions of true rust fungi. Generally rust fungi do affect daffodils, except in one specific scenario and in rare instances. In the Netherlands daffodils can be infected by *Aecidium narcissi*, a rust fungus that can spread from the reed-grass *Phalaris arundinacea* that is sometimes used as a covering material to protect bulbs and shoots from frost and wind-blown sand (van Aartrijk *et al*., 1995). *A. narcissi* does not have an adverse effect on the daffodil crop. Covering crops are not used on daffodils in the UK, and *A. narcissi* is not known to have been reported here. Apart from that, reports of true rust pathogens on daffodils are rare, though *Puccinia schroeteri*, *P. narcissi* and *Coleosporium narcissi* have been reported (Moore *et al*., 1979; Chastagner & Byther, 1985).

Some parallels can be drawn between the initial investigations into daffodil rust and those used in studying chocolate spot of daffodils in the 1960s. (This disorder is distinct from the fungal disease of beans, also called chocolate spot.) Appearing on leaves, its blotches (10mm or more in diameter) and sometimes elongated streaks, both the colour of dark chocolate, its characteristic lesions have long been recognised in the daffodil industry (Moore *et al*., 1979); Swain, 1985). It occurs at a low level and does not appear to have caused any significant damage. It appears some effort was expended looking without success for a causal organism of chocolate spot, according to the account of Moore *et al*. (1979):

> "In two successive seasons (1967/8) several hundreds of cultivars [of daffodils] in the collection at Rosewarne EHS [Experimental Horticultural Station] were examined for signs of [chocolate spot]. Most examples were from Groups 1A and 2A [non-white trumpet and large-cup daffodil cultivars] but not all these cultivars had the markings in both seasons. Histological preparations showed that the colour [was] caused by the death of the epidermal cells but no fungus or bacterium has been isolated consistently from the streaks. Scanning electron micrographs have shown that the cuticle is intact which seems to exclude a causal agent. A viral cause seems to be eliminated because no virus particles have been detected or isolated from narcissus seedlings with symptoms. Casual observations suggest that the appearance of the [chocolate] spots is associated with increasing ambient temperatures."

In the present project chocolate spot lesions were occasionally seen on leaves in the plots, but were not recorded. Samples of stems from all ten sites, with varying levels of rust, were examined for the presence of fungal and bacterial pathogens using standard diagnostic procedures. Microscopic examinations of typical rust lesions showed they consisted of groups of necrotic cells that are fairly superficial, though some more 'streak'-like lesions were deeper. Over two years, an as-yet unidentified *Stemphylium* species was consistently found in cultures from typical rust lesions, but not (where typical rust lesions were absent) in cultures from 'streak' symptoms. The identity of the genus by observation was confirmed by sequencing. At present, however, it is only the association of the pathogen with rust lesions that is proven. The re-inoculation of isolates to fresh daffodil stems is underway, to determine whether or not it is pathogenic, and this will be reported later in 2016.

This appears to be the first report of *Stemphylium* on *Narcissus*. Species of *Stemphylium* do not appear to have been reported previously on any Amaryllidaceous plants except onions, garlic and leeks. *Stemphylium* does not appear to have been reported on other flower bulb crops except gladiolus (for full references see Moore *et al*., 1979). Stemphylium leaf spot of gladiolus, due to *Pleospora herbarum* (conidial stage *Stemphylium botryosum*), has been reported in several European countries (but not the UK), Australia and the USA. It results in round, yellow/green spots, turning necrotic and spreading (Magie, 1948; Schenk, 1955). *S. lycopersici* was also reported on gladiolus (Ellis, 1971).

Several *Stemphylium* species are known to cause leaf spot and similar symptoms on many crop plants; the spots are often, but not always, dark in colour, often becoming necrotic and sometimes spreading. Several examples of damage by *Stemphylium* have been reported in the UK. *S. lycopersici* was noted as a pathogen on tomato foliage, this ‘stemphylium blight’ having serious effects on glasshouse tomatoes (Dickens and Evans, 1973). In this case the pathogenicity of *S. botryosum* was confirmed and different susceptibilities to it found in different tomato cultivars. Cook (1976) investigated black-specking of cabbage, assumed to be a non-parasitic disorder. Small black lesions, often with a chlorotic halo, started with the necrosis of stomatal guard cells. A *Stemphylium* species (and a *Pseudomonas* species) were frequently, but not consistently, isolated from the lesions, so causality could not be linked to *Stemphylium* in this case. ‘Mouldy core’, a storage problem in apple and pear, was investigated by Edney and Morton (1984, 1985). The condition was associated with the presence of a *Stemphylium* species deriving from infection that contaminated the floral parts; *Alternaria*, *Mucor* and *Phytophthora* species also seemed to be associated with ‘mouldy core’. Geeson *et al*. (1988) investigated the storage diseases of carrot and showed that *S. radicinum* was one cause, though it was of secondary importance. Foot rot of green beans (*Phaseolus vulgaris*) was studied by Whalley *et al*. (1991): *S. botryosum* was associated with the condition but in this case it was found to be non-pathogenic, existing as a saprophyte. Benson (2002) reported on an international trial (that included the UK) of asparagus cultivars, including their disease sensitivity to *Stemphylium* species. *Stemphylium* species cause a number of conditions on important crops worldwide, and some recent examples are given in Table 23. The research listed involved disorders already shown to be caused by

Stemphylium species or confirmed as such in the research; in several cases the research included testing fungicides against *Stemphylium*.

Table 23. Some recent examples of diseases caused by *Stemphylium* species

Disease	Host	Pathogen	Reference
Leaf spot	Sweet potato	Stemphylium solani	ALi *et al*. [*sic*] (2015)
Leaf spot	Broad bean	S. globuliferum	Baka (2015)
Stemphylium blight	Onion	S. vesicarium	Bhatia and Devender (2014)
Purple spot	Asparagus	S. vesicarium	Graf *et al*. (2016)
Yellow leaf spot	Sugar beet	*S.* species	Hanse *et al*. (2015)
Leaf spot	Egyptian clover	S. globuliferum	Omar *et al*. (2015)
Brown spot	Pear	S. vesicarium	Puig *et al*. (2015)
Stemphylium blight	Faba bean	S. botryosum and S. vesicarium	Shaikh *et al*. (2015)
Stemphylium blight	Lentil	S. botryosum	Subedi *et al*. (2015)
Leaf spot	Egg plant	S. solani	Sy-Ndir *et al*. (2015)
Grey leaf spot	Tomato	S. lycopersici	XuQing *et al*. (2015)

In the present project the only other fungi isolated in cultures of rust lesions were *Botrytis narcissicola* (occasionally) and *Ramularia vallisumbrosae* (frequently), the causal organisms of smoulder and white mould diseases, respectively. White mould is very common in some Cornish daffodil crops in some seasons, though normally arrested by routine fungicides if applied promptly enough after the symptoms have been seen, but in some cases (as at the Fourburrow site in 2014 and 2015) the crop was seriously affected and little healthy foliage survived to the end of the growing season; *Ramularia* should be expected as a widespread contaminant in such situations. The symptomatology and epidemiology of smoulder and white mould are well known, and they have no similarities to rust lesions.

There is no evidence for a fungal pathogen being responsible for daffodil rust.

Rust: a bacterial pathogen?

Bacterial diseases are very unusual in daffodils (Hanks and Chastagner, in press). Bulb rots due to *Pectbacterium carotovorum* subsp. *carotovorum* have occasionally been seen in bulbs of tazetta cultivars on the Isles of Scilly. A bacterial streak caused by a *Pseudomonas* species has been reported in the USA, with the stems collapsing from the base in the field, or with a soft stem decay starting near the rubber band holding bunches together. In the current project work carried out in parallel with the search for fungi associated with rust lesions found no evidence for bacterial infection.

Rust: a viral pathogen?

As described above, a viral agent was probably not involved in daffodil chocolate spot (Moore *et al*., 1979). When stocks of 'virus-free' or virus-indexed daffodils were being developed in Scotland, it was reported that "chocolate spot was observed in virus-tested [daffodil] clones, and it is suggested that it may be a physiological disorder induced by environmental conditions" (Mowat *et al*., 1983). From a study of chocolate spot in the Netherlands it was reported that an unknown filamentous virus had been found both in apparently healthy daffodil plants as well as in those "with [viral] symptoms of yellow stripe in the leaf-tips, light green leaf discoloration, *chocolate spotting* [author's emphasis] or silver streak" (Kamerman *et al*., 1975). These reports, that chocolate spotting may be found in 'virus-free' and 'apparently healthy' daffodils and those showing typical viral symptoms, argue against a causal virus or viruses. However, in describing virus diseases of daffodils, Bergman *et al.* (1978) and van Aartrijk *et al.* (1995), in the first and second editions of *Ziekten en afwijkingen bij bolgewassen*, include a condition called '*blad bruin'* (brown leaf). A translation from the second edition follows (this is almost identical to that in the first edition, except for a later addition about the effect of sunny days):

> "Spread over the entire leaf are stains with chocolate-brown discoloration visible. From plants with these symptoms NMV [Narcissus Mosaic Virus], LNV [Narcissus Latent Virus], NZSV [Narcissus Late Season Yellows Virus], TRV [Tobacco Rattle Virus] and/or TKV [Tobacco Ring-spot Virus] may be extracted, and none of these viruses can be designated as the main cause of the phenomenon. Very sunny days after flowering give rise to clear development of [these] symptoms."

Recent enquiries established that the description just given was based on work done many years ago, that this area has not been further researched, and that *blad bruin* is considered the equivalent of what in the UK is called chocolate spot (P van Leeuwen, personal communication, 2015). Taken together, this information does not implicate a virus, or combination of viruses, in the chocolate spot or *blad bruin* disorder (nor, of course, does it rule out a viral cause. In these communications the issue of daffodil rust was not raised, but it is tempting to wonder whether a parallel situation – a combined viral and environmental cause - might exist in rust also. Many other physiological disorders occurring in horticultural crops often show as distinct areas of necrotic tissue (Swain, 1985), raising the question whether there might be similarities between daffodil rust and other common physiological disorders. From studies of the physiological disorders tip-burn and cigar-burn of stored cabbage, it was suggested that waterlogged or other adverse conditions contributed to the disorders through interfering with water uptake, with a consequent shortage of calcium in the rapidly growing leaves. It was shown that Turnip Yellows Virus (formerly called Beet Western Yellows Virus) and Turnip Mosaic Virus were primary factors in the development of tip-burn and cigar burn, respectively, both disorders being exacerbated by the presence of Cauliflower Mosaic Virus (Walsh *et al.,* 2004; Walsh, 2008). Although these three aphid-transmitted viruses have not been reported from daffodils, most daffodil stocks are highly infested with several other viruses (Brunt, 1995). The peach potato aphid, *Myzus persicae*, is a common vector of both the viruses implicated in tip-burn and cigar burn and of some important daffodil viruses (Walsh *et al.*, 2004; Brunt, 1995).

In the present project, samples of stems from the ten sites, with and without typical rust symptoms, were examined for the presence of viruses. The expectation was that all samples would be positive for potyviruses at least, since most commercial stocks of daffodils have been shown to be infected with one or more viruses: the key question was whether any viruses were specific, and therefore possibly causal, to the tissues of the rust lesions. The Potyviruses and Nepoviruses are probably the viruses of most interest to daffodil growing, the former including a group of aphid-borne viruses commonly found in daffodils and the latter including several nematode-borne viruses of daffodils (the viruses known to be found in daffodils were recently reviewed by Hanks and Chastagner (in press). Tests were positive for Potyviruses and sub-group A of Nepoviruses. The dominant sequence was that of Narcissus Late Seasons Yellows Potyvirus, one of the most obvious of daffodil viruses, and there was a 76% match to Artichoke Latent Potyvirus which has not previously been reported from daffodils. In the Nepovirus group the dominant sequence corresponded with Arabis Mosaic Nepovirus, which is known to infect daffodils. Tests against sub-groups B and C of the Nepoviruses were negative, as were tests for the Carlavirus and Tospovirus genera. The Tospovirus group had been targeted as they are known to cause leaf-spot symptoms not dissimilar to daffodil rust: for example, Iris Yellow Spot Tospovirus shows a similar symptom on onions (Gent *et al*., 2006) but all the daffodil samples tested were negative for Tospoviruses. During the a putative new potyvirus was identified which was found to be closest in sequence to Artichoke Latent Virus, which generally infects without any obvious symptoms (Minutillo *et al*., 2015); it appears to be widespread as it was detected in all samples tested.

The results were consistent across extracts from typical rust lesions and from stems with no rust lesions: this confirmed the expected general infestation of daffodil stocks by viruses. So far, though, there is no evidence for the involvement of a virus or viruses in daffodil rust.

Rust: plant nutrition?

Nutritional issues are known to be important in many physiological disorders. Deficiencies can be caused directly by the low concentration of a nutrient, by its low mobility (e.g. in the case of boron or calcium), by a pH effect (e.g. for boron, calcium or molybdenium), or indirectly by levels of another nutrient (e.g. of calcium by potassium, magnesium or boron). In general, daffodils do not seem to be responsive to trace elements or even to the concentrations of the major nutrients – early experiments on the nutritional requirements of daffodils were hampered by their unresponsiveness, attributed to the large nutritional reserves present in the bulb scales (Bould, 1939; Hewitt and Miles, 1954; Wallis 1967). Normal practice for the crop is therefore to provide a modest level of nitrogen – excessive amounts have been shown to increase basal rot, even in a usually disease-resistant cultivar (Hanks *et al*., 1998) – and enough phosphate, potash and magnesium to maintain adequate soil fertility (‘*RB 209’*). The only evidence of deficiency that might be expected in a three-year-down daffodil crop is the pale foliage resulting from nitrogen deficiency.

At the ten trial sites base dressings of inorganic fertiliser were applied by the growers before planting, according to their usual protocols. Although their prior agricultural soil analyses gave ADAS indices for nitrogen of 0 to 2, nitrogen was applied at only one site, so the overall soil nitrogen supply was being taken into account. Over the following three years nitrogen concentrations varied quite widely between the sites, but were generally low; foliage colour was not affected, however, and it was considered unlikely any of the plots was deficient in nutrients. In the crops’ first year the ADAS indices for phosphorus and potassium varied between 2 and 4 and 1 and 4, respectively, and by the third year the indices remained in the same range. In a new planting topping-up both P and K would be normally be advised if the indices were 3 or less. Magnesium supplies were adequate throughout, with ADAS indices between 3 and 5 in the first year and 1 and 3 in the third.

Few effects of trace element deficiencies have been reported for daffodils. When Ruamrungsri *et al*. (1996) reported the results of growing daffodil ‘Garden Giant’ in hydroponic culture with various nutrients left out: omitting magnesium resulted in severe interveinal chlorosis near the leaf tips, omitting boron led to the formation of water-soaked areas near the leaf bases, omitting calcium resulted in the fatal browning of the root-tips, and the omission of iron to chlorosis near the base of leaves. Low levels of boron were also reported to reduce the flower yield of daffodils (Emsweller *et al.*, 1938) and increase the incidence of the ‘melting’ disorder of tazetta daffodils (Tompsett, 2002), while calcium and magnesium were also reported as important in maintaining normal bulb growth (Hewitt and Miles, 1954). While many other crops exhibit signs of various trace element deficiencies, particularly of manganese, molybdenum and copper, daffodils do not appear to be particularly liable to them. In the project the soil concentrations of several trace elements varied considerably, though without having any obvious effects. Manganese concentrations were markedly higher at four sites (St Buryan, Rosevidney, Penventon and Fourburrow), while zinc levels were much higher at Goonhavern than elsewhere. High levels of copper cause harmful effects on sensitive crops, especially if the pH is 6.5 or less; copper concentrations reached 50ppm at Mawla and 63ppm at Penventon, putting crops at the latter site, with a pH of 6.3, potentially at risk, although no inimical effects were observed (in fact the crop here was the most vigorous of the ten). The high copper levels at Penventon probably resulted from the organic manure applied at this site (and only at this site), while the daffodil field at Mawla was on the site of the old Tywarnhayle mine, noted for its high yields of copper ore.

In the second and third years of the project nutrient concentrations were also measured in leaf tissue, again with the objective of seeking associations between nutrient levels and the levels of rust. Some quite wide variations in concentrations were found between sites and years. There appeared to be some differences in composition between the three westernmost sites - Kelynack, St Buryan and Tregiffian – and those to the east. Magnesium and sodium levels were higher at the three western sites, as was calcium at Kelynack and Tregiffian (both maritime sites). Manganese levels were low (<20ppm) at Kelynack and St Buryan, and a susceptible crop may have been at risk of deficiency here. Other anomalies were more local: sensitive crops may have been at risk of boron deficiency (<20ppm) at Goonhavern, of molybdenum deficiency (<0.35ppm on an acidic soil) at Fourburrow (pH 5.6–5.9), and of zinc deficiency (<15ppm) at Tregiffian and Fourburrow.

The quite wide ranges of nutrient concentrations found over the project as a whole provided a useful dataset in which to look for associations between soil and tissue nutrient concentrations and rust levels. Simple plots of nutrient concentrations and rust levels showed clearly that any relationships between the two were unlikely. This was confirmed by regression analysis.

There was no evidence for any association between soil or tissue nutrient concentrations and the incidence or severity of rust. Although impossible to prove a negative, this new data is persuasive, and confirms the more tentative conclusions reached as a result of grower information quoted in the Annual Report of project BOF 76 (2013).

Rust: environmental conditions?

Rust symptoms were found at all ten sites in all three years of the project. In almost all cases the symptoms were mild and below a level that would result in the rejection of stems on quality grounds. Rust incidence, on the other hand, was low at flowering in 2013 but considerably higher in 2014 and 2015. There was no consistency between years in the sites that had low or high rust levels, suggesting that rust was not primarily a problem of particular sites, but conditions at the sites were modified by changing environmental forces. Examination of the environmental factors logged at each site indicated that only soil water content (SWC) exhibited major differences between the ten sites, with soil and air temperature, RH and precipitation being comparatively uniform: only SWC was therefore a suitable candidate that might be correlated with the year-to-year and site-to-site differences in rust levels. At the outset of the project the rainfall in winter 2012–2013 was higher than average, and three out of four sites where rust levels were relatively high also had high SWC. The next winter was wetter, with widespread flooding, and although the rainfall in winter 2014–2015 was less severe, the land in many cases remained wet, and in both years a similar pattern of higher rust levels with higher winter SWC was confirmed. Higher SWC resulted in higher rust levels the next spring, though in a minority of cases higher rust levels followed a lower SWC, but low rust levels did not follow a higher SWC. In 16 of the 30 site × year combinations both rust and SWC levels were relatively low, in 10 both were relatively high, and in the remaining 4 rust levels were high despite SWC being low. These findings strongly suggested an association between winter's SWC and spring's level of daffodil rust, with some as-yet unidentified factor interacting with the response in favour of higher rust levels. A rust outbreak seems to be the result of conditions over a period of a few months, rather than a prompt response to a brief environmental trigger, and the finding of putative early-stage rust lesions on the underground parts of stems seems to support this.

When SWC was expressed as the accumulated daily SWC over the 5-month period November–March, regression analysis confirmed some statistically significant associations between SWC and rust level the following spring.

It was shown that several environmental or husbandry factors did not appear to be associated with rust levels.

Conclusion

Apart from the possible involvement of *Stemphylium*, which is yet to be shown to be pathogenic on daffodils, only the effect of high SWC was found to be significantly associated with high rust levels. No pathological, nutritional, husbandry-related or other environmental factors were shown to be involved in the events leading to high rust levels. Because of this, and the nature of daffodil rust symptoms, 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 resulting 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. It seems likely that another, as-yet unidentified factor interacts with SWC to increase rust levels under some conditions, so this does not rule out the involvement of another factor, such as temperature.

Further work

- Continue to investigate the role of *Stemphylium* in daffodils
- Examine how the findings from Cornwall relate to the free-draining soils in eastern England and the Grampians where dust is also a problem
- Investigate what factors interact with SWC by causing higher rust levels when SWC is relatively low; this might be done via pot experiments
- Understand the symptomatology of rust better

Rust management

Controlling physiological disorders brought about by largely unavoidable environmental or climatic conditions may often be impossible, but it should be possible to manage daffodil rust – or oedema – by avoiding planting daffodils, or at least rust-prone cultivars, on low-lying or other poorly draining or compacted areas. It is appreciated that finding suitable daffodil-growing land, especially in west Cornwall, is difficult. The option of not growing 'Golden Ducat' is impractical, because the variety is in demand and, in any case, it appears that many other daffodils can also suffer from the disorder. It is important to state that, generally, outbreaks of rust do remain at a mild, commercially inconsequential level – rust can, to some extent, be lived with. But it is more important to avoid any loss of confidence in the product, especially from export and supermarket customers, and care should be exercised to eliminate any seriously affected stems from the supply chain. It is not practical to introduce further selection during the picking and bunching operation, so it may be up to managers to check the quality of crops shortly before the picking gang goes in – under the right (or wrong) circumstances rust needs only a short window of opportunity to go from inconspicuous to challenging – rust can develop a lot in a week.

Because rust is enhanced by wet soil it is important to avoid compaction, so the problem may, to some extent, revert to looking after the soil. Having time to work the land, when conditions are right for it yet in plenty of time for bulb planting, may be counselling perfection. Yet the problem is real – recent, extensive investigations of soils in southwest England found that over one-third showed high or severe degradation, and half showed moderate damage (Palmer and Smith, 2013). The most damaged fields were those with maize or potatoes or other late-harvested crops, 75% of which showed degradation, and fields with winter cereal crops did not fare much better. The researchers emphasised the use of topsoil lifting and sub-soiling, and avoiding traffic when the soil is too wet. Daffodils have relatively poor root systems, so would be expected to suffer in soils that are worked too shallow or are too compacted.

Technology transfer

- Hanks, G, Collier, R & Jones, J (2013). *Update on project BOF 076: 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 076: 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.
- Anon. (2015). Rust may be linked to wet soil. HDC News, no.211, 8.
- Hanks, GR (2015). Reasons for rust. *AHDB Horticulture Grower*, no. 217, 22-23.
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References

Aartrijk J van, Nes CR van, Peeters, JMM, Raven PWJ & Rooy M de (editors) 1995 Ziekten en afwijkingen bij bolgewassen. 2. Amaryllidaceae, Araceae, Begoniaceae, Cannaceae, Compositae, Iridaceae, Oxalidaceae, Ranunculaceae. IKCL, Lisse, The Netherlands.

ADAS [Soil Scientists Physiology Committee] 1976 *Plant analysis data.* Internal document, June 1976.

ALi C, GongFu D, YanXia S, XueWen X & BaoJu L 2015 Leaf spot on sweet potato (Ipomoea batatas) caused by Stemphylium solani, a new disease in China. *Journal of Phytopathology* 163: 1046-1049.

Baka ZAM 2015 Efficacy of wild medicinal plant extracts against predominant seed-borne fungi of broad bean cultivars. *Acta Phytopathologica et Entomologica Hungarica* 50: 45-65.

Benson BL 2002 Second international asparagus cultivar trial final report. : 159-166.

Bergman BHH, Eijkman AJ, Muller PJ, van Slogteren DHM & Weststeijn G 1978 Ziekten en afwijkingen bij bolgewassen. 2. Amaryllidaceae, Araceae, Begoniaceae, Compositae, Iridaceae, Oxalidaceae, Ranunculaceae. IKC, Lisse, The Netherlands.

Bhatia JN, Devender C 2014 Studies on effectiveness of certain new fungicides in controlling stemphylium blight of onion seed crop. *Agricultural Science Digest* 34: 237-239.

Bould C 1939 Studies on the nutrition of tulips and narcissi. *Journal of Pomology and Horticultural Science* 17: 254-274.

Brunt AA 1995 Narcissus. Pp 322-334 in Loebenstein G, Lawson RH & Brunt AA (editors), *Viruses and virus-like diseases of bulbs and flower crops*. Wiley, Chichester, UK.

Chastagner GS & Byther RS 1985 Bulbs – narcissus, tulips, and iris. Pp 447-506 in Strider DL (editor), *Diseases of Floral Crops*. Praeger Scientific, New York, USA.

Chen T.-C, Li, J-T, Lin, Y-P, Yeh, Y-C, Kang, Y-C, Huang, L-H & Yeh, S-D 2012 Genomic characterization of Calla lily chlorotic spot virus and design of broad-spectrum primers for detection of tospoviruses. *Plant Pathology*, 61: 183-194.

Cook RJ 1976 Black speck on cabbage in Kent. *Plant Pathology* 25: 181-186.

Defra/Welsh Assembly Government 2010-2011 *Fertiliser manual*. 8th edition. Reference book 209. With erratum 2011. TSO, London, UK.

Dickens JSW & Evans SG 1973 A Stemphylium leaf blight of tomato. *Plant Pathology* 22: 70-72.

Digiaro M, Elbeaino T & Martelli GP 2007 Development of degenerate and species-specific primers for the differential and simultaneous RT-PCR detection of grapevine-infecting nepoviruses of subgroups A, B and C. *Journal of Virological Methods*, 141: 34-40.

Edney KL & Morton A 1984 Storage diseases. *Report East Malling Research Station for 1983* : 99-100.

Edney KL & Morton A 1985 Post-harvest diseases of apple and pear. *Report East Malling Research Station for 1984* : 151.

Ellis, MB 1971 *Dematiaceous hyphomycetes*. CAB, Wallingford, UK.

Emsweller SL, Randall GO & Weaver JG 1938 Fertilizer for narcissus bulbs in North Carolina (progress report). *Proceedings of the American Society of Horticultural Science*, 36: 791-795.

Gaspar JO, Belintani P, Almeida AMR & Kitajima C 2008A degenerate primer allows amplification of part of the 3'–terminus of three distinct carlavirus species. *Journal of Virological Methods,* 148: 283–285.

Geeson JD, Browne KM & Everson HP 1988. Storage diseases of carrots in East Anglia 1978-82, and the effects of some pre- and post-harvest factors. *Annals of Applied Biology* 112: 503-514.

Gent DH, du Toit LJ, Fichtner SF, Pappu HR & Schwartz HF 2006Iris yellow spot virus: an emerging threat to onion bulb and seed production. *Plant Disease*, 90: 1468-1480.

Graf S, Bohlen-Janssen H, Miessner S, Wichura A & Stammler G 2016 Differentiation of Stemphylium vesicarium from Stemphylium botryosum as causal agent of the purple spot disease on asparagus in Germany. *European Journal of Plant Pathology* 144: 411-418.

Hanks GR & Chastagner GA in press Diseases of daffodil. In McGobern RJ & Wade HE (editors), *Handbook of Florists' Crops Diseases*. Springer Meteor, USA.

Hanks GR, Carder JH & Rahn CR 1998 *Narcissus: examination of the links between soil nitrogen and basal rot.* Final Report on Project BOF 39. HDC, East Malling, Kent.

Hanse B, Raaijmakers EEM, Schoone AHL & Oorschot PMS 2015 Stemphylium sp., the cause of yellow leaf spot disease in sugar beet (Beta vulgaris L.) in the Netherlands. *European Journal of Plant Pathology* 142: 319-330.

Hewitt EJ & Miles P 1954 The effects of deficiencies and excesses of some mineral nutrients on the growth of tulip and daffodil bulbs in sand culture. *Journal of Horticultural Science*, 29: 237-244.

Kimura M 1980 A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111-120.

MAFF 1986 *The Analysis of Agricultural Materials*. Reference Book 427. HMSO, London, UK.

Magie RO 1948 *Stemphylium* leaf spot of gladiolus in Florida. *Plant Disease Reporter*, 32, 344-345.

Minutillo SA, Marais A, Mascia T, Faure C, Svanella-Dumas L, Theil S, Payet A, Perennec S, Schoen L., Gallitelli D & Candresse T 2015 Complete nucleotide sequence of artichoke latent virus shows it to be a member of the genus macluravirus in the family potyviridae. *Phytopathology*, 105: 1155-1160.

Moore WC with Dickens JSW (editor), Brunt AA, Price D & Rees AR 1979 *Diseases of bulbs.* 2nd edition. Reference book HPD 1. HMSO, London, UK.

Mowat WP 1983 Chocolate spot in Narcissus. Scottish Crops Research Institute Annual Report 1983: 187.

Omar AF, Hafez YM & Emeran AA 2015 Occurrence of a new leaf spot disease on the Egyptian clover (Trifolium alexandrinum L.) caused by Stemphylium globuliferum (Vestergren) in Egypt. *Egyptian Journal of Biological Pest Control* 25: 89-96.

Palmer RC & Smith RP 2013 Soil structural degradation in SW England and its impact on soil-water runoff generation. Soil Use and Management, 29: 567-575.

Pappu SS, Brand R & Pappu HR 1993 A polymerase chain reaction method adapted for selective amplification and cloning of 3' sequences of potyviral genomes: application to dasheen mosaic virus. *Journal of Virological Methods,* 41, 9–20.

Price DJ 1977 Some pathological aspects of narcissus basal rot, caused by Fusarium oxysporum f.sp. narcissi. *Annals of Applie Biology*, 86: 11-17.

Puig M, Moragrega C, Ruz L, Montesinos E & Llorente I 2015 Controlling brown spot of pear by a synthetic antimicrobial peptide under field conditions. *Plant Disease* 99: 1816-1822.

Rees AR 1972 *The Growth of Bulbs*. Academic Press, London, UK.

Ruamrungsri S, Ruamrungsri S & Ohyama T 1996 Visible symptoms of N, P, K, Ca, Mg, Fe and B deficiencies in *Narcissus* L. cv. 'Garden Giant'. *Bulletin of the Faculty of Agriculture, Niigate University 1996*, 49: 41-47.

Ruamrungsri S, Ruamrungsri S, Ooi S, Tanaka Y, Sato T & Ohyama T 1997 A comparative study of mineral elements in bulbs among 6 cultivars of *Narcissus. Bulletin of the Faculty of Agriculture, Niigate University 1997*, 50: 73-81.

Schenk, PK 1955 Een bladlekkenzietke van de gladiool, veroorzaakt door *Stemphylium botryosum* Wallr. *Tijdschr. PlZiekt*., 61: 154-158.

Sheikh F, Dehghani H & Aghajani MA 2015 Screening faba bean (Vicia faba L.) genotypes for resistance to *Stemphylium* blight in Iran. *European Journal of Plant Pathology* 143: 677-689.

Soil Survey of England & Wales 1983 *Soils of England and Wales.* 1:250,000 series. Soil Survey of England & Wales, Harpenden, UK.

Subedi S, Shrestha SM, Kc GB, *et al*. 2015 Evaluation of fungicides against stemphylium blight (*Stemphylium botryosum* Walr) of lentil (*Lens culinaris* Medikus). *Nepalese Journal of Agricultural Sciences* 13: 60-68.

Swain RW 1985 *Plant physiological disorders.*Reference Book 223. HMSO, London, UK.

Sy-Ndir M, Assigbetse KB, Nicole M, Diop TA & Ba AT 2015 Differentiation of Stemphylium solani isolates using random amplified polymorphic DNA markers. *African Journal of Microbiology Research* 9: 915-921.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M & Kumar S 2011 MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*, 28: 2731-2739.

Tompsett AA 1972 Bulbs. Rosewarne Experimental Horticulture Station Annual Report 1971: 15-62.

Tompserr AA 1979 Bulbs. Rosewarne and Isles of Scilly Experimental Horticulture Stations Annual Report 1978: 15-60.

Tompsett AA 2002 *Narcissus tazetta*: boron deficiency as a cause of flower distortion. *Acta Horticulturae,* 570: 141-144.

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M & Rozen SG 2012 Primer3--new capabilities and interfaces. *Nucleic Acids Research*, 40: e115.

Wallis LW 1967 Fertilisers for daffodils. *Daffodil and Tulip Yearbook*, 32: 100-103.

Walsh J 2008 *Reducing losses from virus-induced disorders of processing cabbage*. Final Report on Project FV 160b, HDC, Kenilworth. UK.

Walsh J & Hunter P 2004 *Internal disorders of stored white cabbage.* Factsheet 11/04. HDC, East Malling, UK.

Wei T & Clover G 2008 Use of primers with 5’ non-complementary sequences in RT-PCR for the detection of nepovirus subgroups A and B. *Journal of Virological Methods*, 153: 16-21.

Whalley WM, Mabey M, Turnheim N 1991 The reaction of two green bean (Phaseolus vulgaris) cultivars to Fusarium solani f.sp. phaseoli. *Aspects of Applied Biology* : 133-136.

White TJ, Bruns T, Lee S & Taylor J 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Chapter 38, pp. 315-322 in: *PCR Protocols: a Guide to Methods and Applications* (M. Innis, D. Gelfand, J. Sninsky and T. White, eds.). Academic Press, Orlando, Florida.

XuQing L, ZhongLing T, JiRong Z & JingWu Z 2015 Biological characteristics and fungicide screening of Stemphylium lycopersici causing tomato grey leaf spot. *Acta Agriculturae Zhejiangensis* 27: 1953-1959

Zheng L, Rodoni BC, Gibbs MJ & Gibbs AJ 2010 A novel pair of universal primers for the detection of potyviruses. *Plant Pathology*, 59: 211–220.

Appendix: growth stages (GS) of daffodils

Period	GS	Description[1]	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).